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BACTERIAL PRODUCTION AT DEEP-SEA HYDROTHERMAL VENTS AND COLD SEEPS: EVIDENCE FOR CHEMOSYNTHETIC PRIMARY PRODUCTION

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HISTORICAL PERSPECTIVE

It is well known that the outer surface of the earth is constructed of numerous, semi-rigid plates which together comprise the lithosphere. According to the theory of seafloor spreading (Hess, 1962), new oceanic lithosphere is continuously generated as specialized plate boundaries (spreading centers), which are almost exclusively restricted to ocean basins. Here, molten magma rises from the asthenosphere, cools and accretes to the existing seafloor, and replaces crustal materials displaced laterally and subducted during the spreading process.

Direct measurements of the conductive heat flux at oceanic spreading centers fall short of the expected value predicted from theoretical calculations (Lister, 1980; van Andel, 1981). This discrepancy in the mid-ocean ridge thermal budget led scientists to speculate that convection, in addition to conduction, may be an important process of heat dissipation at active spreading centers. In this model, bottom seawater (~2 °C) penetrates deep into the permeable crust through a complex system of fissures. There it becomes heated and emerges as a warm, buoyant hydrothermal spring or vent. The existence of convective circulation of seawater through newly formed oceanic crust was strongly indicated by measurements of bottom water temperature anomalies at the Galapagos Rift (Williams *et al.*, 1974). It was later confirmed by remote sensing and sampling of the resultant hydrothermal plumes (Klinkhammer, Bender & Weiss, 1977; Lonsdale 1977; Weiss *et al.*, 1977; Bolger, Betzer & Gordon, 1978; Jenkins, Edmond & Corliss, 1978).

Deep-sea bottom photographs obtained at the Galapagos Rift using the unmanned Deep-Tow vehicle (Lonsdale, 1977) revealed oceanic basalts covered with hydrothermal mineral precipitates and an unexpected community of large benthic organisms. The dense

colonies of suspension-feeding molluscs appeared to be intimately associated with the hydrothermal vents, and it was suggested that the high standing stock of macrobenthos resulted from localized increases of food near the hydrothermal plumes.

Typically, life in the deep sea is characterized by sparse populations of metabolically inactive organisms. This is believed to be the result of the extreme conditions imposed on them by food limitation. The availability of planktonic carbon, originally produced in the near-surface seawater through the process of photosynthesis, exhibits a characteristic decrease with increasing water depth. Generally, less than 5% of this photosynthetically produced carbon descends to the seafloor in waters greater than 2000 m (Suess, 1980). The existence of deep-sea hydrothermal vent ecosystems and cold-water seep analogs suggests alternative pathways of primary production to drive the benthic system.

It has been hypothesized that the internal heat of our planet, produced and maintained through the radioactive decay of long-lived isotopes of uranium, thorium and potassium, may represent an alternative energy source to incoming electromagnetic radiation. In this alternative ecosystem model, the intense heating of seawater and subsequent admixture with volcanic solutes and gases yields a liquid medium which could support bacterial primary production either by chemolithoautotrophy (Whittenbury & Kelly 1977; i.e. the reduction of inorganic carbon to carbohydrate at the expense of reduced inorganic compounds, also referred to in this text as chemosynthetic primary productivity by analogy to photosynthetic primary production) or by chemoorganotrophy (i.e. utilization of primordial methane (CH_4) and other abiotically produced organic compounds in the volcanic solutes). On the other hand, the diverse benthic life surrounding hydrothermal vents in the deep sea may originate from, and be sustained by, recycled carbon originally derived from photosynthetic sources. An experimental evaluation of the existence and quantitative significance of these two fundamentally different modes of bacterial nutrition is an objective of the highest priority.

In 1977, a manned-submersible expedition to the Galapagos Rift produced a most remarkable and serendipitous biological discovery. For the first time, hot springs on the seafloor and their associated microbial and macrofaunal communities were directly observed and sampled (Corliss *et al.*, 1979). Subsequent research expeditions to geographically and geologically distinct regions of the seafloor have revealed the presence of similar deep-sea faunal communities.

While the validity of the primary productivity hypothesis has not been substantially challenged, neither has it been (in my opinion) rigorously tested or thoroughly established as fact. On the tenth anniversary of the discovery of the deep-sea hydrothermal vents it is perhaps appropriate to review critically the experimental evidence supporting the novel production hypothesis and, at the same time, to formulate a research prospectus for the next decade.

GEOGRAPHIC LOCATIONS AND DIVERSITY OF GEOLOGIC SETTINGS

Following the initial discovery of microorganisms and macrofauna at the Galapagos Rift, similar hydrothermal vent ecosystems have been discovered at numerous sites along the East Pacific Rise, on the Mid-Atlantic Ridge and at off-axis volcanic island arcs. More recently, animal communities thought to be analogous to the hydrothermal vent ecosystems have been discovered surrounding ambient temperature seeps in the Gulf of Mexico and at the subduction margins of major oceanic plates (Fig. 1). However, major differences might be anticipated between individual 'hot vents' habitats and between the 'hot vents' and 'cold seeps'. This is especially true with regard to the potential source(s) of carbon and energy required to sustain these otherwise morphologically similar communities. The large-scale geologic and small-scale geochemical settings determine, to a large extent, the diversity of potential habitats for the growth and reproduction of chemolithoautotrophic and chemo-organotrophic bacteria. Consequently, a thorough understanding of the chemistry of seawater–basalt interactions and a complete chemical description of the environments are essential for a rigorous evaluation of the chemosynthetic primary production hypothesis.

Hard lava oceanic ridge hydrothermal vents

As a group, these systems are all located at active oceanic spreading centers and consequently have numerous shared characteristics. The circulation of seawater through mid-ocean ridge crustal materials is responsible not only for dissipating a significant amount of heat but also for mediating important water–rock interactions. These interactions constitute a major exchange of ions between the ocean and the igneous basement and provide a mechanism for the transport of selected chemical constituents into the deep sea. Many of these

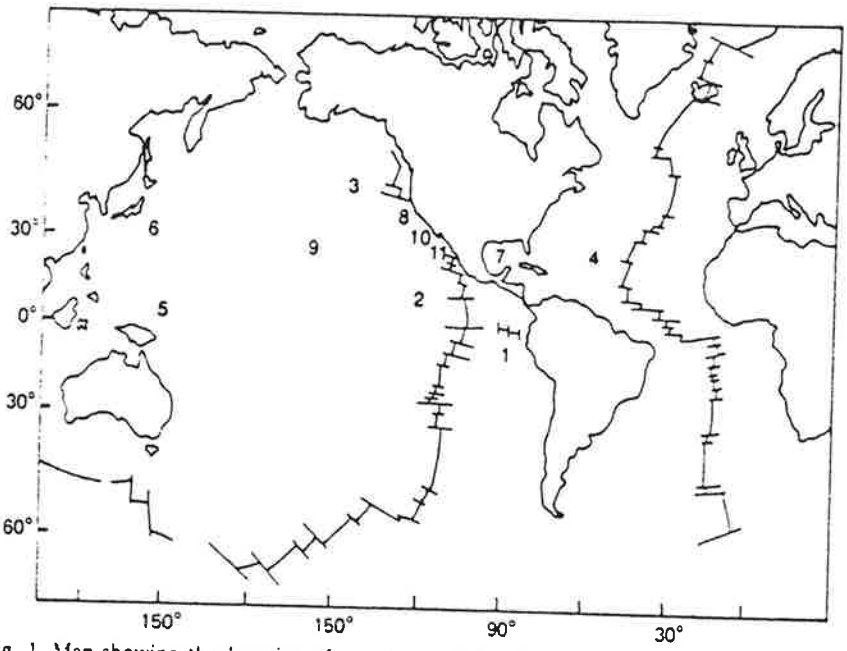


Fig. 1. Map showing the location of a variety of hydrothermal vents and cold-water seeps. (1) Galapagos Rift zone (00°48′N, 86°13′W; 2480 m). (2) East Pacific Rise with vents located at: 10°57′N (2400 m); 12°49′N (2600 m); 20°50′N (2615 m); 18°31′S. (3) Juan de Fuca Ridge including: southern Juan de Fuca Ridge (46°0′N, 130°01′W; 1544 m), northern Juan de Fuca Ridge (46°53′N, 129°17′W; 2370 m), axial seamount – central Juan de Fuca (45°57′N, 130°01′W; 1545 m), Endeavour Ridge (47°57′W, 129°04′W; 2200 m), Explorer Ridge (49°44′W, 130°18′W, 2000 m). (4) Mid-Atlantic Ridge TAG site (26°N; 3200 m) and snake pit site (23°22′N). (5) Manus back-arc basin (3°10′S, 150°17′E; 2500 m). (6) Eurasian–Philippine plate boundary (Tenryu Canyon, Sagami Bay, Japan Trench). (7) Gulf of Mexico including: Orca Basin (26°55′N, 91°20′W; 2400 m), west Florida Escarpment (26°02′N, 84°55′W; 3270 m), Louisiana slope (27°40′N, 91°32′W; 650 m). (8) Oregon subduction zone (44°41′N, 125°18′W; 2036 m). (9) Loihi Seamount (18°55′N, 155°16′W; 1000 m). (10) San Clemente Basin (32°14′N, 117°44′W; 1800 m). (11) Guaymas Basin (27°02′N, 111°22′W; 2020 m).

dissolved ions and solutes (HS^-, NH_4^+, Fe^{2+}, Mn^{2+}, dissolved organic matter (DOM)) and volcanic gases (H_2, CO, CH_4) have the potential to support bacterial chemosynthetic primary production (Table 1).

Initial geochemical investigations of the Galapagos Rift hydrothermal vents indicated that the temperature and bulk fluid chemistry of the water samples were the result of a two end-member mixing process involving: (1) the superheated geothermal fluids produced at depth through the interaction of descending seawater and hot basalt, and (2) ambient bottom seawater (Corliss *et al.*, 1979). If all hydrothermal vents in a given region have end-members of uniform chemical properties, then the concentration of conservative

Table 1. *Potential bacterial metabolic processes at deep-sea hydrothermal vents and cold seeps*

Conditions	Electron (energy) donor	Electron acceptor	Carbon source	Metabolic process
Aerobic	H_2	O_2	CO_2	Hydrogen oxidation
	HS^-, S^0, $S_2O_3^{2-}$, $S_4O_5^{2-}$	O_2	CO_2	Sulfur oxidation
	Fe^{2+}	O_2	CO_2	Iron oxidation
	Mn^{2+}	O_2	?	Manganese oxidation
	NH_4^+, NO_2^-	O_2	CO_2	Nitrification
	CH_4 (and other C-1 compounds)	O_2	CH_4, CO_2, CO	Methane (C-1) oxidation
	Organic compounds	O_2	Organic compounds	Heterotrophic metabolism
Anaerobic	H_2	NO_3^-	CO_2	Hydrogen oxidation
	H_2	S^0, SO_4^{2-}	CO_2	Sulfur and sulfate reduction
	H_2	CO_2	CO_2	Methanogenesis
	CH_4	SO_4	?	Methane oxidation
	Organic compounds	NO_3^-	Organic compounds	Denitrification
	Organic compounds	S^0, SO_4^{2-}	Organic compounds	Sulfur and sulfate reduction
	Organic compounds	Organic compounds	Organic compounds	Fermentation

components that do not react during ascent and mixing (e.g. silica) should conform to a single mixing curve when plotted against temperature (Fig. 2*a*). The chemical variability observed among the individual hydrothermal vents can then be interpreted as evidence for variable mixing between the two end-member fluids. This process is ultimately controlled by subseabed plumbing and by the mechanisms of hydrothermal circulation, and can be expected to vary both spatially and temporally.

Yet the chemical analysis of selected vent water samples indicates that differences may exist in the high temperature end-member fluid composition. For example, the barium concentrations in hydrothermal fluids from the Clambake vent are twice as high (at a given temperature) as the concentrations measured in three other vent fields within a 1.5 km radius (Corliss *et al.*, 1979; Fig. 2*b*). Consequently, while there is a certain amount of chemical uniformity and, therefore, predictability in the Galapagos Rift hydrothermal vents,

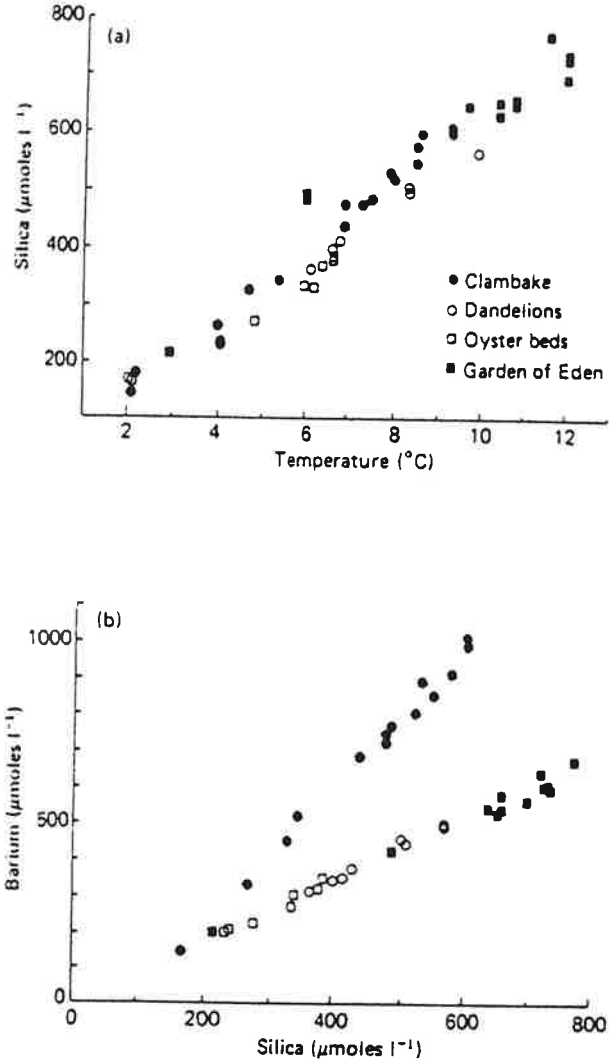


Fig. 2. Compositional trends for selected chemical species at the Galapagos Rift hydrothermal vents. (*a*) Plot of silica concentration against temperature for the four vent fields indicated. (*b*) Plot of barium concentration against silica concentration. (Symbols as in *a*) (From Corliss *et al.*, 1979.)

there also exists considerable variation in both absolute and relative composition of the vent fluids.

Of considerable importance for a critical evaluation of the hypothesis of chemosynthetic bacterial primary production is information on the diversity and concentration of potential electron donors and electron acceptors which might be utilized in these specialized habitats. Although hydrogen sulfide was detected in three of the four

vents initially discovered at the Galapagos Rift (Corliss *et al.*, 1979). its concentration did not vary systematically with vent temperature. Dissolved oxygen (O_2), which is generally considered to be the most efficient electron acceptor for bacterial chemolithoautotrophy, extrapolates, at these sites, to a concentration of zero at a temperature of 8–9 °C (Fig. 3). Nitrate is absent at temperatures above 11–12 °C (Corliss *et al.*, 1979). If these two member mixing models can be considered to be accurate chemical descriptions of hard lava hydrothermal systems in general, then the zone of active aerobic bacterial production must be considered to be a small portion of the total hydrothermal vent habitat (Karl, 1985).

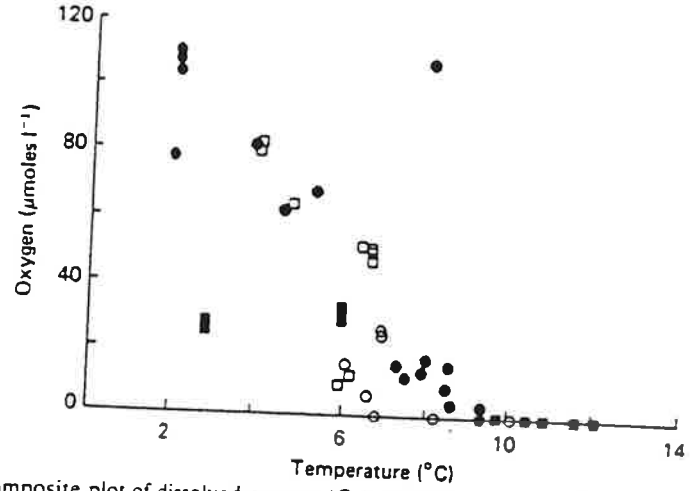


Fig. 3. Composite plot of dissolved oxygen (O_2) against temperature at four Galapagos Rift hydrothermal vent fields. (Symbols as in Fig. 2*a*.) (From Corliss *et al.*, 1979.)

Although the warmest hydrothermal vent waters measured during the initial Galapagos Rift expedition were only ~19 °C, an extrapolation of the silica *versus* temperature data (see Fig. 2*a*) to the quartz solubility curve suggested an end-member fluid temperature of ~350 °C. The discovery of massive sulfide-mineral deposits on the seafloor at the ridge axis of the East Pacific Rise (EPR) spreading center (21 °N latitude), during a 1978 expedition with the French manned-submersible *CYANA*, was strong evidence for the presence of high-temperature solutions (Francheteau *et al.*, 1979; Hekinian *et al.*, 1980). A subsequent cruise to the EPR 21 °N site using the manned submersible *ALVIN* discovered hydrothermal vents with temperatures of 380 ± 30 °C (Spiess *et al.*, 1980) and with the precise end-member fluid composition predicted by Edmond *et al.* (1979)

on the basis of data collected from the low-temperature Galapagos Rift vent water samples. Here, the high-temperature hydrothermal fluids flow from discrete mineralized chimneys at rates of 1–2 $m\,s^{-1}$ (Fig. 4). The end-member fluids are clear until they contact cold ambient bottom seawater at which time they form fine-grain metal sulfide precipitates, hence the term 'black smoker'. The chemical composition of these high temperature end-member fluids has been summarized by Von Damm *et al.* (1985).

The sulfur chemistry in vent fluids is complicated by at least two separate sources of HS^- (seawater sulfate and basaltic sulfur) and by at least two sinks (deposition of sulfate as anhydrite ($CaSO_4$) and the formation of metal sulfide minerals). Sulfide concentrations in the black smoker fluids ranged from 6.7 to 8.7 mmoles kg^{-1} and pH from 3.3 to 3.8 (McDuff & Edmond, 1982; Von Damm *et al.*, 1985). Furthermore, dissolved methane (CH_4) and hydrogen (H_2) measurements indicated concentrations of 50–65 μmol (STP) kg^{-1} and 355–1700 μmol (STP) kg^{-1}, respectively, with considerable variation among the individual vent samples (Welhan & Craig, 1983). The conspicuous lack of significant amounts of C-2 hydrocarbons at 21 °N and the ^{13}C-enriched character of the methane ($\delta^{13}C = -15$ to -17.5 ‰ *vs* PDB; Welhan & Craig, 1983) argue against a thermocatalytic or biogenic origin for methane. The CH_4 flux calculated from the high temperature vents is sufficient to replace the deep-sea CH_4 in ~30 yr, implying a very rapid bacterial consumption in hydrothermal plumes (Welhan & Craig 1979).

The heat flux for a single black smoker is on the order of $4 \times 10^7\,J\,s^{-1}$, a value which is 3–6 times the total theoretical heat flow for a 1 km segment of mid-ocean ridge that extends 30 km on each side (a distance of 30 km is equivalent to a crustal age of 10^6 yr; Macdonald *et al.*, 1980). Clearly, black smokers must be highly ephemeral and probably have an average lifetime of approximately 10 yr (Macdonald, 1982). In 1979, two active black smokers (350 °C) were observed and sampled at the 21°N site designated NGS. However, in 1981 that same vent field was inactive and devoid of macrofauna (Von Damm *et al.*, 1985).

In addition to the black smokers, Galapagos-type warm water vents (≤25 °C), sulfide mound thermal springs and white smoker edifices (<300 °C) were also observed at the 21 °N EPR site (see Fig. 4). Furthermore, much of the hydrothermal fluid effluent appeared to be discharged as a 'line source' along cracks and fissures in the pillow basalt landscape rather than from Galapagos-type 'point

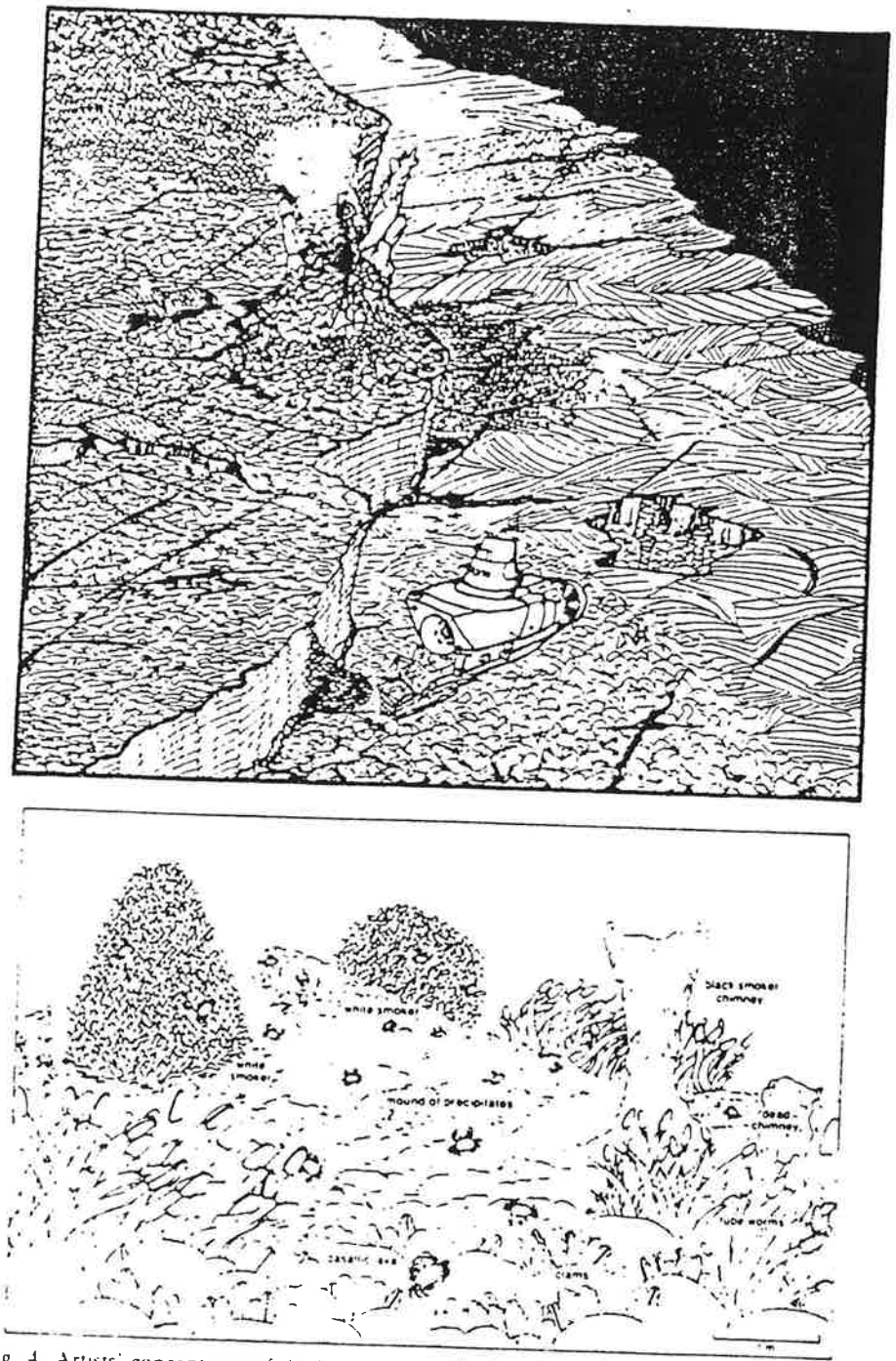


Fig. 4. Artists' conceptions of the large-scale geologic setting of a hydrothermal vent field (11°N, EPR) and the smaller-scale biological communities in the vicinity of a black smoker chimney. Upper drawing from McConachy *et al.* (1986); lower drawing from Haymon & MacDonald (1985).

source' discharges. The similarity of the 21 °N EPR vent fauna to those previously sampled at the Galapagos Rift was the first suggestion that hydrothermal vent communities might be widespread.

Since 1980, direct evidence for the existence of hydrothermal vents has been obtained for numerous hard lava systems (Fig. 1). Additional discoveries and descriptions are likely to follow. For example, Lupton & Craig (1981) have demontrated that the ^3He derived from hydrothermal injections can be detected more than 2000 km west of the EPR spreading center. The extent and magnitude of the ^3He plume between 15 °S and 20 °S are interpreted as strong indications that the largest and most active hydrothermal fields have yet to be discovered (Lupton & Craig, 1981).

Although the hydrothermal vents of the Juan de Fuca–Explorer–Endeavor Ridge system in the northeast Pacific Ocean share certain characteristics with the EPR spreading centers, there are also significant differences in the water chemistry and faunal communities. This was especially evident at the Explorer Ridge where at least one of the high-temperature vents (291 °C) contained no detectable HS^- (Tunnicliffe *et al.*, 1986). This is believed to be the result of HS^- removal by zinc. No animals were present at these 'abiotic' vents (Tunnicliffe *et al.*, 1986). Differences in the faunal community assemblages between the Explorer Ridge and the Juan de Fuca Ridge were attributed, in part, to vent age; the latter displayed geological features of a relatively old system while the former may be newly formed.

Sediment-covered submarine hot springs

In contrast to the hydrothermal vents which have been investigated at sediment-starved mid-ocean ridge spreading centers, hot springs have been discovered or are expected to occur at several sediment-covered locations, including a buried ridge axis, a back arc spreading center and an active hotspot (Fig. 1). In these specialized habitats, hot geothermal fluids interact with the sediments before emerging from the seafloor. This interaction results in a chemical composition which may be quite different from the hydrothermal vents produced by seawater–basalt interactions alone (Edmond & Von Damm, 1985). Thermocatalytic breakdown of sedimentary organic matter results in petroleum, NH_4^+ and other potential substrates for bacterial metabolism. Because these carbon and energy sources ultimately depend upon surface derived organic matter, at least some

of the microbial growth in these regions clearly cannot be considered chemosynthetic primary production.

Cold seeps

Recently, dense populations of hydrothermal vent-type organisms have been discovered in several distinct ambient temperature regions of the deep sea (Fig. 1). Although the geologic setting and geochemical characteristics of these communities are quite different from the hydrothermal vent habitats, the striking similarity of the large epibenthic animals has raised questions regarding the role and nature of bacterial production in these 'cold seep' regions.

At least four separate cold-seep habitats have been described. The first site is an anoxic, hypersaline basin in the northern Gulf of Mexico (Orca Basin; Shokes *et al.*, 1977) with a chemical composition similar to the Red Sea brines and with elevated concentrations of methane and dissolved/particulate organic matter but a low concentration of HS^- (<1 μm; Sackett *et al.*, 1979). A separate hypersaline, HS^- enriched, NH_4^+ enriched (3–4 mM) sedimentary habitat has also been discovered on the passive continental margin at the base of the Florida Escarpment (Paull *et al.*, 1984; Hecker, 1985). Although active fluid flow was not observed, it was inferred from the steep chemical gradients that were measured in sediment pore water profiles. The elevated salinities observed in this region suggest that the fluids are derived from the adjacent Florida carbonate platform rather than from the abyssal plain sediments (Paull *et al.*, 1984). Since the HS^- and NH_4^+ are probably derived from organic matter diagenesis, these substrates would not be expected to support chemosynthetic primary production. Nevertheless, they might support a novel production cycle based on recycled organic matter. A third cold-seep habitat has recently been found at tectonically active plate subduction zones off Oregon, where ancient seawater (i.e. connate water) is discharged as pelagic sediments and then compacted and accreted to the leading edge of the plate (Suess *et al.*, 1985; Kulm *et al.*, 1986). These cold-water seeps are not enriched in HS^- but they do contain substantial concentrations of methane believed to be of biogenic origin (Suess *et al.*, 1985). It has been hypothesized that free-living and symbiotic methane-oxidizing bacteria serve as a localized source of food for this unusual benthic community. Preliminary reports of the existence of similar benthic communities in the region of active accretion between the Eurasian and Philippine

plates (Tenryu Canyon, Sagami Bay and Japan Trench; Swinbanks, 1985*a*, *b*) suggest that this may be a general feature of convergent plate boundaries. Finally, several regions of diffuse oil and gas seepage on the Louisiana continental slope also appear to support the growth of dense populations of macrobenthic animals (Kennicutt *et al.*, 1985).

Perhaps the only general conclusion which can be drawn regarding bacterial production at hard lava oceanic ridges, sediment-covered hot springs and the various cold-seep habitats is that it is unlikely that a single common metabolic process controls the flow of carbon and energy in these diverse deep-sea habitats. Consequently, it is incumbent upon the individual investigators to conduct a careful, region-specific evaluation of the chemosynthetic primary production hypothesis rather than to accept it as fact. Even if the process of chemolithoautotrophy at a particular hot vent or cold seep is established as fact, it may not be appropriate to extrapolate these results to other sites given the above-mentioned diversity in the geophysical properties and geochemical characteristics.

DIVERSITY OF MICROBIAL HABITATS

Deep-sea hydrothermal vents comprise numerous potential habitats for the growth of chemolithoautotrophic microorganisms. While there is a certain degree of chemical similarity among these diverse geomorphological regions, the precise chemical, physical and resultant microbiological signatures of the individual sites are likely to be determined by a complex balance between magma chamber plumbing, hydrothermal circulation and the presence or absence of pelagic sediments. Consequently, it may be inappropriate, if not misleading, to assume that field results from different locations or even between two individual vents from a given site reflect similar microbial processes. Furthermore, since the temporal patterns of fluid discharge have not been studied systematically, it may even be inappropriate to assume constancy for an individual hydrothermal vent over time scales of hours, days or years. While it is now believed that individual hydrothermal vents are ephemeral, with 'life spans' of the order of approximately 10–100 years, it is unknown whether there is a systematic succession of microorganisms which corresponds to vent age. As a result, individual vents must be carefully sampled and properly characterized before a thorough understanding of the complex ecological relationships which are likely to exist can emerge.

To date, this careful sampling and thorough characterization has not been possible.

At least four distinct hydrothermal vent habitats and associated microbial communities have been described: (1) free-living bacterial populations associated with the discharged vent fluids and presumably growing and reproducing within the subseabed system (Karl, Wirsen & Jannasch, 1980; Ruby, Wirsen & Jannasch, 1981; Harwood, Jannasch & Canale-Parola, 1982; Ruby and Jannasch, 1982; Tuttle, Wirsen & Jannasch, 1983); (2) microbial mats growing on rock, chimney, sediment or animal surfaces which are exposed to vent water (Jannasch & Wirsen, 1981; Grassle, 1982); (3) symbiotic associations of microorganisms and vent fauna (Cavanaugh *et al.*, 1981; Cavanaugh, 1985; Desbruyers *et al.*, 1983) such as the vestimentiferan tube worms (*Riftia pachyptila*), giant clams (*Calyptogena magnifica*) and polychaete worms (*Alvinella pompejana*); and (4) microorganisms within deep-sea hydrothermal vent plumes (Winn, Karl & Massoth, 1986; Cowen, Massoth & Baker, 1986).

Due to the absence of rapidly flowing pore waters, cold-seep-region habitats are dominated by sedimentary populations of microorganisms, bacterial mats and possible symbiotic associations with epibenthic and infaunal communities. Although it is likely that sediment-derived nutrients are injected into the overlying water column, the absence of a warm buoyant fluid discharge prevents the formation of a distinct low density plume analogous to those observed in volcanic settings. However, it is conceivable that saline water discharge may result in the formation of a coherent high density plume which would be expected to sink rather than rise. A similar benthic plume might also be expected to occur at high temperature vents that have experienced boiling thereby leaving a residual hypersaline solution.

As most vent water samples are collected at points where the fluid discharge mixes with ambient bottom seawater, we have no quantitative information on the precise chemical and physical properties of the environment (or environments) where microorganisms are likely to proliferate. It is probable that dense microbial mats or lenses grow in response to the gradients created and maintained by subseabed mixing of vent fluids and bottom seawater; however, these environments have been impossible to sample. In order to test adequately hypotheses regarding potential pathways for the flux of carbon and energy, it is imperative to obtain corroborative information on the chemical composition of the suspected source habitats (Karl, 1985).

METHODS OF SAMPLING

The initial discovery and much of the early exploration of hydrothermal vents relied to a large extent upon towed instrument sensors, fine-scale bathymetric mapping, ocean bottom instruments and the collection of water samples from conventional surface vessels. Unfortunately, the zone of neovolcanic activity and, hence, active hydrothermal fluid discharge, is generally restricted to a very narrow portion of the seafloor (1–2 km; Macdonald, 1982) so that sophisticated, manned submersibles (e.g. *ALVIN*, *SEACLIFFE*, *TURTLE*, *CYANA*, *NAUTILE*, *PISCES IV*, *JOHNSON SEALINK*, *MAKALI I*, *PISCES V*, *SHINKAI 2000*) are essential for detailed sampling and instrument deployment. Two major disadvantages of using manned submersibles for hydrothermal vent and cold seep research are their high cost and limited accessibility. The US Navy nuclear powered research and engineering vehicle, *NR-1*, has recently been commissioned for academic research efforts. The unique advantage of *NR-1* is her ability to remain submerged for up to 30 days compared to <12 h for conventional research submarines.

Future research progress is clearly limited by the availability of sample materials for direct *in situ* experimentation and not by the availability of experimental methods, motivated scientists or testable hypotheses. Despite recent and significant improvements in sampling capabilities (Von Damm *et al.*, 1985; Lane *et al.*, 1986; Wirsen, Tuttle & Jannasch, in press), a major technological limitation has been the inability to obtain large-volume samples of undiluted vent water which are suitable for simultaneous chemical and microbiological analyses. Furthermore, the walls and internal surfaces of the hydrothermal vent system, rather than the flowing waters, *per se*, are most likely to be the principal habitats for microbial growth. These are, at best, difficult to sample. Consequently, the results available to date on biomass and metabolic activity of discharged (or dislodged) microbial cells must be taken to represent minimum estimates for the vent ecosystem as a whole.

EVIDENCE FOR BACTERIAL CHEMOLITHOAUTOTROPHY

By analogy to subaerial volcanic systems, it was first suggested that a significant amount of organic carbon utilized by the localized vent habitats could be produced by the process of chemosynthetic primary production (Lonsdale, 1977). It is also possible, however, that bacterial primary production at deep-sea vents may occur via chemo-

organotrophy at the expense of heterotrophic utilization of organic substrates (including methane) produced abiotically during high-temperature rock–water interactions. Nowhere else on earth is there an ecosystem that is not, at least partially, dependent upon photosynthesis. The scientific question of fundamental significance with respect to life at deep-sea vents and cold seeps, then, is whether the energy utilized for growth is derived from geothermal sources or whether microbial production requires allochthonous substrates ultimately derived from photosynthesis. However, irrespective of thermodynamic considerations, the ecological consequences of localized bacterial production of organic matter are indeed profound.

Microscopic analyses of the first deep-sea hydrothermal vent water samples collected during the 1977 Galapagos Rift expedition (analyzed by J. Baross cited in Corliss *et al.*, 1979) revealed the presence of 'high concentrations of sulfur-oxidizing and heterotrophic bacteria (from 10^8 to 10^9 bacteria ml^{-1})' as determined by epifluorescence microscopy of glutaraldehyde-preserved water samples (Corliss *et al.*, 1979). These data supported, although by no means rigorously confirmed, the hypothesis of bacterial chemosynthetic primary production. Since 1977, additional water samples have been analyzed for the abundance and *in situ* metabolic activities of bacterial populations contained within hydrothermal fluids, attached to rock and animal surfaces and associated with hydrothermal vent animals. A summary of the experimental data collected from deep-sea vents is presented below. To date, there have been no systematic microbiological experiments performed at off-axis vent sites or cold water seep habitats.

Distribution and abundance of bacteria in low-temperature (<50 °C) vent waters

When the slightly turbid hydrothermal vent waters are sampled, filtered and directly observed using either bright-field, epifluorescence or electron microscopy, much of the turbidity of the solution can be ascribed to the presence of bacterial cells (Jannasch & Wirsen, 1979; Grassle *et al.*, 1979; Karl, Wirsen & Jannasch, 1980; Fig. 5). An important qualitative observation is the occasional presence of large clumps of bacterial cells and the apparent heterogeneity in cell size and morphology. Cell counts of suspended bacteria in vent waters up to 1 m above the seafloor were 5×10^5 to 10^6 ml^{-1} (Jannasch & Wirsen, 1979; Karl, Wirsen & Jannasch, 1980), approximately

one to two orders of magnitude greater than in control non-vent bottom water. Microscopic examination of bacterial mats, rock/animal surfaces and artificial surfaces deployed *in situ* for a period of 10.5 months has also revealed the presence of dense populations of morphologically distinct bacteria (Jannasch & Wirsen, 1981; Jannasch, 1984).

Quantitative data on adenosine 5′-triphosphate (ATP) concentrations present in the vent waters indicated that the total biomass of microorganisms in the flowing hydrothermal fluids from Garden-of-Eden vent (Galapagos Rift) exceeded that in the control deep water by a factor of >300, and was approximately 4 times greater than the ATP concentration measured for the photosynthetically active surface waters (Karl, Wirsen & Jannasch, 1980). If the ATP data are extrapolated to total living microbial biomass (using a C:ATP ratio of 250 (Karl, 1980), the evidence for a localized bacterial-based food supply is quite overwhelming (Fig. 6). Subsequent microbial biomass investigations of various geographically distinct hydrothermal vent ecosystems at 11° and 21°N on the EPR, the Guaymas Basin in the Gulf of California and the Endeavour Ridge, consistently revealed an elevated presence of microorganisms in discharged vent fluids, relative to ambient bottom seawater (Karl, Burns & Orrett, 1983; Karl *et al.*, 1984; Karl, 1985; Winn & Karl, 1985; Winn, Karl & Massoth, 1986; Table 2). Biomass enrichment factors (ATP in vent water: ATP in ambient deep-sea water) ranged from <3 to >500 for 23 individual vent samples analyzed to date. The maximum ATP concentration of 0.5–1 μg ATP l^{-1} (equivalent to 125–250 μg bacterial C l^{-1}) measured at geographically distinct hydrothermal vents suggests that the potential for biomass production may be similar for a wide variety of hydrothermal vent fields.

These biomass and bacterial cell number data confirm the presence of bacteria in hydrothermal vent fluids and, hence, support the model of localized microorganism production in the deep sea. Alone, however, biomass data can neither confirm the presence nor predict *in situ* metabolic activities of chemolithoautotrophic bacteria.

Enrichment culture isolation of 'target' bacterial species

Microbiologists have traditionally relied upon enrichment culture techniques to obtain pure culture isolates of a given species or physiological group (Schlegel & Jannasch, 1967). Once isolated, these specific bacterial strains can then be used in laboratory experiments

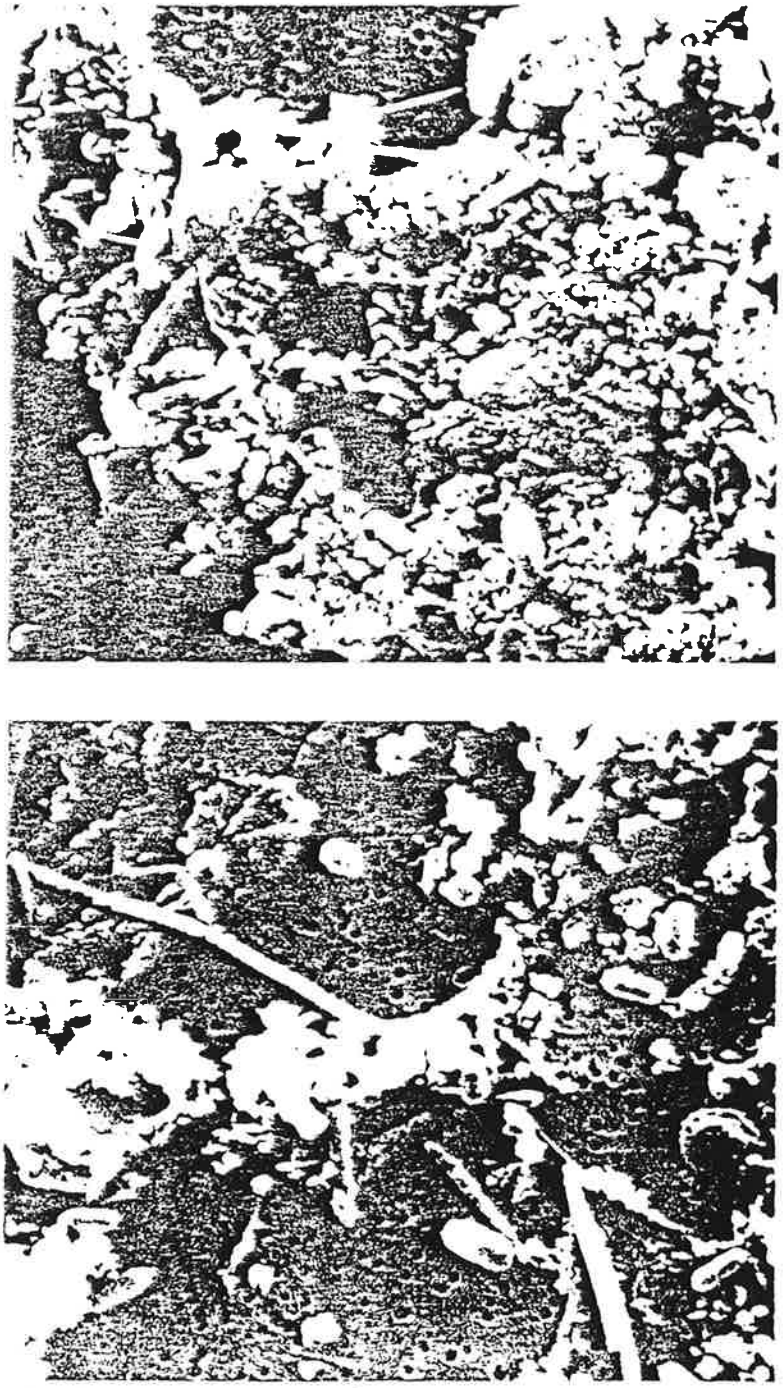


Fig. 5. Scanning electron micrographs of particulate materials from the turbid Galapagos Rift zone vent waters collected on Nuclepore filters. Amorphous particulate material in the upper photo is probably sulfur. Average pore diameter is 0.2 μm.

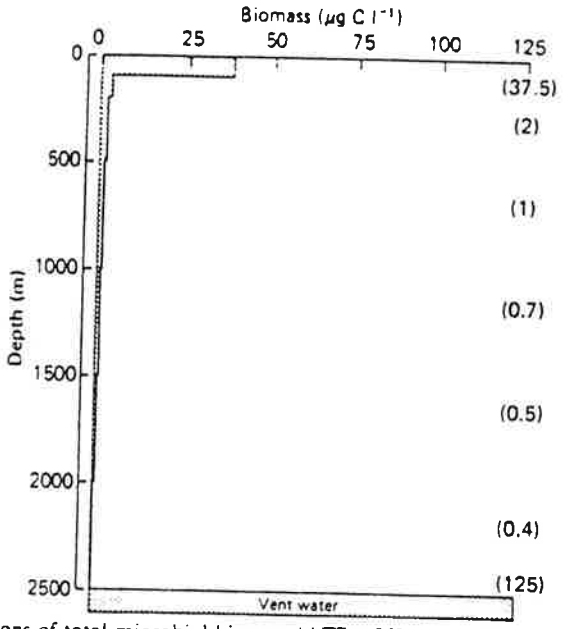


Fig. 6. Distributions of total microbial biomass (ATP × 250) of the water column overlying the Galapagos Rift zone and of a sample collected from the Garden-of-Eden vent (vent water). The samples were collected in Niskin bottles and extracted with boiling phosphate buffer. ATP was measured using the firefly bioluminescence assay system. Values in parentheses correspond to the average value of biomass carbon (μg C l^{-1}) of the depth intervals shown.

designed to study specific physiological, biochemical or genetic characteristics of the isolate.

Successful elective culture of a specific bacterial group is conclusive evidence for the existence of that species in the natural habitat from which the sample was collected. However, a negative result does not necessarily imply that a specific physiological group is absent. In theory, positive enrichment of a particular species or physiological group could result from the presence of a single viable cell or spore. Consequently, unless performed in a semi-quantitative manner (using a vanishing dilution or most probable number (MPN) technique), the ecological significance of elective culture results are of limited value. Furthermore, the mere presence of a given strain does not confirm its *in situ* metabolic activity or capacity for growth and reproduction. These must be evaluated directly under *in situ* conditions.

Elective culture isolation experiments performed using samples collected from deep-sea hydrothermal vents (sometimes, by necessity, under non-sterile conditions) have revealed the presence of

Table 2. *Summary of data on the ATP concentrations measured for water samples collected from geographically distinct hydrothermal vent fields (From D. Karl & C. Winn, unpublished data)*

Vent location	*ALVIN* dive no.	Date	Number of water samples	Range of ATP concentrations (ng l^{-1})
Galapagos Rift				
Clambake	878–9	1–79	11	9.0–52.7
Mussel bed	800	1–79	4	9.0–24.4
Garden-of-Eden	883–4	1–79	20	26–906
Rose garden	983	11–79	3	6.7–30.4
Control, deep water		1–79	3	1.55–1.95
21°N, EPR				
Clam acres	1212	4–82	3	4.4–13.4
Clam acres	1214	4–82	4	6.5–18.8
Clam acres	1216	4–82	1	12.5–15.5
Clam acres	1221	4–82	3	7.0–10.5
Clam acres	1225	4–82	6	20.7–68.9
Clam acres	1228	4–82	1	10.4–17.8
Control, deep water		4–82	4	1.8–2.2
11°N, EPR				
	1381	5–84	8	8.6–26.2
	1385	5–84	1	6.8–11.8
Endeavor Ridge				
	1418–9	7–84	2	24.2–500
Guaymas Basin				
	1603	7–85	2	4.3–25.8
	1610	8–85	1	17.3–46.0
	1615	8–85	1	114.3–208.3

a physiologically diverse microbial community (Table 3). Several target species known to be capable of chemolithoautotrophic growth have been recovered from hydrothermal fluids. The majority of the sulfur-oxidizing bacteria are mixotrophic (i.e. heterotrophic bacteria which exhibit growth stimulation upon the addition of reduced sulfur compounds) rather than the putative obligate chemolithoautotrophs (Ruby, Wirsen & Jannasch, 1981; Jannasch & Wirsen, 1985; Wirsen, Tuttle & Jannasch, 1986). Extinction dilution experiments using a thiosulfate enrichment medium indicated the presence of a viable sulfur-oxidizing population that was one to three orders of magnitude less than that determined by direct microscopy (Wirsen, Tuttle & Jannasch, 1986). The isolation of additional groups, suspected to

Table 3. *Enrichment culture isolation of specific groups of microorganisms in samples collected from deep-sea hydrothermal vents*

Vent location	Sample source	Isolate	Comment	Reference
Galapagos Rift	Mussel bed vent	Free-living spirochete	Obligately anaerobic, heterotrophic, low growth rate and cell yield; can grow in presence of HS^-	Harwood, Jannach & Canale-Parola, 1982
Galapagos Rift	Mussel bed vent water	*Thiobacillus* sp. (strains TB49r, TB49s TB49c)	Chemoautolithotrophic $S_2O_3^{2-}$ oxidizer	Jump & Tuttle, 1981
Galapagos Rift	Rose-garden vent from mussel periostracum	*Thiomicrospira* sp. (strain L-12)	Obligately chemolithotrophic sulfur oxidizer, microaerophilic, tolerant of HS^- up to 300 μM; growth optimum at 25 °C and and 1 atm, barotolerant to 250 atm	Ruby & Jannasch, 1982
Galapagos Rift	Various	95 strains of sulfur-oxidizing bacteria (including 12 obligate chemoautolithotrophs and 83 heterotrophs)	Obligate chemoautolithotrophs were microaerophilic and used HS^-, S^0, $S_4O_6^{2-}$ or $S_2O_3^{2-}$ as an energy source; some heterotrophs produced acid and others produced base when grown on $S_2O_3^{2-}$ + yeast extract; CO_2 incorporation rate for heterotrophs was <5% that measured for autotrophic growth	Ruby, Wirsen & Jannasch, 1981

Galapagos Rift	From glass contact slides left *in situ* for 10 mo at mussel bed vent and from periostracum of a mussel	Manganese oxidizer	Utilize Mn^{2+} with an inducible enzyme system; oxidation requires O_2; showed ATP synthesis at expense of Mn^{2+} oxidation; no evidence for autotrophic growth; they appear to be mixotrophs	Ehrlich, 1983
Galapagos Rift	Scrapings from mussel bed organisms	*Hyphomonas hirschiana*, *Hyphomonas jannaschiana*	Aerobic, heterotrophic prosthecate bacteria; reproduce by budding	Weiner *et al.*, 1985
21 °N, East Pacific Rise	Base of white smoker chimney	*Methanococcus jannaschii* (DSM #2661)	Extremely thermophilic methanogen; optimum growth at 85 °C; obligately anaerobic; grow autotrophically on 80% H_2 + 20% CO_2 with CH_4 formation, doubling time of 26 min at 85 °C	Jones *et al.*, 1983
21 °N, East Pacific Rise	Scrapings from vestimentiferan tube worm	*Thiomicrospira crunogena* (ATCC #35932)	Obligate chemolithoautotrophic sulfur oxidizer, oxidizes $S_2O_3^{2-}$, HS^- and S^0; CO_2 is primary source of carbon; oxidation requires O_2; potentially high growth rate ($0.8\,h^{-1}$); will grow from 4 °–38.5 °C, 1–250 atm	Jannasch *et al.*, 1985
Juan de Fuca	Hydrothermal fluids from sulfide mounds and chimneys	Thermophilic, heterotrophic bacteria	Grown on acetate–GELRITE medium at temperatures up to 120 °; strictly anaerobic	Deming & Baross, 1986

be present and active under the defined environmental conditions, has been unsuccessful. These groups include the large, filamentous mat-forming sulfur bacteria (e.g. *Beggiatoa*, *Thiothrix*, *Thiovulum*) which have been collected but not isolated, the putative bacterial symbionts of the vestimentiferan tube worms and giant clams (see section on symbiosis) and other more exotic or novel microbes (e.g. facultative sulfur-respiring heterotrophs, anaerobic methane-oxidizers, sulfur-respiring methanogens). Successful isolation of members of the latter groups will probably require more creative approaches and a large element of chance. Relatively few microbiologists would regard the isolation of new organisms as a primary goal of their research (Williams, Goodfellow & Vickers, 1984). This is especially true for ecological studies at deep-sea vents where the most fundamental hypotheses can be tested only by direct *in situ* experimentation.

At the present time it is neither known, nor easily determined, whether the species which have been isolated from hydrothermal vents are important components of the natural ecosystem. Future experiments using fluorescent antibodies produced against the pure culture isolates or the analysis of 16S ribosomal RNA nucleotide sequences might help to characterize the natural microbial assemblages. Furthermore, the vent water isolates could also be used in 'caged' culture experiments to evaluate their ability to grow and to reproduce in the suspected natural habitats.

Microbial production and assimilation of carbon dioxide

Critical 'proof' for the hypothesized productivity of chemolithoautotrophic bacteria might be provided by direct measurements of the uptake and assimilation of radiolabeled $^{14}CO_2$ (Jannasch & Wirsen, 1979; Karl, Wirsen & Jannasch, 1980). These technically simple and straightforward experiments are often difficult to interpret. This is primarily because the dark assimilation of $^{14}CO_2$ cannot be equated readily with chemolithoautotrophic metabolism because all microorganisms assimilate $^{14}CO_2$ during normal growth and metabolism (Tuttle & Jannasch, 1973). Consequently an analytical method for uniquely determining $^{14}CO_2$ uptake by chemolithoautotrophic microorganisms must be devised before the measurement of $^{14}CO_2$ uptake can be related quantitatively to chemosynthetic primary production. It is not unexpected that $^{14}CO_2$ is assimilated by hydrothermal vent microbial populations (Table 4).

Table 4. *$^{14}CO_2$ assimilation capacity of deep-sea hydrothermal vent microbial populations (GRZ = Galapagos Rift zone; 21 °N = East Pacific Rise at 21 °N)*

Location	Incubation conditions	Substrate addition	Sample no.	CO_2 incorporated (nmol l^{-1} d^{-1})	Reference
Mussel bed (GRZ)	Dark, *in situ* syringe sample incubation, 23 days	None	1	76.1	Tuttle *et al.*, 1983
		None	2	299.1	
		0.1 mM $S_2O_3^{2-}$	1	271.1	
		0.1 mM $S_2O_3^{2-}$	2	384.2	
		1 mM $S_2O_3^{2-}$	1	233.2	
		1 mM $S_2O_3^{2-}$	2	284.4	
Rose Garden (GRZ)	Dark, *in situ* syringe sample incubation, 6.2 d	None	1	3.4, 33.2[a]	Tuttle, Wirsen & Jannasch, 1983
		None	2	398.1	
		0.1 mM $S_2O_3^{2-}$	1	12.2, 9.2	
		0.1 mM $S_2O_3^{2-}$	2	10.9, 2.5	
		1 mM $S_2O_3^{2-}$	1	79.6, 134.2	
		1 mM $S_2O_3^{2-}$	2	70.2, 97.2	
Rose Garden (GRZ)	Dark, *in situ* serum bottle incubation, 6.2 d	None	1	146.1	Tuttle, Wirsen & Jannasch, 1983
		None	2	2.7	
		None	3	26.7	
		0.5 mM Na_2S	1	140.8	
		10 mM $S_2O_3^{2-}$	1	154.0	
Rose Garden (GRZ)	Dark, *in situ* (3.6°, 245 atm) or on ship (3 °C, 1 atm); all samples 6.2 d	None	1 (*in situ*)	21.0	Tuttle, Wirsen & Jannasch, 1983
		None	2 (*in situ*)	21.3	
		None	3 (ship)	35.7	
		1 mM $S_2O_3^{2-}$	1 (*in situ*)	21.7	
		1 mM $S_2O_3^{2-}$	2 (*in situ*)	37.3	
		1 mM $S_2O_3^{2-}$	3 (ship)	41.0	
		1 mM $S_4O_6^{2-}$	1 (*in situ*)	7.7	
		1 mM $S_4O_6^{2-}$	2 (*in situ*)	8.3	
		1 mM $S_4O_6^{2-}$	3 (ship)	7.0	
Holger's hole (21°N)	Dark, 1 atm, 23 °C, 100 h	None	1	40	Tuttle, 1985
		1 mM $S_2O_3^{2-}$	1	140	
		1 mM $S_2O_3^{2-}$	2	140	
White smoker (21°N)	Dark, 1 atm, 23 °C, 24 h	None	1	210	Tuttle, 1985
		1 mM $S_2O_3^{2-}$	1	190	
		1 mM $S_4O_6^{2-}$	1	430	
White smoker (21°N)	Dark, 1 atm, 3 °C or 23 °C; all samples 24 h	None	1 (3 °C)	10	Tuttle, 1985
		None	2 (23 °C)	230	
		1 mM $S_2O_3^{2-}$	1 (3 °C)	10	
		1 mM $S_2O_3^{2-}$	2 (23 °C)	340	
		1 mM $S_4O_6^{2-}$	1 (3 °C)	20	
		1 mM $S_4O_6^{2-}$	2 (23 °C)	380	

[a]Replication between subsamples from the same syringe

One approach which has been used to evaluate the presence and relative significance of chemolithoautotrophic bacterial production is to measure rates of $^{14}CO_2$ uptake in the presence of added amounts of known chemolithoautotrophic substrates (Tuttle *et al.*, 1983; Tuttle, 1985; Jannasch & Mottl, 1985; Wirsen, Tuttle & Jannasch, 1986; Table 4). Experiments performed to date have employed, exclusively, the addition of reduced sulfur compounds even though the experimental design is amenable to evaluate any organic or inorganic substrate. In general these experiments have yielded inconsistent and variable results which are difficult to interpret within the framework of the chemolithoautotrophic hypothesis. Lack of stimulation of $^{14}CO_2$ uptake following the addition of appropriate growth substrates could result from: (1) the initial presence of an excess concentration of substrate in the natural habitat (i.e. the population is not substrate limited); (2) the selection of an inactive or less preferred substrate in the experimental treatment; or (3) the absence of a significant population of target bacteria. Stimulation of $^{14}CO_2$ uptake, on the other hand, is not rigorous proof for *in situ* chemosynthetic primary production, because the addition of a specific exogenous substrate to a natural assemblage of microorganisms is equivalent to performing an enrichment culture experiment. Long-term incubation with $S_2O_3^{2-}$ has yielded, not unexpectedly, an increase in the number (and presumably activity) of thiosulfate-oxidizing bacteria (Tuttle & Jannasch, 1977). Consequently, it is important to keep the incubation period short (<0.1 of the potential doubling time) and also to monitor the kinetics of $^{14}CO_2$ assimilation. Furthermore, it is essential that the added substrates are highly purified since even a 1% contamination by reduced organic carbon (not unusual in many analytical grade reagents) could represent an addition of potential heterotrophic substrates that would obviously influence the interpretation of the experimental results.

The absence of a systematic stimulation of CO_2 uptake upon the addition of selected chemolithoautotrophic substrates (Table 4) may be interpreted as direct evidence against the role of reduced sulfur compounds in the metabolism of hydrothermal vent microorganisms. Karl (1985) has previously concluded, based on a totally independent line of reasoning, that the role of sulfur compounds may be less important than currently perceived.

Tuttle & Jannasch (1979) have emphasized that the measurement of net assimilation of carbon dioxide can be assumed to represent chemolithoautotrophic growth, but also that ^{14}C tracer experiments

represent non-isotopic equilibrium systems which cannot adequately resolve gross uptake (the combined activities of both heterotrophs and autotrophs) from strictly chemolithoautotrophic metabolism. However, if simultaneous and independent measurements were available for the rate of net microbial carbon production and for the rate of total carbon dioxide assimilation (both expressed in units of carbon produced per unit volume per unit time) one could then determine whether carbon dioxide assimilation represented a significant percentage of the total productivity. In heterotroph-dominated systems the ratio of carbon dioxide assimilated to total carbon produced should be <0.10 (Romanenko, 1964; Overbeck, 1979) whereas in autotroph-dominated systems the ratio would be expected to approach 1.0. The use of ATP (as a measure of total microbial biomass; Karl, 1980) and [^{3}H]-adenine (as a measure of total microbial production; Karl & Winn, 1984) are uniquely suited for such investigations because these experimental methods do not discriminate among physiologically diverse groups of microorganisms.

To date, these two methods of estimating bacterial production have not been systematically coordinated. However, preliminary results obtained for water samples collected from the 21 °N hydrothermal vent site indicate that carbon dioxide assimilation (~100–200 nmoles C l^{-1} d^{-1}; Table 4) represents only a small percentage of the total carbon production estimate based on DNA synthesis (~6000 nmoles C l^{-1} d^{-1}; Karl, 1985). These data suggest a potential for the assimilation of carbon sources other than carbon dioxide and are consistent with the substantial chemoorganotrophic metabolic activity which has been measured at the Galapagos Rift zone (Tuttle, Wirsen & Jannasch, 1983). In the future, simultaneous measurements of carbon dioxide assimilation (using $^{14}CO_2$) and total microbial production (using [^{3}H]-adenine) could be achieved in a single sample using dual-labeled procedures.

In vitro enzyme activities

Ribulose-1,5-bisphosphate carboxylase (RuBPCase) and phosphoenol pyruvate carboxylase (PePCase) are key enzymes that catalyze carboxylation reactions in microbial cells. Many (but not all) chemolithoautotrophic bacteria contain RuBPCase which is the primary carboxylating enzyme in the Calvin–Benson cycle (e.g. reductive pentose phosphate cycle). By comparison, PePCase is present in all autotrophic and heterotrophic organisms. It is suggested that the

presence of RuBPCase might, therefore, indicate the presence of selected groups of chemolithoautotrophic bacteria (e.g. sulfur oxidizers, ammonium oxidizers) while the ratio of RuBPCase to PePCase might indicate the relative importance of such autotrophic bacteria in a mixed microbial assemblage (Morris *et al.*, 1985). The results of laboratory experiments using marine nitrifying bacteria revealed a positive correlation between *in vitro* RuBPCase activity and the rate of *in vivo* chemoautotrophic carbon dioxide fixation (Glover, 1983); however, the amount of carbon dioxide fixed per unit of enzyme activity was species-specific. The *in vitro* activity of PePCase, on the other hand, was independent of the rate of carbon dioxide fixation. In applying this method to natural ecosystems it is essential to bear in mind that the enzyme activity measured represents the contribution from growing cells, metabolically active but non-proliferating cells, intact dead cells, cell fragments and free enzymes adsorbed onto particulate matter.

Tuttle (1985) has adapted this approach for application to deep-sea hydrothermal vent microbial populations. At the onset of these experiments it was first established that sulfur-oxidizing bacteria isolated from hydrothermal vent waters did, in fact, contain RuBPCase activity when grown under autotrophic conditions in the laboratory. On the basis of these experiments, Tuttle (1985) proposed the following reference data: (1) 0.06 units of RuBPCase activity is equivalent to $\sim 10^9$ cells of *Thiomicrospira* sp. or *Thiobacillus* sp. in late exponential phase; (2) RuBPCase in autotrophically grown cells exceeded PePCase by a factor of ~40; and (3) heterotrophs, lacking RuBPCase, exhibited high but variable amounts of PePCase activity which often exceeded the RuBPCase activity of an equivalent number of autotrophic sulfur oxidizers. The field results revealed the presence of both RuBPCase and PePCase in discharged hydrothermal vent fluids. The variability observed in these experiments makes it difficult to formulate any general conclusions. However, based on the results available to date there does not appear to be overwhelming evidence for the existence of a free-living microbial community dominated by chemolithoautotrophs (Tuttle, 1985). In fact, the ratios of RuBPCase to PePCase suggest the presence of a substantial population of heterotrophic bacteria in the hydrothermal vent waters. These data are consistent with direct measurements of the heterotrophic uptake of acetate and glucose in samples collected from the Galapagos Rift vents (Tuttle, Wirsen & Jannasch, 1983; Wirsen, Tuttle & Jannasch, 1986) and with the previously mentioned disparity

between total microbial carbon production and carbon dioxide assimilation. The observed stimulation of chemoorganotrophic activity upon the addition of $S_2O_3^{2-}$ is indicative of the presence of mixotrophic sulfur bacteria and this result is consistent with the results of enrichment culture isolation experiments (Ruby, Wirsen & Jannasch, 1981).

In contrast to the freely flowing vent waters, sample materials collected from various tissues of *Calyptogena* and *Riftia* revealed a preponderance of RuBPCase activity (Tuttle, 1985). The RuBPCase:PePCase ratios ranged from 1.47 for *Calyptogena* stomach tissue to 50.3 for a sample collected from the anterior portion of the trophosome. The potential role of symbiotic chemolithoautotrophic bacteria will be discussed in a subsequent section of this review.

Stable isotope data

The measurement of the naturally occurring stable isotope abundances in samples collected from deep-sea vents and cold seeps provides a potential mechanism for elucidating the sources and utilization pathways for dietary carbon (Rau & Hedges, 1979; Rau, 1981*a*), nitrogen (Rau 1981*b*) and sulfur (Fry, Gest & Hayes, 1983). Since thermodynamic properties of substances depend upon their mass (Craig, 1953), isotopic fractionation is expected to occur during chemical, physical and biological processes. For example, carbon isotopes are known to be fractionated during the process of photosynthesis (Park & Epstein, 1960). Although the data are much more limited, chemolithoautotrophic bacteria appear to use $^{12}CO_2$ preferentially to an even greater extent than do photoautotrophs, thereby synthesizing organic matter with a relative ^{13}C content (referred to as $\delta^{13}C$, measured relative to a PDB (Pedee belemnite) marine carbonate standard) which can be 30‰ lower than that of available inorganic carbon pool (Rau, 1985).

Similarly, the ^{15}N content of animal tissues is known to increase (i.e. $\delta^{15}N$ becomes more positive) as materials flow from primary producers through primary consumers and to higher trophic levels (Minagawa & Wada, 1984). Consequently, it may be possible to use $\delta^{13}C$ and $\delta^{15}N$ measurements to distinguish localized chemolithoautotrophic bacterial production from a food web based on secondary heterotrophic metabolism.

Rau & Hedges (1979) published the first $\delta^{13}C$ results from hydrothermal vent animal tissue which demonstrated a striking depletion

Table 5. *Relative ^{13}C abundances of inorganic and organic carbon pools from various source materials and hydrothermal vent animal tissues*

Sample source	^{13}C (‰)	Reference
Dissolved inorganic carbon	−1.7 to −3.5	Williams *et al.*, 1981
Particulate organic detritus	−20 to −25 (mean −22)	Williams *et al.*, 1981
Non-vent marine organisms	−8 to −25	Rau & Hedges, 1979
Non-vent molluscs	−16 to −18	Rau & Hedges, 1979
Dissolved inorganic carbon, Galapagos Rift (end-member)	−5.1 to −5.9	Craig *et al.*, 1980
Dissolved inorganic carbon, 21 °N site (end-member)	−7	Craig *et al.*, 1980
Thermogenic methane	>0 to −55	Frank, Gormly & Sackett, 1974
Biogenic methane	−60 to −110	Whiticar, Faber & Schoell, 1986
21 °N Methane (end-member)	−15.0 to −17.6	Welhan & Craig, 1983
Guaymas Basin methane	−40 to −50	Welhan & Craig, 1983
Guaymas Basin methane	−69 to −73	Simoneit *et al.*, 1979
Galapagos Rift mussel tissue	−32.7 to −33.9	Rau & Hedges, 1979
Galapagos Rift mussel tissue, 1 m from vent	−33.8 to −33.9	Williams *et al.*, 1981
Galapagos Rift mussel tissue, 8 m from vent	−32.8	Williams *et al.*, 1981
Galapagos Rift clam tissue	−32.0 to −32.1	Rau, 1981*a*
Galapagos Rift vestimentiferan tube worm tissue	−10.8 to −11.0 (muscle) −10.9 to −11.1 (trophosome)	Rau, 1981*a*
Galapagos Rift vestimentiferan tube worm tissue	−10.8 to −11.0	Williams *et al.*, 1981
Galapagos Rift crab tissue	−13.7 to −17.6	Rau, 1985
21 °N, EPR clam tissue	−32.6 to −32.7	Rau, 1981*a*
21 °N, EPR pompeii worm tissue	−11.2	Desbruyeres *et al.*, 1983

of ^{13}C when compared to other known sources of oceanic organic matter. These data were consistent with the hypothesis that a localized source of chemolithoautotrophically produced bacterial carbon was the basis for the Galapagos Rift hydrothermal vent food web. Subsequent measurements of clam tissues collected from the Galapagos Rift and from the 21 °N hydrothermal vent field confirmed these depleted ^{13}C values (Rau, 1981*a*; Table 5). In contrast to the bivalves, samples of several tissues from the vestimentiferan tube worm had isotope ratios that were significantly higher than those previously measured for open ocean biota. This large carbon isotopic dissimilarity between the tube worms and the large clams, which inhabit the

same vent field, was interpreted by Rau (1981*a*) as evidence for at least two independent food source materials.

Subsequent measurements by Williams *et al.* (1981) confirmed these $\delta^{13}C$ values for hydrothermal vent animal tissues. A novel aspect of their study, however, was the measurement of ^{14}C (a radioactive isotope of carbon) in addition to the concentrations of ^{12}C and ^{13}C. Since the $\Delta^{14}C$ content (expressed as the deviation [‰] from the ^{14}C activity of nineteenth-century wood) of the dissolved inorganic carbon (DIOC) derived from magmatic sources is much different from either DIOC of ambient bottom seawater ($\Delta^{14}C = -1000$ and -233‰, respectively) or particulate matter derived from surface waters ($\Delta^{14}C = +20$‰), it is possible to calculate the relative contribution of each individual carbon source to the production of animal tissue. Their results indicated that the principal source of dietary carbon for both mussels and tube worms was derived from the DIOC pool in the vent effluent waters. Since the tissue ^{14}C values were lower than the ambient deep-water DIOC value, at least some of the carbon incorporated into animal tissue must have been derived from magmatic sources (Williams *et al.*, 1981). The nearly identical ^{14}C activities of a sample of vestimentiferan tissue (-270 ± 20‰) and two separate samples of mussel tissue (-263 ± 8‰ and -270 ± 6‰) suggest that the tube worms are utilizing the same DIOC sources even though the $\delta^{13}C$ value of the tube worm tissue was 23‰ greater than the mussel tissue (Williams *et al.*, 1981; Table 5).

Similar carbon isotope measurements have recently been conducted at three cold-water seep sites (Table 6). Kennicutt *et al.* (1985) have reported $\delta^{13}C$ values for animal tissues sampled from the Louisiana seep that are nearly identical to the values previously measured at the Galapagos Rift and 21 °N hydrothermal vents. Although the authors use their data to suggest the presence of a local chemolithoautotrophic food source, other interpretations (e.g. heterotrophic oxidation of isotopically light hydrocarbons or methane) seem equally plausible. Measurements of samples collected from the Florida Escarpment cold-seep site revealed animal tissues with an extremely negative ^{13}C value (Paull *et al.*, 1985; Table 6). The authors attribute these observations to a localized chemosynthetic food source. It is hypothesized that the assimilation of isotopically depleted biogenic methane is partially responsible for these isotope-depleted animal tissues. Measurement of ^{14}C activity, however, indicates that a majority (~60%) of the tissue carbon is 'modern' which

Table 6. *Relative ^{13}C abundances in animal tissues collected from cold-water seep habitats*

Sample source	^{13}C (‰)	Reference
Florida Escarpment		
Mussel tissue	−74.3 (±2.0; n = 10)	Paull *et al.*, 1985
Gastropod tissue	−59.9 (±0.7; n = 2)	Paull *et al.*, 1985
Vestimentiferan tissue	−42.7 (±0.7; n = 3)	Paull *et al.*, 1985
Sedimentary organic carbon	−80.4 (±0.4; n = 3)	Paull *et al.*, 1985
Louisiana Slope		
Clam tissue	−31.2 to −35.4	Kennicutt *et al.*, 1985
Tube worm (tissue)	−27.0	Kennicutt *et al.*, 1985
(tube)	−28.1	
Various fish species	−17.2 to −17.9	Kennicutt *et al.*, 1985
Mussel gill tissue	−51 to −57	Childress *et al.*, 1986
Methane	−45	Childress *et al.*, 1986
Orca Basin		
Dissolved inorganic carbon	−15 to −19	Sackett *et al.*, 1979
Dissolved organic carbon	−23 to −27	Sackett *et al.*, 1979
Particulate organic carbon	−17 to −22.6	Sackett *et al.*, 1979
Methane	−72 to −74.5	Sackett *et al.*, 1979
Oregon Subduction Zone		
Clam gill tissue	−51.6	Kulm *et al.*, 1986
Clam periostracum	−35.7	Kulm *et al.*, 1986
Tube worm (tissue)	−31.9	Kulm *et al.*, 1986
(tube)	−26.7	

restricts the potential contribution of fossil methane sources. This source limitation is inconsistent with the $\delta^{13}C$ data as currently interpreted. Consequently, a fairly complex carbon flux scenario must be devised to accommodate the two independent data sets (Paull *et al.*, 1985).

The results of these carbon isotopic data, as well as the data on $\delta^{15}N$ and $\delta^{34}S$ abundances not explicitly discussed here, clearly indicate a localized source of food for the hydrothermal vent and cold-seep habitats that appears to be separate from organic matter derived from photosynthesis. A major limitation of these investigations concerns the uncertainty in the isotopic composition of all the potential sources of carbon (e.g. vent water DOC, carbon monoxide, methane) supporting microbial production. Furthermore, it seems obvious that one needs to measure directly the $\delta^{13}C$ (and $\delta^{15}N$, $\delta^{34}S$) of the presumed food (i.e. the discharged bacterial cells) but, to date, this has not been possible. Furthermore, it is conceivable that

several independent microbial metabolic processes occur simultaneously which may result in an isotopic signature which is not characteristic of any simple food web. An example of such antagonistic metabolic reactions might be the simultaneous production and oxidation of methane. Both processes fractionate carbon isotopes but in opposite directions. Finally, it must be kept in mind that we have not yet measured, directly or indirectly, the extent to which vent- or seep-associated bacteria fractionate either carbon, nitrogen or sulfur isotopes during metabolism and growth. These experiments could be performed by allowing well characterized vent isolates to grow *in situ* (cage or dialysis-type culture) and by measuring directly the relative isotopic content of the biomass that is produced.

Chemosynthetic symbiosis

During the initial anatomical and histological investigations of the giant vestimentiferan tube worm *Riftia pachyptila* elemental sulfur crystals were discovered in the trophosome. It was suggested that the sulfur deposits might be the result of the metabolism of symbiotic sulfur-oxidizing bacteria (Jones, 1981). Subsequent microscopic, biochemical and enzymatic evidence has been presented confirming the presence of chemolithoautotrophic (RuBPCase-containing) bacteria (Cavanaugh *et al.*, 1981; Felbeck 1981; Cavanaugh, 1985). Since this animal lacks a mouth and a digestive system it was hypothesized that the endosymbiotic bacteria provided organic compounds at the expense of inorganic substrates (HS^-, oxygen and carbon dioxide) supplied by the host, thus providing the first example of a chemolithoautotrophic bacterial–animal symbiosis. Since that initial discovery, many diverse examples of 'chemosynthetic symbiosis' have been reported from reducing sediments and hydrothermal vent habitats (Cavanaugh, 1985). More recently, Childress *et al.* (1986) have demonstrated the existence of a methane-based bacterial symbiotic association with a Louisiana slope mussel. It is likely that other substrate-specific symbioses will also be discovered.

In spite of the apparent widespread occurrence of this phenomenon we know relatively little about the nature of the bacteria associated with the host tissues, their ability to fractionate carbon, nitrogen or sulfur isotopes or the detailed mechanism of the presumed transfer of carbon and energy between host and symbiont.

While these bacterial–animal associations are clearly an example of symbiosis ('living together') the evidence for mutualism is less convincing. Relevant data, previously summarized (Jannasch & Nelson, 1984; Jannasch 1984*b*, 1985; Jannasch & Taylor 1984; Cavanaugh, 1985; Jannasch & Mottl, 1985; Jannasch & Wirsen, 1985), will not be reviewed here. Until the primary symbiont is available for pure culture laboratory study it may be inappropriate to speculate on the quantitative role of this presumed bacterial chemolithoautotrophic symbiosis to the total metabolism of the specific animal host. A recent report of the ability of *Solemya reidi* (a clam) mitochondria to assimilate HS^- and generate ATP (Powell & Somero, 1986) may require that we re-evaluate the nutritional role of these bacterial endosymbiotic associations.

Black smokers and hydrothermal plumes

Ever since the high-temperature vents (>100 °C) were first discovered in 1979 at the 21 °N site, important questions have been raised regarding the possible presence of bacteria in these superheated seawaters. Although the data available thus far are, to a certain extent, contradictory, there does appear to be strong evidence for the existence of thermophilic bacteria in and around the black smoker habitat (Baross, Lilley & Gordon, 1982; Baross & Deming, 1983; Jones *et al.*, 1983; Lilley, Baross & Gordon, 1983; Karl *et al.*, 1984; Deming & Baross, 1986). Perhaps the most important question, however, regards the exact temperature at which thermophilic bacteria from these environments are capable of growing.

Both the *in situ* field experiments and laboratory cultivation at temperatures in excess of 100 °C are technically very difficult to perform. Interpretation of measurements on *in situ* collections of high-temperature vent water samples is further complicated by the difficulty of obtaining pure vent water samples which are undiluted by ambient seawater. The latter contains a diverse assemblage of microorganisms and clearly represents a potential point source of 'contamination'. Likewise, the reported cultivation of vent-derived organisms at 250 °C (Baross & Deming, 1983) has generated much controversy (Trent, Chastain & Yayanos, 1984; White 1984) which, in my opinion, remains unresolved at the present time.

Lane *et al.* (1986) have recently conducted an experiment at the Guaymas Basin hydrothermal vent field wherein a titanium growth

chamber (referred to as 'vent cap') was placed over two separate high-temperature hydrothermal vents (156 and 300 °C). The results of this *in situ* growth experiment indicated that bacteria were able to grow in close proximity to, but not directly in, the high-temperature waters. Similar conclusions were obtained by Karl, Wirsen & Jannasch (1986) based upon field experiments performed during the same cruise. In addition, the data presented by Karl, Wirsen & Jannasch (1986) indicated that undiluted water samples collected from eight separate high-temperature (150–350 °C) vents contained no recognizable bacterial cells and no measurable ATP. Paired water samples collected at a distance of 20–30 cm above the point of hot fluid discharge, however, contained a large number of bacteria, had ATP concentrations in excess of 300 ng l^{-1} and were metabolically active over a wide range of incubation temperatures (25–80 °C; Karl, Wirsen & Jannasch, 1986). These results indicate that the origin of the bacteria associated with high-temperature vent plumes is probably via entrainment of cells from peripheral habitats rather than from the hot geothermal fluids. The entrained microorganisms associated with these hydrothermal fluids may be responsible for the removal of selected metals, reduced gases and dissolved organic matter as the warm buoyant plumes rise and move off-axis (Winn, Karl & Massoth, 1986; Cowen, Massoth & Baker, 1986). Measurement of the growth and metabolic activities of these hydrothermal plume-associated microorganisms represents an important area for future research.

SUMMARY AND FUTURE RESEARCH PROSPECTS

In preparing this review I have been encouraged by the enormous progress which has been made toward elucidating the basis for life at deep-sea hydrothermal vents and cold-seep habitats. Given the fact that we have only recently discovered these novel ecosystems and considering the difficulties in obtaining samples for *in situ* experimentation, I consider the published record of research achievement to be quite remarkable. However, at the same time I have also been impressed by the many important, fundamental scientific questions which remain unresolved or at best only partially answered. First and foremost among the list of future research objectives is the need for a comprehensive and systematic evaluation of the chemolithoautotrophic production hypothesis and for a detailed chemical description of the suspected microbial habitats.

One might arbitrarily conclude that geothermal energy-based bacterial processes are the most logical explanation to account for the observed high bacterial productivity. However, at the present time we must conclude, based on the experimental data available, that the hypothesis of chemosynthetic primary production has not been rigorously tested and, therefore, cannot be accepted as the principle mode of bacterial nutrition at the deep-sea vents and cold seeps. Although chemolithoautotrophic bacteria are clearly present in both habitats, their quantitative contribution to total microbial production is, in my opinion, unknown at the present time. Considering the preponderance of heterotrophic sulfur-oxidizing bacteria (mixotrophs) and the evidence for the importance of anaerobic metabolism, it is probable that the role of free-living chemolithoautotrophic sulfur-oxidizing bacteria has been overestimated. Future experiments should also evaluate the importance of additional potential substrates including hydrogen, methane, reduced organic compounds and, perhaps, pyrophosphate (Liu, Hart & Peck, 1982).

The discussion of bacterial productivity has thus far revolved around carbon assimilation when, in fact, carbon rarely, if ever, limits the productivity of natural microbial assemblages. Future field experiments should focus on the utilization of nitrogen (especially dinitrogen fixation), phosphorus and other essential nutrients. Irrespective of the amount of geothermal energy available to the microbial community, the production of new cellular materials will ultimately be controlled by the availability of these essential nutrients. Under certain conditions of energy (electron) availability accompanied by growth nutrient limitation it is conceivable that the microorganisms present in that particular environment will produce excessive amounts of carbon-rich storage products or excrete nitrogen- and phosphorus-poor soluble organic compounds. Excretion of DOM is known to occur during the normal, energy-sufficient growth of sulfur-oxidizing bacteria. It is interesting to speculate that bacterial–animal symbioses evolved at the vents in response to a condition of inorganic nutrient limitation. In any case, it appears likely that the endosymbiotic bacteria neither grow rapidly nor produce large amounts of biomass but, rather, convert carbon dioxide to soluble reduced carbon compounds for the nutrition of the host animal. The specialized role of endosymbiotic bacteria may help explain our lack of success at culturing these bacteria in spite of their ubiquitous occurrence.

From a thermodynamic perspective it is essential to identify the source of both electron acceptors and electron donors before commenting on the relative importance of chemolithoautotrophic bacterial production. If reduced inorganic substrates are not important as electron donors at deep-sea vents and cold seeps, then what are the alternatives? Lonsdale (1977) discussed the potential importance of thermal advection as a means of focussing photosynthetically derived suspended particulate matter near hydrothermal vent sites. While this hypothesis has enjoyed modest support (Enright *et al.*, 1981), it probably does not represent a process of quantitative significance (Williams *et al.*, 1981). Furthermore this advection hypothesis does not explain the presence of animal communities at numerous cold-seep habitats which exist in the absence of thermal plumes. The potential role of chemoorganotrophic bacterial metabolism, both primary chemoorganotrophy at the expense of abiotically produced organic compounds and the more 'conventional' utilization of recycled photosynthetically derived organic substrates, has not been evaluated carefully or systematically. The possibility of chemical evolution (abiogenic synthesis of organic substrates) at deep-sea vents has been hypothesized (Corliss, Baross & Hoffman, 1981) but has not been tested. However, water samples collected from the geothermally active Red Sea brines have revealed high concentrations of thiocyanate (2.4×10^{-5} M) and glycine (1×10^{-6} M) suggesting an abiotic origin (Ingmanson & Dowler, 1980). A second, less exotic but at the present time equally plausible, source of chemoorganotrophic substrates is the large pool of DOM in the deep ocean (~0.3 to 0.5 mg C l^{-1}). It is conceivable that DOM transported into the vent habitat by percolation of seawater through the fractured landscape represents a major supply of carbon and energy for vent microorganisms. Clearly this ecosystem model, if confirmed, would represent a significant departure from the current views of the vent ecosystem as being independent from photosynthesis. While I do not necessarily claim this to be fact, it is interesting to point out that the productivity and biomass of bacterial cells which have been measured at deep-sea vents (Fig. 6 and Table 2) are not inconsistent with this production mechanism (i.e. maximum biomass of bacteria in vent waters is ~100–200 μg C l^{-1} compared to 300–500 μg C l^{-1} total DOM). In this regard, the measurement of $\delta^{13}C$ or $\Delta^{14}C$ may be essential, provided all potential source pools are also measured. It is also conceivable, and highly probable, that mixotrophic organisms simultaneously utilizing both organic and inorganic electron

donors have evolved at deep-sea vents and cold-seep habitats. The possibility of additional novel pathways for energy metabolism and biosynthesis should not be ignored in the design of future *in situ* experiments. Once the question of bacterial production is resolved, it will be necessary to focus further on the remainder of the food web in order to evaluate the relationship (if any) between microbial biomass and the higher trophic levels.

A major limitation, which is likely to continue to plague *in situ* microbiological studies of deep-sea vents and cold seeps, is the difficulty in obtaining samples representative of the source habitats. This general concern for reliable collection of samples can also be viewed in a much broader context when one considers the effort and expense invested in the exploration of these novel ecosystems. Future microbiological studies should also include the simultaneous investigation of more accessible shallow-water marine geothermal springs and hydrocarbon seeps, at least to the extent that these latter ecosystems can be considered true 'analogs'. The development of additional, unmanned autonomous submersibles with improved sampling and remote manipulation capabilities should also be encouraged. Finally, the construction of a long-term ocean bottom observatory (J. Delaney, personal communication) at an active spreading center, seamount or cold-seep habitat would provide an opportunity to conduct a comprehensive investigation of the spatial and temporal variability of the microbial habitat and an in-depth study of microbial colonization, production and succession.

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