EOS
EOS, TRANSACTIONS, AMERICAN GEOPHYSICAL UNION

VOLUME 84 NUMBER 12
25 MARCH 2003
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Probing for Life in the Ocean Crust with the LEXEN Program

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The oceanic upper crustal reservoir is a mosaic of environments with different thermal and hydrological characteristics that may form distinct biospheres. The upper 500 meters of oceanic crust is porous, permeable, and the site of extensive circulation of warm sea water—from the axis out to the sea floor that is more than tens of millions of years in age. This upper crustal section represents an enormous reservoir of hydrothermal fluid that has been suggested as a microbial incubator of global proportions. In the axial crustal formation zone at mid-ocean ridges, local environments near high temperature hydrothermal vent fields can harbor unique microbial populations [*Summit and Baross*, 2001; *Huber et al.*, 2002]. The geographic distribution of these microbial populations is not known, and they may exist only in the immediate proximity of individual vent fields, or they may be more extensively distributed within the near-surface rocks along the entire spreading axis.

Igneous ocean crust can be arbitrarily categorized as either 'normal' crustal sections or seamounts, with both types appearing to evolve and change with age. Examining these two crustal environments for unique microbial communities and their chemical signatures was a primary goal of our Life in Extreme Environments (LEXEN) program. Our strategy was to sample fluid for microbial and chemical analyses from paired sites, including a young and an old seamount and young and old normal crust. In addition to microbiologic and organic/inorganic chemical studies of the crustal fluid, we planned to monitor fluid vents from each site for changes in flow rate and fluid temperature caused by tides, local and remote earthquakes, and crustal strain.

A recent cruise to the northern Juan de Fuca Ridge with the new Remotely Operated Vehicle JASON-II used novel extraction techniques to sample hydrothermal fluid for just such analyses.

At Baby Bare Seamount, a 2000-kg coring weight was used to drive two stainless steel probes several meters into the summit of a lightly-sedimented bare rock outcrop, and the hollow probes immediately began artesian venting of warm crustal fluid. Hydrothermal fluid was also sampled using more traditional methods from the crustal reservoirs underlying the Juan de Fuca Ridge axis and ridge flanks, including the Main Endeavour Hydrothermal Field, Axial Seamount, and ODP Hole 1026b. Preliminary microscopic counts show numbers of micro-organisms within the fluid of the upper crustal reservoir at all sites exceed those of background sea water. Anaerobic micro-organisms adapted to growth at 70°C and 90°C were cultured from fluids extracted both from the 3.5-My old Baby Bare Seamount site and ODP Site 1026b. The temperature growth range for these organisms exceeds the maximum exit fluid temperatures of 26°C, indicating that their habitat for this ridge-flank site may be in the lower crustal rocks where the projected upper crustal reservoir temperatures are near 63°C.

By H. P. Johnson and the LEXEN Scientific Party

Baby Bare Seamount

Sampling uncontaminated fluid from the sub-sea floor beneath the summit of Baby Bare Seamount presented the greatest challenge. At this site, the warm, lightly-sedimented igneous rocks with high-temperature gradients within the surface sediment layer (estimated with a 0.5-m probe at 40°C/meter) and abundant surficial macrofaunal populations posed a high potential for severe contamination of the microbiological samples and dilution of the fluid with sea water. Sampling fluid from the sub-seafloor aquifer was successful when we drove steel sampling probes several meters (1.5 meters for Probe #3 and 3 meters for Probe #4) into the exposed rocks at the summit using a 2000-kg piston coring weight (Figure 1). Once driven below the surface, the two hollow stainless steel spikes began immediate venting of warm (17°C to 21°C) hydrothermal fluid. We hypothesize that the probes penetrated a thin coating of impermeable surface sediments and alteration minerals, allowing flow to initiate.

Immediately after penetration, the fluid from the probe was clouded with fine sediment, but became clear (except when disturbed) after several days of continuous flow. Nozzles had been installed at the exposed end of the sampling probes to provide the necessary tight coupling for the sampling of fluid, and to allow time-series monitoring of flow and temperature. Fluid from both probes was extensively sampled during the cruise with JASON-II, and flow and fluid temperature are presently being monitored over a year-long period with autonomous instruments.

Although we extensively surveyed the summit of Baby Bare with JASON-II during a September 2002 cruise, no free-flowing springs of hydrothermal fluid were observed, even at sites (with markers) of previous sampling cruises [*Mottl et al.*, 1998; *Wheat et al.*, 2002]. This lack of natural springs in 2002 where they had existed previously suggests that the seamount summit may have a pervasive outer shell or crust of low permeability material that must be broached by some process such as earthquakes or coring in order to form temporary free-flowing artesian springs. Because our shortest probe (1.5 meters) created a free-flowing spring, any low permeability "shell" must be thin and very close to the surface.

Young Crust–axial Seamount and the Endeavour Segment

In contrast to the older seamount, at mid-ocean ridge spreading centers, hydrothermal fluid vents freely from crustal aquifers through cracks and fissures in the outer basaltic carapace. These ragged, uneven fractures are the sites of inorganic chemical reactions and complex microbial activity. Uncontaminated fluid sampling requires either that a sampling tube be deeply inserted into the fissure below the near-surface mixing zone, or that a sealed sampling chamber that completely excludes bottom sea water must be placed over the vent. At Axial Seamount, we used both sampling tubes inserted deeply into the fissures and a sampling box that had been previously grouted with cement onto the sea floor [*Hutnak and Johnson*, 1999]. At the Main Endeavour Field (Figure 2), we deployed a "portable" version of the cemented box constructed of coated nylon. An effective seal between the fluid sampler and the ragged sea floor surface was established using a heavy, liquid-filled rubber tube, forced into conformity with the uneven rock surface by lead weights.

ODP Hole 1026b

Accessing the crustal biosphere by drilling into igneous sea floor has many inherent problems, as most drill holes penetrate underpressured crustal sections. Unless the drill hole is sealed, sea water will flow into the crust through these holes after drilling is completed. ODP Hole 1026b on the east flank of the Juan de Fuca Ridge is a rare exception, penetrating an over-pressured crustal reservoir and continuously venting 63°C crustal fluid since 1997 [*Davis et al.*, 1997]. Although not proven, the high flow rates out of the hole argue that much of the initial drilling contamination may have been flushed from the system since that time. At this site, fluid extraction tubes were simply lowered into the top of the 7.5-cm diameter pipe at the wellhead, and inserted well below the sea water mixing zone at the orifice. Samples of the artesian fluid flowing out of ODP Hole 1026b were transferred by a peristaltic pump into sample bags mounted either on the ROV JASON-II or on independent elevator samplers.

Results

The primary goals of our LEXEN program are to ascertain if different crustal environments harbor distinct indigenous microbial communities, and whether the diversity and physiology of these communities are linked to the inorganic and organic chemical characteristics of crustal fluid. Microbial and inorganic chemistry samples from all 4 sites were obtained using a multi-element Teflon bag sampler mounted on the stern of JASON-II. Using a novel large-volume fluid sampler design that interfaced with the reservoir, large volumes (80 to 250 liters for single samples) of fluid were recovered from each hydrothermal environment, and processed to isolate both dissolved and particulate organic matter. It will take some time to complete these microbial and chemical analyses, and the flow/temperature monitors and a new time-series fluid sampler will not be recovered until summer 2003. However, even the preliminary chemistry data are intriguing.

For example, the ammonia concentrations (Table 1) of Baby Bare fluids from the newly deployed probes resemble basement fluids flowing from Hole 1026b [*Wheat et al.*, 2000]. The source of ammonia is not known, but it may derive from contact with a sedimentary source. Preliminary values for silica are also elevated over sea water, and indicate that both flank and axial hydrothermal venting may contribute to the general elevation of this element that has been observed in the northeastern Pacific bottom water.

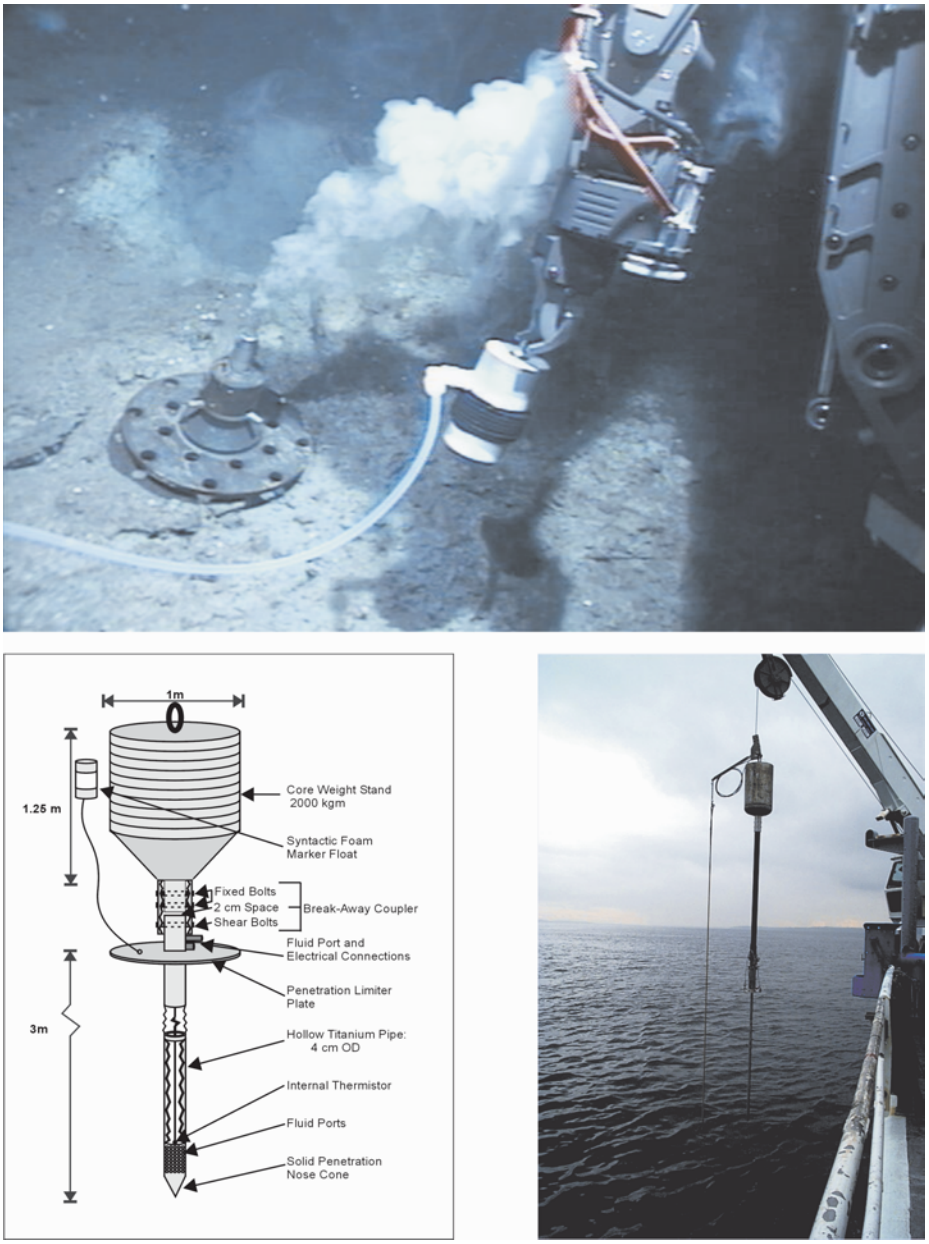

Fig. 1. Insertion of the fluid sampling probes into the summit of Baby Bare Seamount. Lower left: Diagram showing key elements of the probe. Lower right: Probe deployment at sea, using the Oregon State University coring facility equipment. Notice the long black pipe between the coring weight and release, which was added when pre-cruise tests showed that the original configuration was gravitationally unstable. Top: Probe venting warm hydrothermal fluid after insertion into the seafloor. White "smoke" appears to be entrained fine-rained sediment, which cleared after several days of venting. The JASON-II manipulator (right side) is preparing to install fluid sampler.

These preliminary chemistry data argue that our sampling strategies have been successful, and that the micro-organisms detected in fluids from these environments originate from a crustal habitat, and not from contaminating bottom sea water. While the numbers of micro-organisms determined by epifluorescence microscopy for four of the five sampling sites are somewhat elevated over seawater (Table 1), the cell count data alone cannot reveal the physiological diversity and source of these organisms. For example, the low numbers of microbes from Hole 1026b fluids may indicate that organisms indigenous to deep crustal environments grow primarily on mineral surfaces as attached biofilms which are only occasionally sloughed off into fluids [*Huber et al.*, 2002].

An exciting early result from this study shows how microbes can contribute to our understanding of crustal habitats. Preliminary characterization of cultured microbes from Baby Bare fluids indicates the presence of anaerobes adapted for growth at 70°C and 90°C. The full temperature growth range for these organisms has not yet been determined. However, it is likely that they could grow as low as 63°C—the upper crustal temperatures observed at Hole 1026b [*Davis et al.*, 1997] and projected to exist only a few meters below the water/rock surface of Baby Bare [*Wheat et al.*, 2002]. These data substantiate the crustal reservoir origin of fluid at Baby Bare Seamount and further indicate the existence of a hot, anaerobic biosphere in ridge flanks crust. Ongoing physiological characterization of these organisms will further our understanding of how microbes interact with the chemistry of fluids and help constrain the temperature range of their habitat.

Although our preliminary results are very promising, more extensive chemical and microbiological analyses are just getting underway. Our currently deployed time-series sampling and monitoring instruments are scheduled to be recovered with JASON-II in July 2003. However, there does not seem to be much question that, on the Juan de Fuca plate at least, hydrothermal fluid circulating in the upper oceanic crust that is younger than 4 My harbors a complex microbial community. A similar sub-sea floor biosphere in crust older than 4 My is plausible, but has not yet been sampled.

Determining the taxonomic and physiological diversity of this community and the extent to which this is unique to crustal habitats continue to be the goals of this project. An important caveat, however, is that the existence of the sub-sea floor biosphere cannot yet be extended to the full global scale. If we have learned nothing else about the sub-sea floor in recent decades, it is that what may be true in one environment does not necessarily apply to another, even just over the horizon.

Acknowledgments

The LEXEN Program is supported by National Science Foundation/Life in Extreme Environments grant OCE0085615 to H. P. Johnson.

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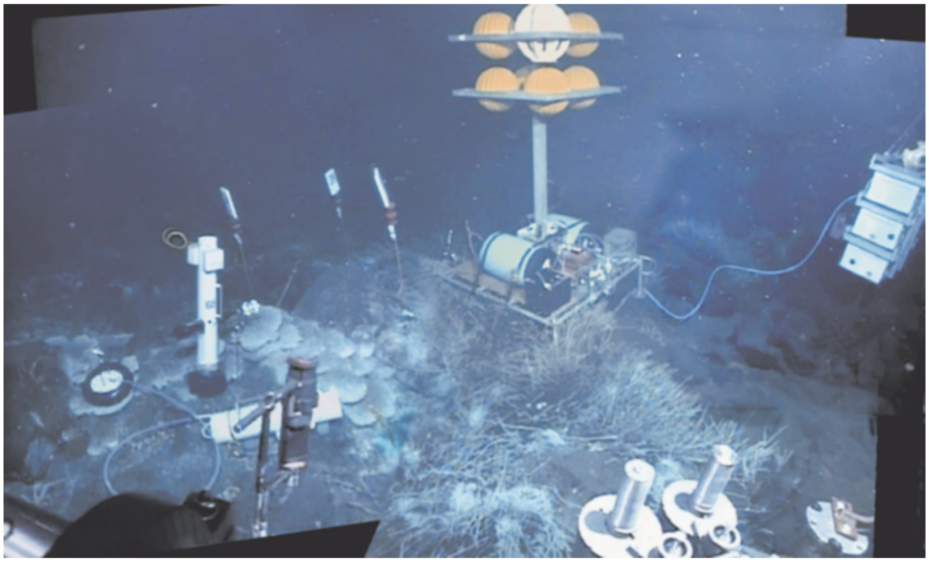

Fig. 2. Easter Island vent field and deployed samplers and markers at the Main Endeavour Field. Yellow floats support the Barrel Sampler, capable of taking 100 liters of fluid per sample. White rectangular elevator on right is a newly developed time-series fluid sampler, presently deployed at another site for a year. Vertical white tube is a MAVS current meter. Horizontal white tube is a flow meter/temperature monitor with the black ring of heavy liquid that presses the collector into conformance with the uneven seafloor. Images were taken from JASON-II and mosaic is by J. Howland.

Table 1. Microbial cell counts and preliminary (NH^3 and Si) chemistry from five sites sampled during the LEXEN field program. Comparison is with bottom water values from a non-hydrothermal site (CTD cast) away from the axial zone.

Site	**Temp °C**	**Cells/ml**	**± SD**	**NH^3 μmol/liter**	**Si, μmol/liter**
Baby Bare, Probe #3	18.9	1.0×10^5	1.3×10^4	90	350
Baby Bare, Probe #4	19.7	1.8×10^5	2.2×10^4	90	400
ODP Hole 1026b	62.5	7.5×10^4	1.0×10^4	90	1200
Easter Island, MEF	23.3	1.1×10^5	1.5×10^4	24	1280
Bag City, Axial Seamount	18.3	1.5×10^5	2.6×10^4	4	390
local deep seawater	1.95	4.0×10^4	6.0×10^3	< 1.0	180

Precision of NH^3 and Si data is 4%.
Cell count ± SD values are the 95% confidence level.
Fluid temperatures are those when samples were taken.

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