Underwater Domains in Yellowstone Lake Hydrothermal Vent Geochemistry and Bacterial Chemosynthesis

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Abstract

Reduced inorganic compounds of geothermal-origin hydrogen sulfide (H_2S), iron (Fe[II]), and methane (CH_4) were common but not ubiquitous components of hydrothermal vent fluids of Yellowstone Lake at concentrations capable of supporting chemolithoautotrophic (geochemical-oxidizing, carbon dioxide (CO_2)-fixing) bacterial growth. Closely linked to the presence of reduced geochemicals was abundance of chemosynthetic bacteria and dark CO_2 fixation activity. Pronounced productivity at vent sites in the northern basin (Mary and Sedge Bays, Storm and Steamboat Points, and east of Stevenson Island) was accompanied by reduced sulfur stimulation in near-vent receiving waters, while none of these characteristics were found in West Thumb vent fields. Per-liter bacterial productivity at vents (to 9.1 µgC/L/hour) could reach algal photosynthesis in surface waters (to 8.9 µgC/L/hour). Thermophilic (heat-loving) sulfur- and methane-oxidizing bacteria were isolated from vent orifice waters, and CO_2 fixation incubations at 50°C indicated that the majority of chemosynthesis within the vents themselves was optimal at high temperatures. Receiving waters had much less activity at 50°C than at ambient temperature (4–20°C), distinguishing populations of mesophilic (moderate-temperature) bacteria that had also responded to the input of geochemicals from vents. Strong evidence for mineral-dependent bacterial productivity was obtained, with limited data suggesting an influence of lake stage or outflow on vent and productivity characteristics.

Introduction

For decades the colorful mats of bacteria and algae surrounding bubbling vents and fumaroles at Yellowstone National Park have been a focus of both touristic and scientific interest. It is with no small wonder that people look upon the growth of microorganisms in the often very hot, very corrosive fluids. Yet the interaction of biology with geothermal and geochemical energy may be more ancient than any other ecology. Prior to the mid-1970s, many scientists favored the theory of organic matter formation in the atmosphere and initial biological activity in surface brine pools using lightning energy as the primary catalyst (c.f. Miller 1953; Oro et al. 1990). Following the discovery of deep-sea hydrothermal geoecosystems in the mid-1970s, an additional hypothesis was developed, invoking organic matter formation and biological assembly in the high-temperature (to

350°C), high-pressure (>200 atm) deep-sea vents and surroundings. Both theoretical and experimental evidence supporting each theory exist, and in fact the two concepts are not mutually exclusive.

Early life certainly was microbial, at least tolerant of high temperatures, and predominantly made use of chemical energy for metabolic needs. At present, the highest temperatures for growth range to 113°C (Stetter 1999) and the isolated organisms are involved in methane and sulfur transformations. Yellowstone National Park offers a variety of habitats from hot (but <96°C), dissolved geochemical-laden (often to saturation with silicate or carbonate) surface springs and geysers with high microbial diversity (Barns et al. 1994) to hotter (to 130°C), dissolved geochemical-rich (but not saturated) waters and gases of Yellowstone Lake underwater vents and fumaroles. From a biogeochemical and ecological point of view, Yellowstone Lake is appealing because observed maximum vent-fluid temperatures range around or just above the limits for microbial life (Huber et al. 1989; Jørgensen et al. 1992), yet many of the same physical and geochemical characteristics of marine vents are preserved. Other freshwater hydrothermal sites have been identified, including massive sulfide deposits in Lake Tanganyika, East Africa (Tiercelin et al. 1989, 1993); hot-water vents in Lake Baikal, Russia (Crane et al. 1991; Shanks and Callender 1992), and deep microbial mats in Crater Lake, Oregon, USA (Dymond et al. 1989). Given the geochemically derived source of nutrition and the typically harsh physicochemical habitats in which they thrive, it is understandable that the bacteria known as lithotrophs (literally “rock eaters”) are usually the dominant forms of life in such environments. While they provide further rationale for the study of freshwater systems, few are as tractable as Yellowstone Lake for accessibility to study.

The Yellowstone caldera underlies the northern half of Yellowstone Lake, while the Yellowstone River inflow and the southern half of the lake lie outside the caldera boundary. Within the caldera, geothermally heated subsurface water percolating through hot rocks above the magma chamber becomes enriched in carbonate, silicate, and chloride, with some locations additionally rich in methane, iron and sulfide. The park is world-renowned for its geothermal activity. This provides a significant opportunity to delineate vent geochemical effects on bulk lake water composition, because enrichment occurs far from the most significant surface inflow, which is the Yellowstone River in the Southeast Arm (Figure 1). The northern half of Yellowstone Lake is strongly influenced by underwater geothermal hot springs and gas fumaroles. These features release water with high concentrations of silicate and bicarbonate as well as reduced materials of mineral origin, including hydrogen sulfide, Fe[II], methane, and, more rarely, ammonia into the bottom waters. While the vents of Yellowstone Lake resemble deep-sea hydrothermal systems in some important respects, the nearly closed nature of the basin and the relatively small volume of receiving waters provides additional opportunities for process research. Because riverine inputs and outputs may be estimated, Yellowstone Lake geothermal and biogeochemical activities are amenable to budgeting by mass balance (inputs + change = outputs).

Figure 1. Map of Yellowstone Lake showing areas of underwater hydrothermal features sampled by ROV. West Thumb samples ring the entire basin, and Mary Bay, Sedge Bay, Steamboat Point, and Storm Point samples were also within 300 m of shore. Stevenson Island collections were made in the deep canyons just east of the island. Southeast Arm samples were taken midway down the arm (65–90 m water depth). Yellowstone River inlet samples were taken by NPS personnel well upstream of the mouth.

Work over the last 10 years on the development of remotely operated vehicle (ROV) survey and sampling technology (Marocchi et al. 2001; Remsen et al., this volume) demonstrated the absolute necessity of remote sampling of the deep, hot, seemingly inhospitable fluids of Yellowstone Lake vents. Starting with a simple Mini-Rover system consisting of video and still cameras and a claw with small pump-driven sipper tube, photographic surveys and water samples suitable for limited dissolved geochemical (Cl^-, SiO_2, $SO_4^=$, Na^+, etc.) and dissolved gas (CH_4, CO_2, ^{222}Rn) analysis were obtained (Klump et al. 1988). Combining the submersible results with surface-collected samples from the inlet at Southeast

Arm and the outlet at Fishing Bridge, it became apparent that aqueous species and gases found in vent fluids were also significantly enriched in lake water relative to surface inflows (Table 1) and in some cases comparable to marine vent-

Table 1. Mineral content of mid-Atlantic Ridge seawater and marine vents compared with Yellowstone Lake inflow, outflow, and freshwater vents, 1994–1998 sampling results.

Parameter	Units	Maximum or Minimum	Marine Hydro-thermal Fluids (TAG, 26°N)[a]	Mid-Atlantic Seawater[a]	Yellowstone River Inflow	Yellowstone Lake Vent Fluids	Yellowstone River Outflow
Temperature	°C	Maximum	365	2	22	120	20
Acidity	pH	Minimum	3.35	7.8	7.05	4.92	7.29
Dissolved oxygen	mg/L	Minimum	0	7.6	9.0	0	8.5
Hydrogen sulfide	mM	Maximum	3.5	0	0.0004	0.9	0.0006
Sodium	mM	Maximum	537	464	0.078	3.360	0.341
Potassium	mM	Maximum	17.1	9.8	0.022	0.076	0.034
Calcium	mM	Maximum	0.031	0.01	0.093	1.032	0.136
Magnesium	mM	Minimum	0	52.7	0.057	0.025	0.087
Silica	mM	Maximum	20.75	0.2	0.333	1.283	0.223
Chloride	mM	Maximum	636	541	0.007	1.146	0.126
Sulfate	mM	Minimum	0	27.9	0.012	0.054	0.070
Manganese (II)	µM	Maximum	680	0	<0.20	0.87	<0.20
Iron (II)	µM	Maximum	5590	0.0015	<0.02	15	0.05

[a] Marine data from summary of Humphris and McCollom 1998.

ing systems. Although near-surface groundwater may contribute to enrichment, exceptionally strong signals from such geochemical indicators as radon-222 (derived from deep-rock degassing) and high flux rates of methane across the air–water interface imply a major role for submarine vents and fumaroles.

Visual evidence of a long history of submarine geothermal activity is abundant in West Thumb, Mary Bay, Sedge Bay, Steamboat Point, and even in the very deep waters (120 m) off Stevenson Island, all within the caldera boundary (Marocchi et al. 2001). “Vent hole with white ppt. (323'); large relic pipe (176'); sponge attached to relic structure (176'); sulfide seeps, white ppt. (106'); bacterial mat on relic (110'); hot water vent with leeches (143'); sulfide fumaroles with white ppt. (143'); shimmering water with zooplankton swarm (310'); fish near hot water vent (128'); probe in 120°C hot vent—black smoker! (131')” are a few of the annotations from still and video images catalogued from the last few years (Remsen et al., this volume).

Submersible observations reveal some significant similarities and some major differences between the freshwater Yellowstone Lake hydrothermal systems and marine deep-sea hydrothermal vents (Humphris et al. 1995). Both show powerful, highly localized geochemical process signals in solid-phase deposits and dis-

solved chemical species. Both demonstrate finite lifetimes through existence of relic vent fields. Both act as focal points for biological activity (Page et al. 1991; Toulmond et al. 1994; Nelson et al. 1995), particularly in the microbial community (Cary et al. 1993; Cavanaugh 1994; Stetter 1999), with biomass significantly higher than surrounding areas and of distinct composition (Jannasch and Mottl 1985). Low hydrostatic pressure and hence lower maximum temperature, freshwater source material, and continental basement rock composition result in substantially different mineral content of emanating fluids at Yellowstone Lake, however. Biological community development is also far less complex because of the evolutionarily-short existence of the system. One of the most important differences is that Yellowstone Lake has definable, measurable inflows and outflows (compared with, for example, the eastern Pacific Ocean).

Biogeochemical reactions both form and consume reduced minerals, and as the term implies both biotic (microbiological) and abiotic (chemical) mechanisms are involved. Because the reactions have negative free energy, they may be accomplished spontaneously, often under conditions of extreme temperature, pressure, and reactant concentration, or they may be facilitated by enzymes contained within the cytoplasm of the microorganisms known for these reactions. Biogeochemical transformations and a model net reaction are given in Table 2, along with a representative microbial genus or genus prefix that biologically undertakes the transformation (cf. Brock and Madigan 1991).

Biological transformations of dissolved inorganic nutrients occur almost exclusively in the domain of microorganisms (algae, bacteria, fungi) and plants.

Table 2. Biogeochemical transformations, model net reactions, and representative microbial genus or genus prefixes.

Reductive-component model reactions	
Methanogenesis	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ (*Methano** spp.)
Sulfate reduction	$SO_4^{=} + 4H_2 \rightarrow S^{=} + 4H_2O$ (*Desulfo** spp.)
Iron reduction	Fe[III] + $2e^- \rightarrow$ Fe[II] (heterotrophic respiration; e.g., *Shewanella* spp.)
Manganese reduction	Mn[IV] + $4e^- \rightarrow$ Mn[II] (as iron above)
Oxidative-component model reactions	
Methane oxidation	$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$ (*Methylo** spp.)
Reduced sulfur oxidations	$H_2S + \frac{1}{2}O_2 \rightarrow S^0 + H_2O$ (*Thio** spp., *Beggiatoa* spp.) $H_2S + 2O_2 \rightarrow H_2SO_4$ $2S^0 + 3O_2 + H_2O \rightarrow 2H_2SO_4$ $S_2O_3^{=} + 2O_2 + 2H^+ \rightarrow 2H_2SO_4$
Iron oxidation	Fe(II) + $\frac{1}{4}O_2 \rightarrow$ Fe(III) + H_2O (*Ferroglobulus* spp., *Gallionella* spp.)
Ammonia oxidation	$NH_3 + 1\frac{1}{2}O_2 \rightarrow HNO_2 + H_2O$ (*Nitroso** spp.)

* Denotes multiple genera in a group.

In most aquatic environments, microbial activity is restricted to photoautotrophs (photo = energy from light, auto = cellular carbon from CO_2 fixation; algae) and heterotrophs (hetero = organic matter decomposition providing both energy and cellular carbon; bacteria and fungi), with chemolithoautotrophy (chemo = energy from reduced inorganic chemicals, litho = chemicals of geologic origin, auto = CO_2 fixation; bacteria) restricted to the very bottom waters and upper few cm of sediments (Jørgensen and Fenchel 1974). In hydrothermally influenced systems, injection and mixing of relatively stable reduced geochemicals (e.g., CH_4, Fe[II], NH_4^+, H_2S, and intermediate sulfur oxidation products) provides an opportunity for accentuated chemosynthesis and population growth of bacteria responding to the available energy sources (CH_4: Distel and Cavanaugh 1994; Cheng et al. 1999; Fe: Cowen et al. 1986; Hafenbradl et al. 1996; Emerson and Moyer 1997; Mn: Mandernack and Tebo 1993; H_2: Brysch et al. 1987; Nishihara et al. 1990; H_2S: Nelson et al. 1989; Hallberg and Lindstrom 1994). Lithotrophic bacteria require the same inorganic nutrients for biomass production as photoautotrophs and many heterotrophs, and hence compete with them in nutrient cycling. The elemental stoichiometries (mol:mol) of tissue are approximately the same in all these microbes, i.e., $C_{106}\ N_{16}P_1\ S_{0.5}$.

Bacterial growth and metabolism occurs in proportion to the amount of usable nutrients in the environment, while the presence of bacteria depends upon previous access to nutrients. In the context of this work, both the presence and activity of specific bacterial types (e.g., nitrifiers, sulfur oxidizers, methane oxidizers) indicate that the respective nutrient substances are available. By utilizing an appropriate suite of metabolic measurements coupled with enumeration of specific bacterial populations, an independent confirmation of hydrothermal contributions to lake geochemistry is possible, and the extent of biological transformations in geochemical cycling may be elucidated. This paper summarizes efforts to characterize microbial community function specifically in underwater hydrothermal emanations of the Greater Yellowstone Geoecosystem.

Sampling Locations and Methods

Underwater hydrothermal vents have been sampled in Yellowstone Lake for over 15 years (Marocchi et al. 2001; Remsen et al., this volume). Three areas have been repeatedly studied: the West Thumb basin; the northern basin, including Mary Bay, Steamboat Point, Storm Point, and Sedge Bay; and the deep waters just west of Stevenson Island. Suspected vent areas were identified by observations of bubbling, hot-water upwelling, shimmering water, presence of bacterial mats or apparent mineral precipitates, or inappropriately warm water at depth. Due to the limited amount of ROV dive time and weather difficulties on the lake, most effort was focused on reliable vent areas around the above-mentioned features. On occasion, surveys with the ROV delved into unexplored flanks of active regions.

Vent samples have been collected using traditional water sampling bottles over visible bubblers, by wading with hand-held sample bottles, by SCUBA diving with sample bottles and syringe arrays (Buchholz et al. 1995), and by ROV

equipped with a variety of water and solid-phase sampling implements (Klump et al. 1992). For SCUBA samples, divers identified features of interest, then opened the cap of a sample bottle as close to the orifice as possible. In some cases, 60- or 140-cc syringes were filled from the emanating water. For the ROV samples, a progressively more refined mechanism has been developed over the years (Marocchi et al. 2001; Remsen et al., this volume). Initially, the submarine's claw arm held a piece of tubing leading to a peristaltic pump on the surface vessel. The inlet was placed close to a feature and pumped water collected for chemical analysis. Later, a more independent, multiple-closed-loop sampling system was deployed (Klump et al. 1988), yielding more and deeper samples, but of limited volume (a few mL). Subsequently, larger samples were collected using multiple-syringe arrays. Syringes imparted the additional benefit of reducing sample contamination with atmospheric gases and lake water. As a result, measurement of more difficult analytes (e.g., reduced iron, hydrogen sulfide, methane, etc.) could be undertaken. Prior to each day's sampling, the entire sipper system was flushed with ultra-pure deionized water; residual dead-volume was about 30 mL. In a 8 x 140-mL sample this represented only 2–3% dilution, not intolerable for most geochemical analyses or even biological rate measurements, but somewhat more problematic for redox-sensitive analytes (iron and sulfur compounds in particular) and pure culture isolations.

On board the surface vessel, the National Park Service *R/V Cutthroat,* subsamples for sensitive analytes (dissolved gases, sulfur compounds, reduced metals, biological rate parameters, etc.) were taken by syringe (60 or 140 cc) without exposure to air or any non-plastic parts. When possible, derivatization or other means of sample preservation were taken aboard the vessel.

Chemical Analyses

Principal dissolved inorganic compounds were measured by flow injection analysis (FIA; SiO_2, NO_3^-, NO_2^-); ion chromatography (IC; Cl^-, $SO_4^=$); spectroscopy ($HPO_4^=$, NH_4^+), gas chromatography (GC; CH_4), or atomic absorption spectroscopy (AAS; Ca^{++}, Mg^{++}, Na^+, K^+, Fe, Mn) according to standard methods (APHA 1992). Iron was also determined by the ferrozine spectrophotometric method of Stookey (1970) with (total Fe) and without (Fe^{++}) reductant extraction. Total CO_2 was analyzed by the Teflon-membrane FIA method of Hall and Aller (1992). Beginning in 1997, reduced sulfur compounds (H_2S, $S_2O_3^=$, $SO_3^=$) were quantified by a scaled-up modification of the micro-bore high-performance liquid chromatographic (HPLC) method of Vairavamurthy and Mopper (1990) using dithio-bis-nitropyridine (DTNP) derivatization. Much of the analytical equipment was transported to the park, and all labile species were analyzed on site, usually within one day of sampling and preparative stabilization.

Biological Measurements

Bacterial isolates were obtained from vent water samples by enrichment, dilution, and growth on liquid or solidified media using inorganic nutrient supplements (CH_4, Fe[II], H_2S, $S_2O_3^=$, polysulfide) according to a variety of standard

approaches. Enrichment and growth were accomplished at three temperature ranges reflecting types of bacteria expected in these geochemically and geothermally altered habitats (cf. Henry et al. 1994). Mesophiles (bacteria growing at temperatures lower than 40°C) were cultured at room temperature (18–25°C), while thermophiles (best growth at 60–70°C) and extreme or hyperthermophiles (growth at 80–110°C) were incubated in ovens at elevated temperatures (50°C and 80°C respectively).

Reduced sulfur-oxidizing bacteria were a particular focus of attention for several reasons: (1) Yellowstone National Park vents and geysers include representatives unmistakably rich in reduced sulfur, especially hydrogen sulfide (which is odorous) and elemental sulfur (which exhibits a halo of yellow, and sometimes crystalline precipitate, around orifices). The sulfur provides an energy source for chemolithotrophic bacteria to fix carbon dioxide as the principal building-block of tissue. (2) Sulfur-oxidizing bacteria are well represented in, or even dominate, marine hydrothermal vent systems with characteristics comparable to the vents of Yellowstone. (3) Many of the thermophilic and extremely thermophilic bacteria (i.e., growth at very high temperature) described from marine hydrothermal systems are sulfur oxidizers. (4) Certain metabolic characteristics, particularly carbon dioxide fixation in the dark, make possible an assessment of chemolithotrophic growth, including that of sulfur oxidizers, in the presence of other more common heterotrophic (organic matter-degrading) bacteria.

Chemolithotrophic activity (dark) and photosynthetic activity (light) were both assessed by an incubation method in which acid-volatile ^{14}C-bicarbonate was biologically converted into acid-stable organic-^{14}C (CO_2 fixation). All rate measurements were made in acid-washed 20-mL liquid scintillation vials using a temperature-controlled block, with ^{14}C-bicarbonate (ICN Corporation, Costa Mesa, California) added to 1 µCi/mL final activity. Dark fixation incubations extended for 9–12 hours, while photosynthesis measurements used 1.5–3 hour incubations in a light gradient (Back et al. 1991). Supplements and inhibitors were added at 1:100 or higher dilution to minimize inoculation artifacts. Incubation was terminated by addition of 2N H_2SO_4 to pH <2; capped vials were purged of unincorporated ^{14}C at the senior author's home institution in Milwaukee by shaking for 12–24 hours in a fume hood. Liquid scintillation cocktail (Hydrofluor; National Diagnostics, Manville, New Jersey) was added and samples counted in a Packard 1500 liquid scintillation counter (Packard Instruments, Meriden, Connecticut) for 20 minutes or 1% error, whichever came first. Zero time blanks were <200 DPM from additions of 2×10^7 DPM at inoculation. Rate calculations took into account the concentration of total CO_2 measured on site, with controls assayed in triplicate to quintuplicate depending on availability of sample and desired treatment matrix. Areal photosynthesis was modeled with the programs of Fee (1990).

Results and Discussion

Geochemical characteristics of hydrothermal fluids. Several products of hot water–rock interaction have been reliably enhanced in both marine and fresh-

water vents (Table 1). Comparing mid-Atlantic deep water and TAG hydrothermal vent fluids, Humphris and McCollom (1998) listed key geochemicals influenced by hydrothermal processes. Reliable increases have been documented for temperature, acidity, hydrogen sulfide, silicate, manganese, and iron (Table 1) in most marine vents, and Yellowstone Lake vents adhere to these same characteristics when compared with Yellowstone River inlet waters. On the removal side, marine systems completely remove magnesium and sulfate from their source waters, deep in the geothermal system, while this characteristic is muted in Yellowstone Lake vents (Table 1). One complicating factor is that the source concentrations of these components were small at Yellowstone, making such decreases difficult to demonstrate if they indeed occur. More significantly, it is apparent from many analyses that hydrothermal vent fluids at Yellowstone were diluted with lake water deeper in the conduits than we have been able to sample, at least in recent years. Comparison of current findings with much earlier data from Yellowstone Lake (Klump et al. 1988) suggests that vent geochemistry may have changed significantly over a decade. Because vent sites had not been marked until 2001, it is difficult to quantitatively compare among years, except on the broad scale of basin regions (e.g., Mary Bay, West Thumb) and observed extreme values. From the perspective of microbiology, however, geochemical processes were found to increase concentrations of reduced geochemicals supportive of chemosynthetic bacterial productivity in both marine and freshwater hydrothermal systems.

Dark carbon dioxide fixation—measurements of bacterial chemosynthesis. Extensive chemolithotrophic activity by bacteria in Yellowstone Lake was supported by utilization of geochemically reduced compounds and detected via dark $^{14}CO_2$ fixation in water, vent, and microbial mat slurry samples. In addition to outright chemosynthesis under favorable conditions, potential chemosynthesis was sought with the aid of incubation supplements, and microbial activity was verified through the use of specific metabolic inhibitors. In general there were three response patterns to the measurement matrix.

Active bacterial productivity using mineral-derived energy was demonstrated in many vent-orifice samples from the northern and north-central domains. The 1997 experimental design is exemplified by an active vent at Steamboat Point (Figure 2). Unamended control rates of dark CO_2 fixation were often 10–100 times higher than those of samples taken from the open lake and showed substantial inhibition by the prokaryotic protein synthesis inhibitor chloramphenicol (CAP). Methanol (MeOH) used to dissolve CAP had no effect. Addition of ammonium did not enhance chemosynthesis either by stimulating ammonia-oxidizing bacteria or by relieving possible nitrogen limitation of growth during the 9- to 12-hour incubations. Thiosulfate, a model reduced sulfur compound known to support growth of many sulfur-oxidizing bacteria, yielded a 60% stimulation of activity in this case (vent $[H_2S]$ = 34 µM) in the presence or absence of added ammonium. Stimulation also was eliminated by CAP, again indicating bacterial involvement. Collectively these data documented substantial bacterially mediated carbon dioxide fixation in habitats containing utilizable concentrations of

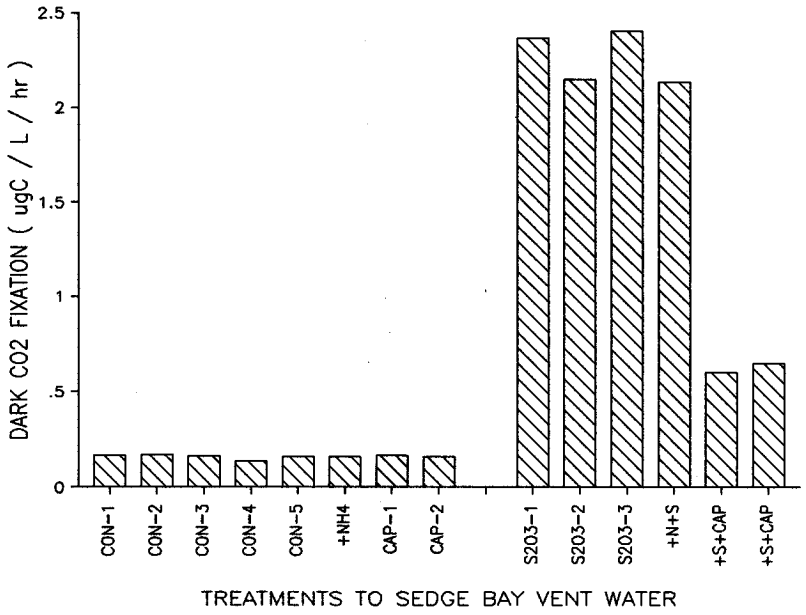

Figure 2. Response of 1998 Sedge Bay shallow-vent dark $^{14}CO_2$ fixation to added poten - tial stimulants (e.g., 5 mM thiosulfate, S_2O_3= or S; 1 mM ammonium, NH_4+ or N; and a protein synthesis inhibitor (20 µg/mL chloramphenicol, CAP) alone or in combination (final concentration given). Individual replicates are shown.

reduced geochemicals.

Potential chemosynthesis was frequently encountered in the immediate vicinity of active vents and fumaroles, particularly where vigorous turbulent mixing of the water column was common (e.g., shallow nearshore areas) or where vent fluids were injected into confined volumes (e.g., narrow canyons). This response is well documented by a SCUBA-collected sample from a shallow (3 m deep) fissure in Sedge Bay, shimmering with warm water but readily exchangeable with overlying lake water (Figure 3). Controls and nitrogenous supplements yielded rates only 3–4 times higher than values in water taken from the open lake, but thiosulfate addition increased CO_2 fixation by over fifteenfold to levels competitive with photosynthesis. Thiosulfate stimulation was greatly reduced by CAP, as before, but the inhibitor had little effect on control activity. Often when dark fixation was low, the growth-oriented inhibitor CAP had little influence, but growth response to stimulation remained sensitive. Again ammonium addition was without effect. In these circumstances it was clear that when reduced geochemicals became available, bacterial populations were present and capable of immediate growth resumption. The spatial distribution of potential production most likely reflected the recent history and magnitude of reduced geochemical emanations.

The third type of finding was the absence of chemosynthetic activity (Figure 4), which is normal in non-geothermally influenced waters but provides an

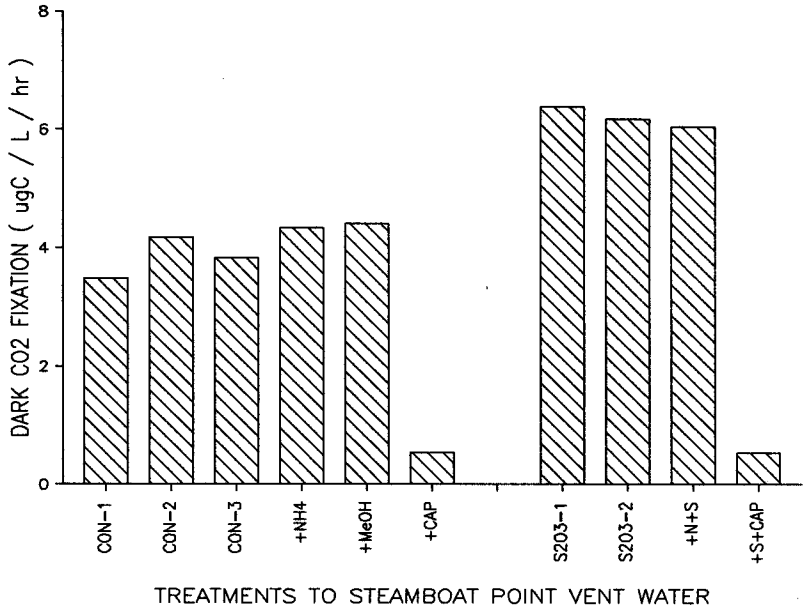

Figure 3. Response of 1998 Steamboat Point shallow-vent dark $^{14}CO_2$ fixation to added potential stimulants, as in Figure 2. Control for CAP addition was methanol (MeOH), the solvent. Due to limited sample availability, all samples did not receive all treatments.

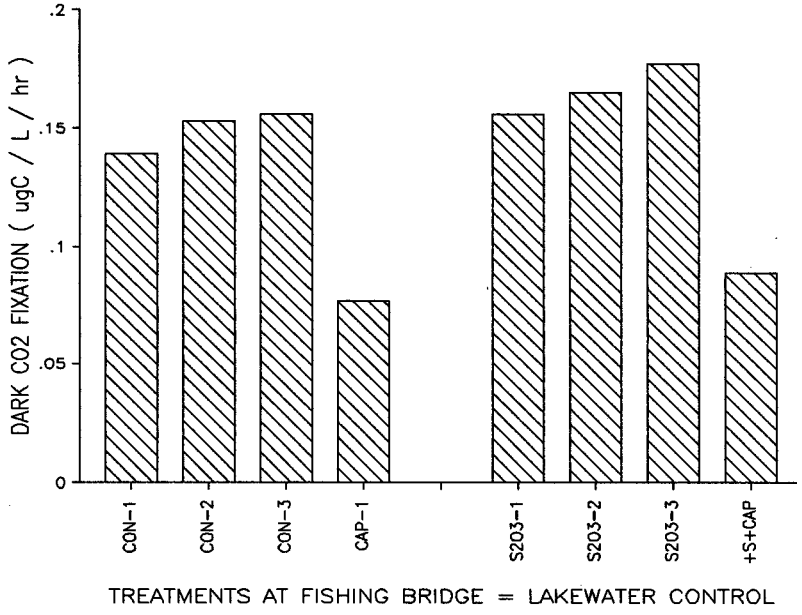

Figure 4. Response of 1998 surface water of the Yellowstone River outflow at Fishing Bridge dark $^{14}CO_2$ fixation to added potential stimulants, as in Figure 2.

important control in Yellowstone Lake. In this example, using the Yellowstone River outflow during 1997, unamended controls showed very low rates of dark CO_2 fixation (note scale expansion relative to Figures 2 and 3). Furthermore, addition of thiosulfate was not stimulatory and CAP exerted only moderate inhibition. While the absolute rates vary from extreme lows in the Southeast Arm to slightly higher values in the surface waters of the northern basin, the characteristics of non-stimulation by reduced sulfur and weak CAP effect are consistently demonstrable.

With the advent of large-volume ROV sampling (approximately 1 liter per sample) in 1997 came opportunities to measure bacterial productivity rates as well as aqueous chemistry on vent samples. Previously, only samples collected by divers or in the proximity of vents (with manual water samplers) could be tested for chemosynthesis processes to complement enrichment, isolation, and pure culture work. The 200 mL or more required for worthwhile rate measurement effort was simply too dear given the great value of interannual chemical analysis comparisons. Of the hundred samples from vents, fumaroles, water column profiles, and other lake sites, the vast majority fit one of the three above response styles. We now apply these results to understanding biogeochemical interaction of microorganisms and reduced compound emanations in specific vent fields and overlying waters.

Photosynthesis—the basis for comparison. In lakes, primary production (i.e., CO_2 fixation into organic matter) is usually carried out by photosynthetic organisms (algae, rooted plants) using light energy, in contrast to chemosynthetic CO_2 fixation described above and below. To place the bacterial contribution in perspective, a survey of photosynthesis was undertaken each year. A vertical profile of CO_2 fixation vs. irradiance was obtained with a photosynthetron (Lewis and Smith 1983) and areal productivity ($mgC/m^2/day$) calculated using the programs of Fee (1990). Both volumetric potential (µgC/L/hour) and most probable areal rates are relevant for comparison. Annual surveys exemplified by 1996 results provided representative photosynthesis rate ranges (Figure 5) for the main regions of Yellowstone Lake. In this approach, the light dependence of photosynthesis was measured in a light gradient, and the results used in conjunction with light penetration profiles to calculate whole water column photosynthesis ($mg/m^2/day$). Also relevant for comparison with chemosynthesis was the maximum volumetric rate of photosynthesis (µgC/L/hour), approximated by data between 250–800 µmol photosynthetically active radiation (PAR; 400–700 nm) photons/m^2/sec (10–30% of full sunlight).

Lowest volumetric photosynthesis was always found in the Yellowstone River inlet at the tip of Southeast Arm, with similarly low rates in the open waters of Stevenson Island and Mary Bay (1–2 µgC/L/hr). Intermediate volumetric productivity was attained in enclosed basins of West Thumb and the central Southeast Arm (3–4 µgC/L/hr), while the highest rate was found in the Yellowstone River outlet (5 µgC/L/hr). Chemosynthesis was certainly on a par with photosynthesis in the above examples, demonstrating that chemical energy

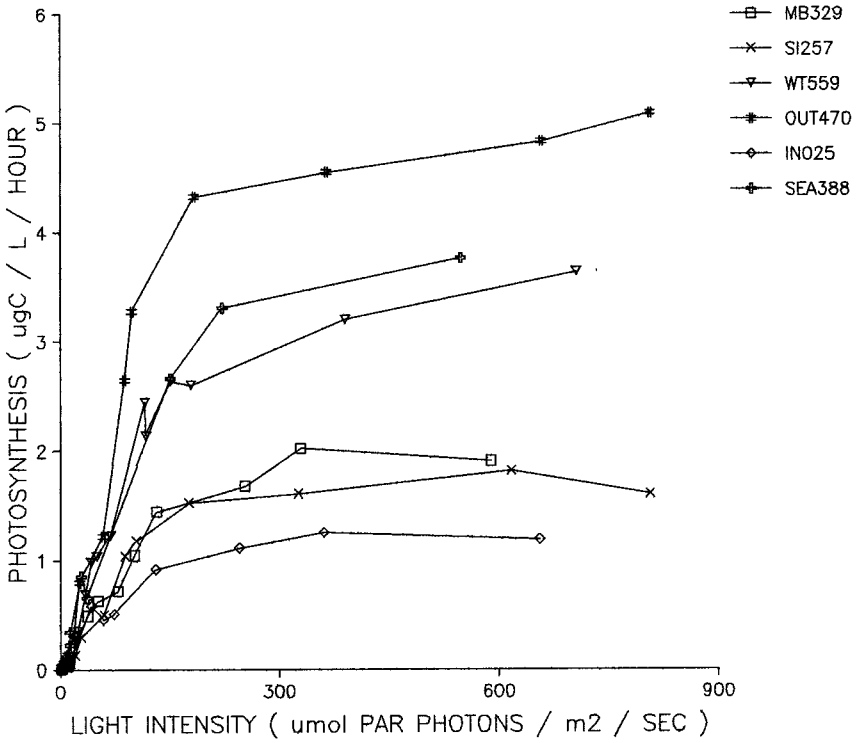

Figure 5. Light dependence of photosynthesis varied among locations in Yellowstone Lake. The three-digit number in the legend is the areal production (mgC/m2/day) calcu - lated from these curves for 1996. MB, Mary Bay; SI, Stevenson Island; WT, West Thumb; OUT, Yellowstone River outlet at Fishing Bridge; IN, Yellowstone River inlet at Southeast Arm; SEA, Southeast Arm mid-basin.

could be as effective as light energy in promoting CO_2 assimilation into organic matter. Thus, active vents could attain sufficient production to support at least some degree of bacterial-based food web activity.

Areal production, integrated over the depth of the water column (for chemosynthesis) or over the depth of PAR light penetration (for photosynthesis), is a measure of ecosystem-level contribution. In the low-water year 1994, northern-basin chemosynthesis off Stevenson Island (3930 $mgC/m^2/day$) was significantly greater than photosynthesis (1620 $mgC/m^2/day$). Dark CO_2 fixation rates were only 2.2 µgC/L/hour but were uniform over a 75-m water column and the 24-hour day, while photosynthesis maxima were higher at 6–9 µgC/L/hour but decreased rapidly with depth (hence light) for the 14-hour light-day. Subsequent higher-water years demonstrated decreased areal photosynthesis and greatly reduced water column chemosynthesis. In the 1996 example (Figure 3), calculated areal production ($mgC/m^2/day$) by water column algae was highest in West Thumb (559) and Yellowstone River outflow (470) samples; intermediate in

Southeast Arm (388), Mary Bay (329), and Stevenson Island (257); and extremely low in the Yellowstone River inlet (25). Water column chemosynthesis was very low during high-water years, so areal chemosynthesis was dominated by near-vent production. At an average of 5 µgC/L/hour, vent haloes alone could account for over 100 mgC/m^2/day, a significant but limited contribution to total water column biomass production.

Biogeochemical domains of chemolithotrophy and a role for dissolved minerals. Reactions of rock and hot water at high hydrostatic pressure result in both passive geochemical leaching (e.g., chloride, silicate, carbonate) and active mineral transformation (e.g., reduction of carbon dioxide to methane, sulfate to sulfide, Fe[III] to Fe[II], Mn[IV] to Mn[II], often using hydrogen gas as reductant). In the areas of West Thumb and northern Yellowstone Lake, thermal features on shore appear to descend directly into the lake, and in fact underwater vents are abundant in those and other areas (Marocchi et al. 2001; Remsen et al., this volume). Biogeochemical domains (that is, characteristically coherent regions) appear to be important in both surface- and underwater venting systems.

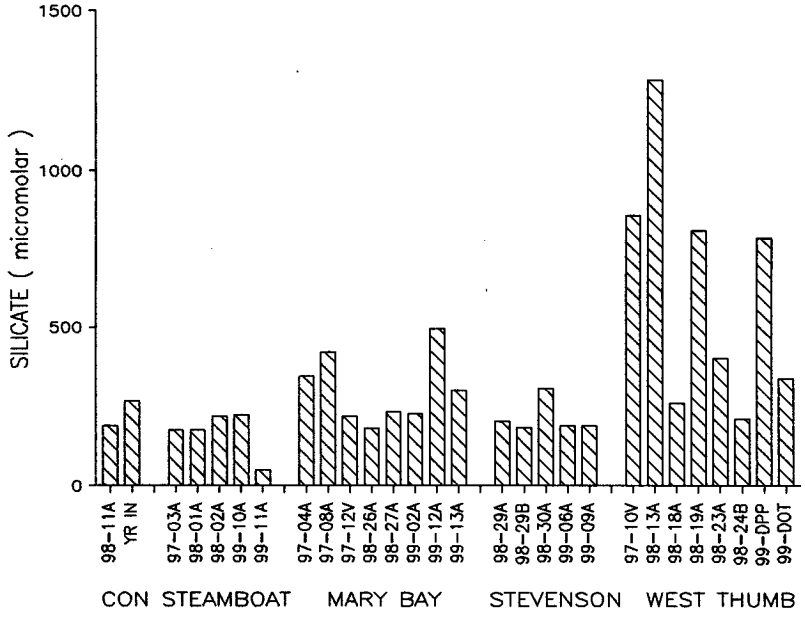

Figure 6. Domains of biogeochemistry were apparent at underwater hydrothermal vents in Yellowstone Lake, 1997–1999, as demonstrated by selected geochemical concentrations (Figures 6–10) and dark $^{14}CO_2$ fixation (Figure 11) in vent waters. Silicate showed strong enrichment in West Thumb vents. Left column, 98-11A, was a control bottom sample (35 m) taken with the ROV in a cold (10°C) inactive relic vent field in Mary Bay. YR is the Yellowstone River inlet control. From left to right, vent samples from Steamboat Point (5), Mary Bay (8), Stevenson Island (5), and West Thumb (8) are shown for each parameter. 1999 DPP (Pumice Point) and DOT (Otter vent) samples from the West Thumb area were collected by Jim Bruckner using SCUBA diving. Results are shown for all analyses, with low values appearing as blank. Missing samples (CH_4 only) have no identification label.

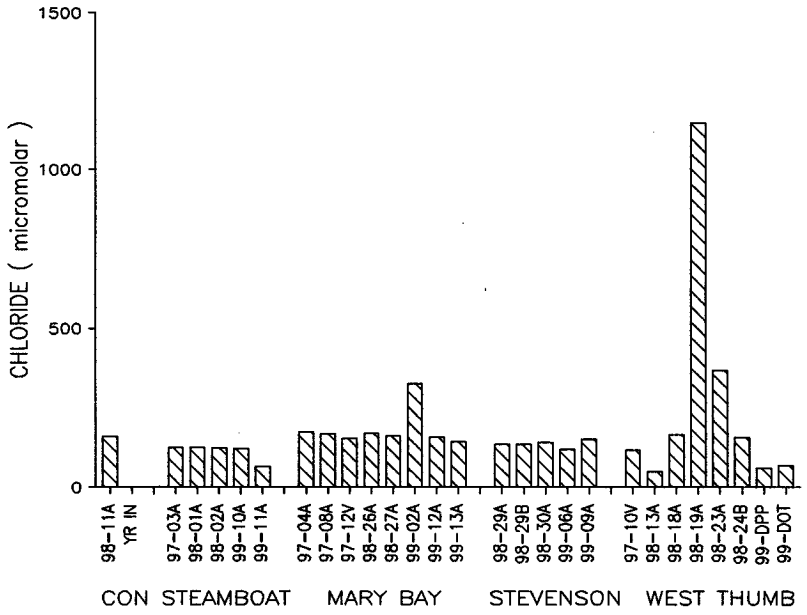

Figure 7. Chloride enrichment was less frequent in 1997–1999, but occurred in West Thumb vent waters.

Both silicate (Figure 6) and chloride (Figure 7) in hydrothermal vent fluids from West Thumb were frequently enhanced over lake water mean values of about 200 µM and 100 µM, respectively. In these and subsequent figures, vent 98-11A (far left) is a sampling control taken by the ROV sipper system very near the bottom in a deep but inactive relic vent field in Mary Bay, and represents one type of lake water control value. The Yellowstone River inlet is another important control sample. Though not all vents in West Thumb displayed elevated SiO_2 and Cl^-, only vents in this area reliably did so during 1997–1998 sampling efforts. Only slight increases in SiO_2 (less than twofold) were seen in 1997 Mary Bay and one 1998 Stevenson Island vent. The fact that only one area demonstrates high solute levels, yet all areas contain vents reaching extreme temperatures (up to 120°C), suggests that very different source reservoirs or vent conduit systems exist in the western vs. northern parts of the lake.

Three other geochemical indicators of water–rock interaction obeyed different domain specificity. Total CO_2 (lake water mean 0.6 mM) was variably but reliably enriched in all domains (Figure 8) with the most extreme values all in the Mary Bay region. Collection and handling of these samples was very important in obtaining accurate results because of degassing. Many of the vent samples formed visible bubbles with time in bottles on deck even though they were initially as warm or warmer than surface waters. For sensitive samples, however, we collected sub-samples in rubber-free syringes minutes after the submarine was out of the water. $\sum CO_2$ was found to decrease with a half-life of about 20 min-

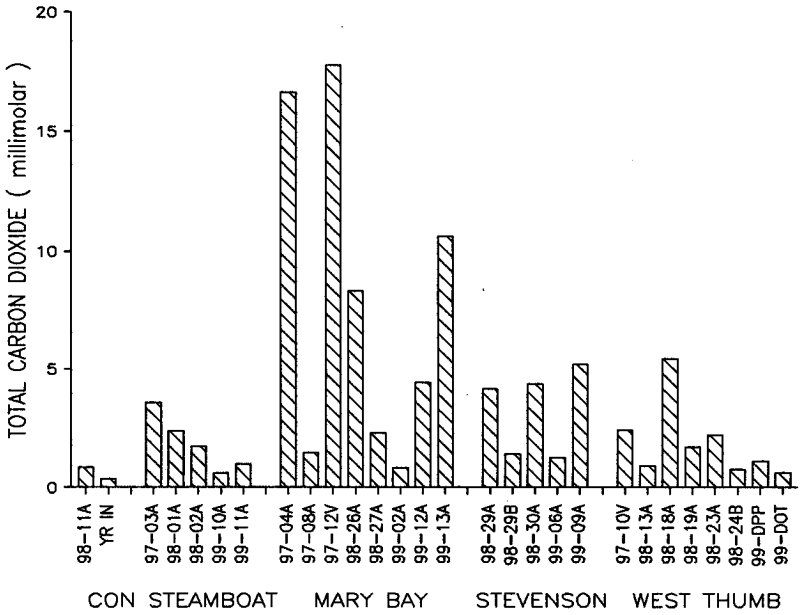

Figure 8. Total CO_2 enrichment was widespread and strong in many deeper vents regard - less of location.

utes in a beaker, but persisted undiminished for 4 to 6 hours in capped syringes (data not shown). All reported measurements of vent water $\sum CO_2$ were analyzed directly in capped syringes and represent the best (i.e., slightly less than to equal) estimate of *in situ* $\sum CO_2$. This has a strong bearing on calculations of chemosynthetic dark CO_2 fixation rates described below.

In contrast to $\sum CO_2$, both methane (lake water mean <1 µM; Figure 9) and hydrogen sulfide (lake water mean <0.5 µM; Figure 10) were well represented in Mary Bay and Stevenson Island vents, while they were rarely detected in West Thumb. Sulfide was also regularly found off Steamboat Point, though at a lower concentration (Figure 10). Thus, the northern and north-central domains were high in carbonate and reduced compounds, whereas the western domain did not stand out. These three components share one characteristic that differentiates them from chloride and silicate: they can exist and be transported in the gas phase. At acid pH all three are significantly or dominantly volatile, and may be distilled from reservoir fluids into a chloride- and silicate-free steam. By this mechanism the domains to the north could have origins in the same reservoir as the West Thumb vents, yet display vastly different geochemical features.

There is a strong association between the domains of reduced inorganic compound emanation and those of bacterial geochemical utilization, as exemplified by chemosynthesis measurements (Figure 11). Both extreme northern regions of the lake (Steamboat Point and Mary Bay) persistently had dark CO_2 fixation rates far above those of open lake water (approximately 0.05 µgC/L/hr) and often

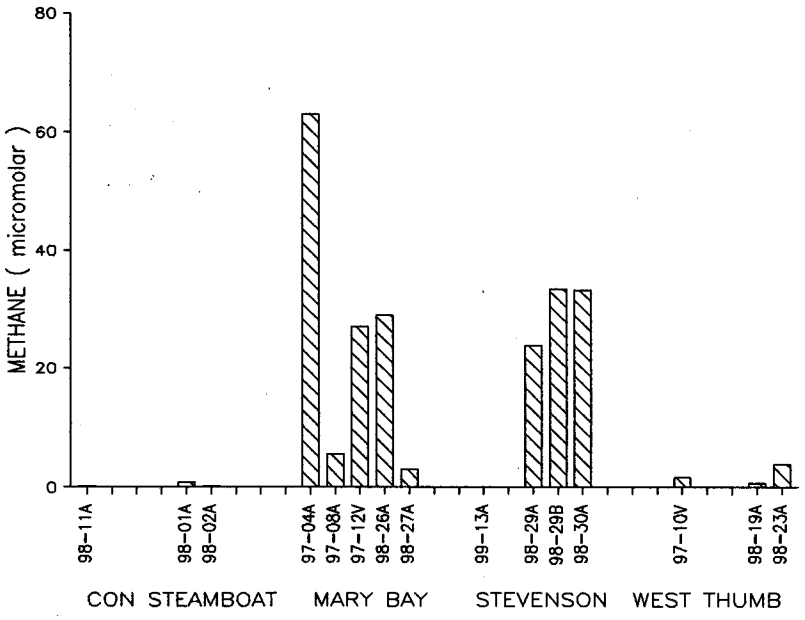

Figure 9. Methane occurred predominantly in Mary Bay and east of Stevenson Island. Analytical difficulty for this parameter in the field is apparent in missing values.

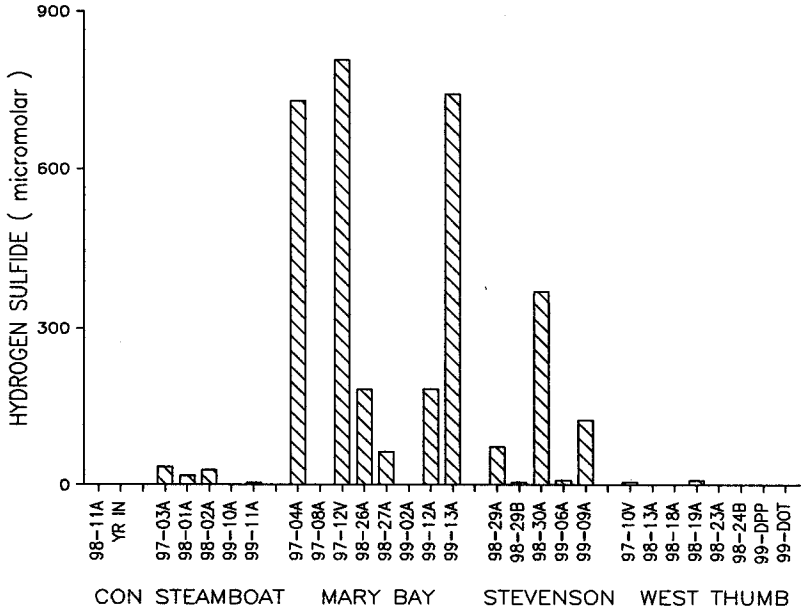

Figure 10. Hydrogen sulfide was frequently enriched in Mary Bay and east of Stevenson Island but was never of consequence in West Thumb.

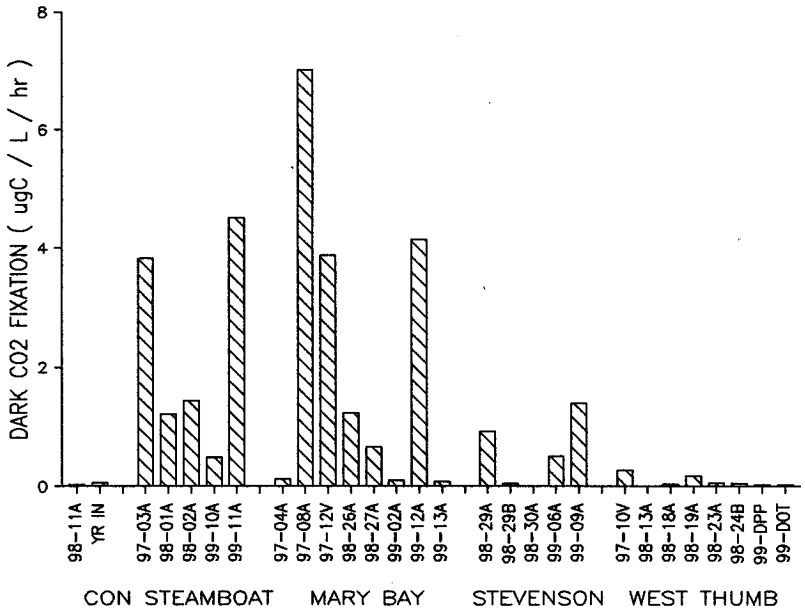

Figure 11. Bacterial chemosynthetic dark $^{14}CO_2$ fixation was common in all northern basin domains, but nearly absent in West Thumb.

exceeding photosynthesis at the surface (approximately 3 µgC/L/hr). Because CO_2-fixing bacteria require one or more reduced mineral-derived substances (e.g., H_2S, Fe[II], etc.) for growth and subsequently remove the nutrient, it is not necessary that high sulfide and high chemosynthetic rates be well correlated at a point in space and time. Hence, high levels of sulfide may presage bacterial vigor, while lower levels may be the result of consumption. In fact, in domains where H_2S was reliably present there tended to be an inverse relationship between standing concentration and bacterial productivity. However, where H_2S was rarely found, as in West Thumb, chemosynthesis was rarely found.

Temperature and microbial productivity in hydrothermal vent waters. Hydrothermal vent systems press the limits of life both through corrosive or otherwise toxic aqueous and gas phase composition, and through imposition of high temperatures. In marine habitats, sulfide and reduced iron often reach concentrations of several millimolar, with additional metals (zinc, copper, cadmium, etc.) often having concentrations in the tenths of millimolar or higher—levels rapidly fatal to most organisms of any kingdom. Toxicity of the chemical solutions is further exacerbated by vent fluid temperatures as high as 350°C in deeper, high-hydrostatic-pressure (>200 atmospheres) locations. Among the more common organisms known to humankind, thermally induced death occurs at temperatures of 42–45°C. This is a distinguishing characteristic of the mesophiles (mid-temperature-loving organisms), including virtually all plants, animals, fungi, and the overwhelming proportion of bacteria. While some organisms can survive higher

temperatures, especially for short periods, the ability to thrive and grow at elevated temperatures belongs exclusively to a limited group of prokaryotic (without true organelles) bacteria and archaebacteria. These organisms, the thermophiles (heat-loving; 45–70°C) and extreme- or hyperthermophiles (70–113°C to date) are the sole inhabitants of hot, chemically inhospitable hydrothermal environments that may reflect conditions more widely distributed on early Earth or other planets (e.g., the Martian polar cap) and moons (e.g., ice-covered Europa, a moon of Jupiter). Even present-day extremophiles are restricted to the periphery of marine hydrothermal vent conduits and seeps where superheated, geochemical-laden fluids are cooled and diluted with cold ocean-bottom waters. From this perspective, Yellowstone Lake vents provide an ideal study site because the shallow depths (<150 m), resultant low hydrostatic pressure (<15 atmospheres), and in-transit mixing with lake water keep maximum vent temperatures in the vicinity of the limit currently known for growth.

Two approaches to studies of thermophily in Yellowstone Lake microbial ecology both make use of elevated temperature incubations to exclude common mesophilic bacteria for elucidation of extremophile activity. Growth, isolation, characterization, and molecular analysis of populations and strains have been a principal focus. Using growth at 50°C for thermophiles and 80°C for hyperthermophiles, enrichments and isolates for three major groups of chemolithotrophic bacteria have been successful. A thermophilic sulfate reducer has been characterized (Henry et al. 1994) and thermophilic methane- and sulfur-oxidizing bacteria have been obtained. Recently, a sulfur-oxidizing bacterium capable of growth at 80°C has also been grown. The laboratory organisms and publicly available molecular genetic database have been used as the basis for molecular probing of cultures and populations.

Presence of appropriate species of bacteria in a viable (living) state is necessary but not sufficient for expression of chemosynthetic productivity. Physical and geochemical conditions must also be supportive of growth; when they are not, bacteria may enter dormant phases that remain culturable but are actually inactive. It is partly through this mechanism that populations disperse to take advantage of either sporadic or newly established habitats (e.g., intermittent venting, opening of new geothermal features). As a first step toward corroborating molecular and culture investigations, measurements of chemosynthetic dark CO_2 fixation were sometimes paired: one at the temperature of receiving waters (4–25°C) and one at 50°C. At the elevated temperature, mesophilic bacteria are excluded, while both thermophiles and hyperthermophiles retain positive (though perhaps suboptimal in the latter case) activity in excess of their growth in bottom-water conditions.

Stimulation of chemosynthesis by 1.6–3 times during 50°C incubation was observed for three of the four vent samples tested in 1999 (Figure 12), and the fourth retained 67% of control (bottom-temperature) activity in northern and north-central basin samples. Under the same conditions, replicates of near-zero activity at 50°C were obtained at West Thumb and Southeast Arm (data not shown). Water samples sipped simultaneously from 0.5 m above the vent orifice

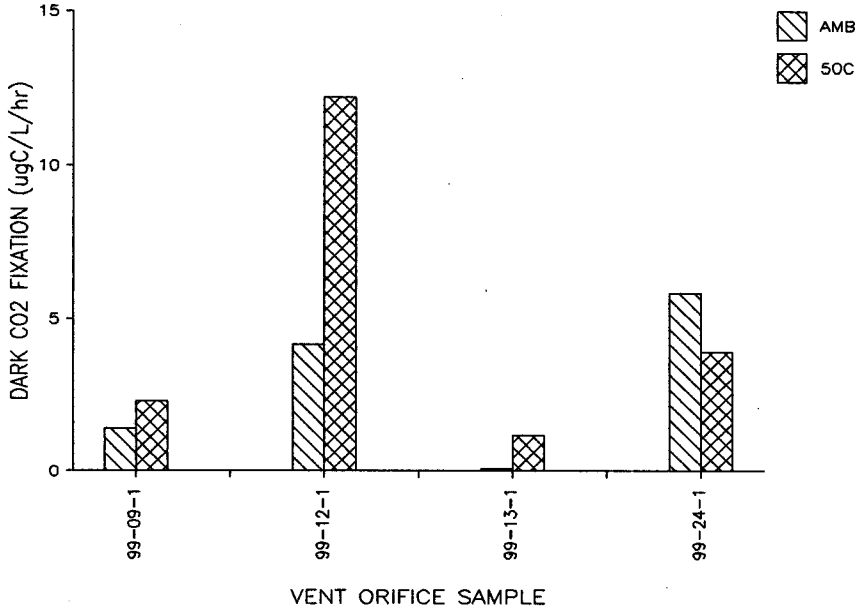

Figure 12. Elevated temperature supported or stimulated thermophilic bacterial dark $^{14}CO_2$ fixation in water samples collected within the hydrothermal vent orifice in Yellowstone Lake during 1999 sampling. Location of vents: 99-09, Stevenson Island (110 m); 99-12 and 99-13, Mary Bay Canyon (53 m); and 99-24, Pelican Roost (approximate - ly 20 m; southeast of Mary Bay). Replicate samples (standard deviation <5%) were incu - bated in a temperature-controlled block at receiving water temperature (<10°C) and in an oven at 50°C.

displayed opposite behavior: more than 80% of control activity was knocked out by high-temperature incubation (Figure 13). Though measurements are few in number as yet, the method was unequivocal in selection against mesophilic bacteria. The results were consistent with growth of thermophilic bacteria within the vent conduits and their transport and expulsion into receiving waters of Yellowstone Lake. Even close to the orifice, population composition was adapted to the use of reduced mineral-derived substrates under mesophilic circumstances, leaving enrichable thermophile populations but at low proportion to total chemosynthetic bacteria. Thus, it is likely that very favorable habitats for detailed study of *in situ* living extremophile communities are present in the northern part of Yellowstone Lake. Ease of access relative to deep sea vents and a closer approximation to optimum growth conditions are significant factors when considering studies for early evolution and/or exobiological applications.

Microbial mats as persistent sources of chemolithotrophic activity. Though somewhat less tractable to quantitative analysis than vent water samples, visual evidence of microbial mats surrounding vents and fumaroles has been both ubiquitous (Marocchi et al. 2001; Remsen et al., this volume) and persistent from

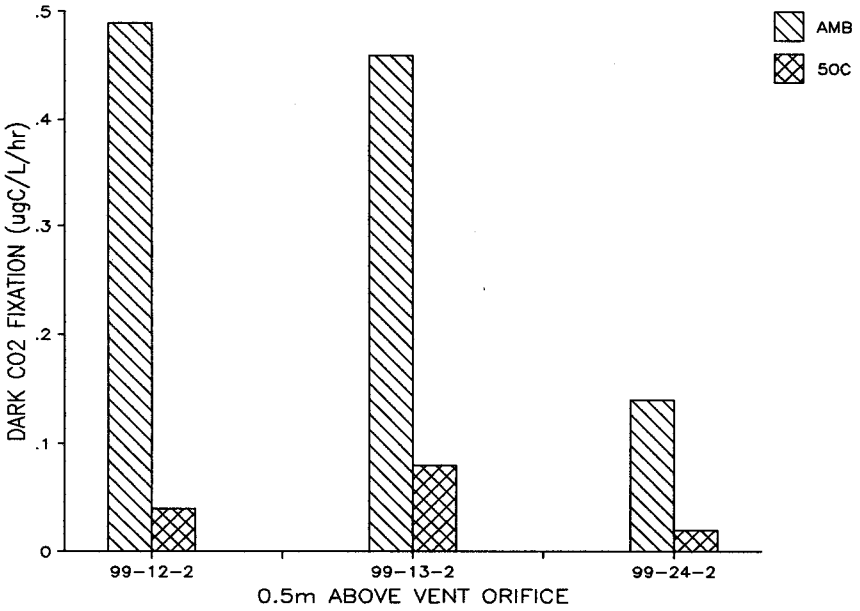

Figure 13. Elevated temperature greatly decreased bacterial dark $^{14}CO_2$ fixation in water samples collected at the top of the ROV arm, 0.5 m above the vent. Samples were incu - bated in parallel with vent orifice samples in Figure 12.

year to year. Microbial mats may be found on the sediment surface, on rock ledges overhanging vents, encrusting rooted plants in shallower water, or wherever a solid surface and dissolved mineral-laden waters come together. The very presence of entwined filaments of geochemical-oxidizing bacteria was often a clue to nearby vents and directed sampling efforts, particularly in the deeper canyons of Stevenson Island and Mary Bay. Because of their growth habit, mats could not be readily sampled with the ROV, but in 1994 SCUBA divers Lori Buccholz and Joel Kostka collected mat material in sterile Whirl-Pak bags from under an overhang of a Sedge Bay vent in late July. The mats were mildly homogenized to facilitate replicate sampling, and dark $^{14}CO_2$ uptake was measured in the presence of a variety of stimulants, primarily inorganic biomass nutrients (nitrogen, N as nitrate; and phosphorus, P as phosphate) and substrates of chemosynthesis (sulfur as thiosulfate, $S_2O_3^{=}$; nitrogen as ammonium, NH_4^+). As with some water samples, thiosulfate strongly stimulated chemosynthesis, while biomass nutrients or ammonium had no or only a minor effect on dark CO_2 fixation respectively (Figure 14). Although visible biomass was present in the samples, the rates of dark CO_2 fixation were also tenfold higher than most unamended vent water samples and were almost doubled by addition of a reduced sulfur compound, thiosulfate.

Summary: Photosynthesis and Chemosynthesis in Yellowstone Lake

The biogeochemical setting of Yellowstone Lake with its several areas of pro-

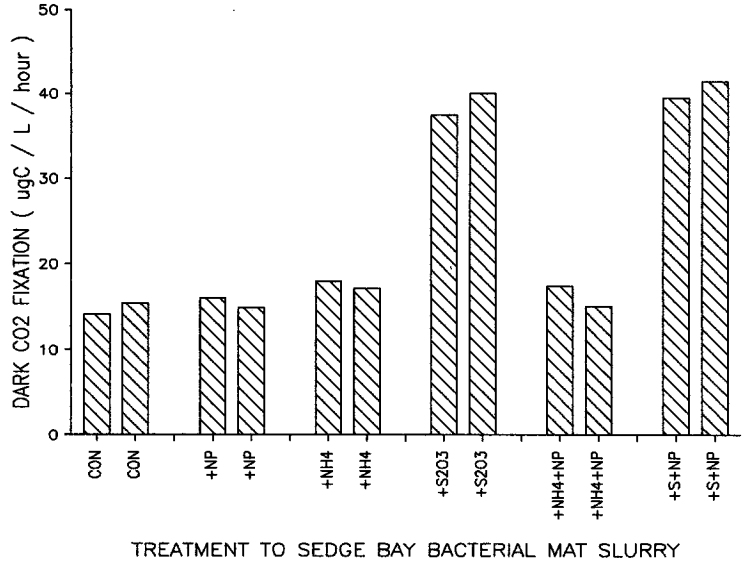

Figure 14. Vigorously chemosynthetic Sedge Bay bacterial mat slurries were stimulated further by thiosulfate addition. Supplements with inorganic growth nutrients nitrate + phosphate (+NP), ammonium (+NH_4), and combinations had little further effect.

nounced and persistent underwater hydrothermal venting provides an ideal setting for growth of mineral-oxidizing bacteria. They include representatives, many thermophilic, of the hydrogen-, reduced sulfur-, iron-, manganese-, and methane-oxidizing bacteria. Nitrifiers (ammonia oxidizers) may also be active, though few vents have been found to contain substantial concentrations of NH_4^+ in recent years. All but the methane-oxidizing bacteria assimilate carbon dioxide as the sole source of carbon for tissue. Using this assay for collective chemosynthetic activity, it was demonstrated that (1) both geochemical emanations and chemosynthetic bacterial activity were not ubiquitously distributed among Yellowstone Lake hydrothermal vents, but rather were focused in distinct regions; (2) a portion of bacteria in the vents themselves had thermophilic characteristics (enhanced or persistent production at 50°C); (3) bacteria growing in the immediate proximity of vents or in overlying waters often could be stimulated by addition of reduced sulfur compounds; and (4) slurries of white mat aggregates surrounding vents had very high rates of chemosynthetic production. Summarizing maximum rates of productivity for five years of sampling (Table 3), it was apparent that in most years vent water samples could attain rates of primary productivity (i.e., carbon dioxide assimilation into biomass) similar to that of surface photosynthesis by algae. Although access to enough vent samples for analysis of biological parameters was limited until 1997 when the syringe sampler was installed, the results still suggest that geochemical energy was sufficient to promote active, if sometimes localized, growth of bacterial populations.

Table 3. Summary of maximum rates of photo- and chemosynthesis in Yellowstone Lake, 1994–1998.

Parameter	Units	1994 Mid-July	1995 Early June	1996 Late July	1997 Mid-July	1998 Mid-July
Maximum surface photosynthesis	µgC/L/hr	6.9 (n = 1)	3.7 (n = 6)	4.8 (n = 9)	8.9 (n = 4)	4.6 (n = 17)
Maximum vent chemosynthesis	µgC/L/hr	3.2 (n = 1)	9.1 (n = 4)	N.D.	7.0 (n = 5)	1.6 (n = 13)
Maximum surface chemosynthesis*	µgC/L/hr	2.3 (n = 3)	0.11 (n = 6)	0.23 (n = 7)	0.23 (n = 5)	0.09 (n = 8)
Open water thiosulfate stimulation	X Control	50	7	2	20	4
River outflow discharge†	1000 ft³/sec	<2	>4	>4	>5	>4

* Includes dark CO_2 fixation by algae.
† Data from United States Geological Survey Website: Gauging Station 06186500 at the Yellowstone River outflow, Fishing Bridge.

Most intriguing was the short visit in 1994, one of the two lowest-water years in the last decade (1992 being the other). During 1994, the entire basin north of Stevenson Island smelled strongly of H_2S, the beach at Mary Bay was nearly too hot to walk on, and fumarole bubbles rising through the water column off Stevenson Island broke on the surface to leave a yellow-white ring of presumed elemental sulfur from oxidation of bubble-borne H_2S. In surface samples from Mary and Sedge bays and in vertical profile at open-water Stevenson Island, dark CO_2 fixation was ten or more times that of typical dark rates for surface samples, and demonstrated strong thiosulfate stimulation. Only one vent was sampled (Sedge Bay), but it showed that under permissive conditions, chemosynthetic activity in the water column could be stimulated through physical mixing of vent-derived geochemicals to levels similar to near-vent samples. In years of high outflow, vents still provided oases of productivity capable of supporting limited animal-consumer biomass, even in deep waters where they would otherwise be absent.

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