Appendix A: Workshop Announcement with Agenda

2009 DARK ENERGY BIOSPHERE INSTITUTE (DEBI) RESEARCH COORDINATION NETWORK (RCN) MEETING

darkenergybiosphere.org

Subseafloor Ocean Biosphere and Borehole Observatory Science

October 19-21, 2009

Fairmont Orchid at Mauna Lani Resort, Hawai'i

The Dark Energy Biosphere Institute (DEBI) is pleased to announce the meeting "Subseafloor Ocean Biosphere and Borehole Observatory Science" to be held 19-21 October 2009 at the Fairmont Orchid at the Mauna Lani Resort, on the island of Hawai'i. This meeting is a component of the NSF Research Coordination Network (RCN) on the Deep Biosphere with co-directors Katrina Edwards and Jan Amend and the mission to help coordinate and support deep biosphere research, and to aid in the transfer of information regarding the deep biosphere's role in the global Earth system.

The scientific conference will comprise a series of keynote speakers covering aspects of Circulation Obviation Retrofit Kit (CORK) subseafloor observatory history and design, technical specifications and options for future modifications, drilling and other operational considerations, monitoring and contamination concerns, key results from earlier subseafloor experiments using observatories, and future directions for subseafloor biosphere science involving observatories. Our intent is to integrate lecture, discussion, and poster formats so as to encourage engagement and development of collaborative opportunities between individuals and groups that have not previously worked together or have had difficulty visualizing the overlap among each other's disciplines.

A provisional agenda of the oral presentations, breakout session themes and training workshop topics has been drafted here. Breakout session discussions are planned to address themes of The Big Science Questions, Technology Innovations and Limitations, and CORK Scenario Planning. All participants are asked to present during a poster session, highlighting work completed or in progress involving subseafloor observatories and/or the deep biosphere, or conceptual proposals describing how to move the science forward through novel application of observatory technology for passive (monitoring) or active experiments. The training workshop at this meeting will consist of a series of presentations and exercises on hydrogeology modeling and computation, electrochemistry measurement and borehole microbial observatory construction, crossing over between microbiological sampling and monitoring and marine hydrogeology. It is important to note that this is a technologically challenging area of research and one that is extremely new to science and in particular to microbiology.

Meeting steering committee:

Jim Cowen (Chair), University of Hawai'i
Brian Glazer, University of Hawai'i
Andy Fisher, University of California, Santa Cruz
Jan Amend, Washington University in St. Louis

REGISTRATION AND HOSPITALITY

Please check in for the meeting on Sunday (10/18) in the Paniolo Room between 2:00 PM and 6:00 PM. Registration and hospitality will also be available in the pre-function area of the amphitheatre on Monday (10/19) between 7:30 AM and 3:00 PM; on Tuesday (10/21) between 7:30 AM and 3:00 PM; and on Wednesday (10/22) between 8:00 AM and 11:00 AM.

The University of Hawai‘i at Hilo Conference Center is overseeing the local needs of our meeting. Mahalo nui loa to Judith, Jules, and Mary Ann.

ACCOMMODATIONS AND MEETING VENUE

The Fairmont Orchid is the meeting venue and is offering a special room rate for our meeting for October 13-26, 2009. Nestled on 32 oceanfront acres on the Kohala Coast of the Big Island of Hawai‘i, The Fairmont Orchid, Hawai‘i is one of the most luxurious and spacious resorts in all of Hawai‘i. Located just 20 miles north of the Kona International Airport (KOA) in the beautiful Mauna Lani Resort community, The Fairmont Orchid resort offers indoor meeting space and outdoor function space. Enroll in the Fairmont President's Club to receive express check-in, complimentary high-speed Internet access, free local calls, daily delivery of either local or national newspapers and more during your next stay.

HOTEL SHUTTLE

We will provide shuttle service between Kona International Airport (KOA) and the Fairmont Orchid before (Sunday) and after (Wednesday afternoon) the meeting. Other options include Speedi Shuttle or taxi service.

POSTERS

Promenade III will be available for poster set up beginning Sunday (10/18) at 12:00 PM. Please have your poster set up by 3:00 PM on Monday (10/19). The poster session will be on Monday evening at 6:00 – 9:00 PM. Please take down your poster by 12:00 PM on Tuesday (10/21). Any posters left up will be discarded.

EMERGENCY CONTACT

Contact Rosalynn Lee at 1-706-338-4518.

SUNDAY: OCTOBER 18, 2009

Pre-workshop Activities

12:00 P – 6:00 Poster set-up [Promenade III]

2:00 – 6:00 Registration & hospitality [Paniolo Room]

6:00 – 8:00 Opening reception [Pool Deck]

MONDAY: OCTOBER 19, 2009

7:30 A – 8:30 Continental breakfast [Brown's Beach House]

Introduction [Amphitheatre]

8:30 – 9:00 Welcome and RCN introduction (Jan Amend)

Circulation Obviation Retrofit Kit (CORKs) Technology and Hardware for Deep Biosphere Research [Amphitheatre]

9:00 – 9:45 CORK design: Foundation in hydrogeology (Keir Becker)

9:45 – 10:15 Coffee break [Wailana Gardens]

10:15 – 11:15 CORK technology: Geochem/biosphere observatories (Geoff Wheat)
11:15 – 12:00 Juan de Fuca case study and subsurface biosphere applications (Jim Cowen)

12:00 P – 1:00 Lunch [Brown's Beach House]

1:00 – 1:45 North Pond future directions (Katrina Edwards)
1:45 – 2:45 Basement-related potential projects (Open floor: single slide presentations)

Breakout Session I: The Big Science Questions

2:45 – 2:50 Breakout assignments [Amphitheatre]

2:50 – 3:15 *Refreshment break [Wailana Gardens]*

3:15 – 4:15 Breakout session IA [Amphitheatre, Promenade I, Promenade II]
4:15 – 6:00 Breakout session IB [Amphitheatre, Promenade I, Promenade II]

Poster Session [Wailana Gardens & Promenade III]

6:00 – 9:00 Dinner & poster session

TUESDAY: OCTOBER 20, 2009

7:30 A – 8:30 Continental breakfast [Brown's Beach House]

Breakout Session I: The Big Science Questions (continued) [Amphitheatre]

8:30 – 10:00 Plenary breakout session I: Report and discussion

10:00 – 10:15 Coffee break [Wailana Gardens]

Breakout Session II: Training Sessions [Amphitheatre, Promenade I, Promenade II]

10:15 – 11:45 Simultaneous and repeating 30-minute training sessions

a. Hydrogeology modeling and computation (Andy Fisher)
b. Electrochemistry measurement and application (Brian Glazer)
c. Borehole microbial observatory construction (Beth Orcutt)

Breakout Session III: Technology Innovations and Limitations

11:45 – 12:00 Plenary: Breakout assignments [Amphitheatre]

12:00 P – 1:00 Lunch [Brown's Beach House]

1:00 – 2:30 Breakout session III [Amphitheatre, Promenade I, Promenade II]
2:30 – 3:00 Plenary breakout session III report [Amphitheatre]

Plenary Discussions [Amphitheatre]

3:00 – 3:30 Group discussions focused on breakout sessions I, II, III and basement-related potential projects

3:30 – 3:45 Refreshment break [Wailana Gardens]

Breakout Session IV: CORK Scenario Planning

3:45 – 5:30 Breakout session IV [Amphitheatre, Promenade I, Promenade II]

7:00 – 9:00 Closing dinner [Coconut Grove]

WEDNESDAY: OCTOBER 21, 2009

7:30 A – 8:30 Continental breakfast [Brown's Beach House]

Breakout Session IV: CORK Scenario Planning (continued)

8:30 – 9:30 Plenary breakout session IV reports and discussion [Amphitheatre]

Final Plenaries [Amphitheatre]

9:30 – 10:00 Insight on the IODP process (Andy Fisher)
10:00 – 10:30 Workshop recap and future direction

10:30 – 11:00 Workshop close with coffee break [Wailana Gardens]

Appendix B: Participant List

2009 DEBI MEETING PARTICIPANT LIST

Steering Committee

Cowen	Jim	jcowen@soest.hawaii.edu	Professor	University of Hawaii
Fisher	Andy	afisher@ucsc.edu	Professor	University of California, Santa Cruz
Glazer	Brian	glazer@hawaii.edu	Assistant Professor	University of Hawaii
Amend	Jan	amend@levee.wustl.edu	Associate Professor	Washington University, St. Louis

Invited Speakers

Becker	Keir	kbecker@rsmas.miami.edu	Professor	University of Miami
Wheat	Geoff	wheat@mbari.org	Research Professor	University of Alaska, Fairbanks
Orcutt	Beth	borcutt@usc.edu	Postdoctoral Research Associate	University of Southern California

Participants

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Siefert	Janet	siefert@rice.edu	Professor	Rice University
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Woodruff	Quinn	qsw0917@ecu.edu	Graduate Student	East Carolina University
Ziebis	Wiebke	wziebis@usc.edu	Assistant Professor	University of Southern California

Appendix C: Poster Abstracts

2009 DEBI MEETING POSTER ABSTRACTS

In-Situ Voltammetry at Kilo Nalu Nearshore Cabled Observatory: Techniques for Studying Redox Cycling
In-Chieh Chen, Jennifer L. Murphy, and Brian T. Glazer

Using High-Resolution Secondary Ionization Mass Spectrometery (SIMS) to Probe Environmental Controls on Microbial Sulfur Cycling
David A. Fike

A Natural Subsurface Laboratory in the NW Bohemian/Vogtland Area (Czech Republic/Germany)
Jens Kallmeyer (unable to attend)

Functional Gene and ^{34}S-Isotopic Evidence of Microbial Methane and Sulphur Cycling in 3.5 Million-Year-Old Buried Ridge Flank Basalt
Mark A. Lever, Olivier Rouxel, Jeff Alt, Nobu Shimizu, Shuhei Ono, Fumio Inagaki, and Andreas P. Teske

Microbes Produce Thermogenesis-Like Methane in Deep Subsurface
Li-Hung Lin, Yu-Chen Ling, Pei-Ling Wang, Chih-Hsien Sun, and Yue-Gau Chen

An *In-Situ Electrochemical Analyzer* for Studying Redox Disequilibria in Subseafloor Basement Fluids at IODP CORK Observatories
Michael T. Matzinger, Brian T. Glazer, James P. Cowen, and Michael S. Rappe

Microbial Communities in Serpentinizing and Ultramafic Environments
Jennifer Kepp, **D'Arcy Meyer-Dombard**, and Dawn Cardace

Seeking Common Ground: Deep Continental and Marine Subsurface Habitats
Duane P. Moser

Mariana Forearc Serpentine Mud Volcanoes Harbor Novel Communities of Extremophilic *Archaea*
Andrea C. Curtis, C. Geoffrey Wheat, Patricia Fryer, and **Craig L. Moyer**

Colonization of Subsurface Microbial Observatories Deployed in Young Ocean Crust
Beth Orcutt, Wolfgang Bach, Keir Becker, Andrew Fisher, Brandy Toner, Geoff Wheat, and Katrina Edwards

Is Pressure a Variable that Should Considered in the Design of Long-Term Colonization Experiments for the Subseafloor Biosphere?
Matt Schrenk

Bacterial Community Composition in Hydrothermal Plume Environments is Heterogeneous and Distinct
Jason B. Sylvan and Katrina J. Edwards

In Situ Colonization Systems in Serpentinizing Microbial Habitats
Quinn Woodruff and Matt Schrenk

***In-Situ* Voltammetry at Kilo Nalu Nearshore Cabled Observatory: Techniques for Studying Redox Cycling**

In-Chieh Chen, Jennifer L. Murphy, and Brian T. Glazer

An *In-Situ* Electrochemical Analyzer (ISEA-III, Analytical Instrument Systems, Inc.) was connected to the Kilo Nalu Nearshore Cabled Observatory network and deployed in a water depth of 10 m. This was the first application of *in-situ* voltammetry from a cabled observatory. Vertical profiling and fixed-depth time series voltammetric measurements were used to determine the concentration of redox-reactive chemical species (including oxygen) under varying diurnal and tidal cycles and wave activity. Water column voltammetric measurements of oxygen concentration were corroborated by optode-derived oxygen measurements. In the sediment, variations in oxygen penetration depth were affected by both significant wave height and diurnal cycles. For profiles in which oxic-suboxic-anoxic zones were delineated, the width of the suboxic zone was affected by diurnal cycles. The passage of sand ripples and the speed of sand ripple migration also affected sediment dissolved oxygen concentration. Voltammetry is a viable tool for making *in-situ* observations of redox cycling.

Using High-Resolution Secondary Ionization Mass Spectrometery (SIMS) to Probe Environmental Controls on Microbial Sulfur Cycling

David A. Fike

The stable isotopic compositions ($\delta^{34}S$) of porewater and solid-phase sulfates and sulfides in marine sediments are useful for understanding active microbial metabolic activity and for reconstructing paleoenvironmental conditions associated with the deposition of ancient sediments. In many modern microbially-dominated sedimentary environments (e.g., microbial mats or deep-sea methane seeps), redox gradients can be steep with a transition from oxic to sulfidic (reducing) condition over just a few mm. In these environments, it is frequently difficult to sample at a sufficiently high resolution to capture the geochemical and microbiological details associated with these redox transitions. We build upon earlier work [Visscher et al. 2000] to demonstrate the ability to analyze aqueous sulfide captured as silver sulfide using secondary ionization mass spectrometry (SIMS) to characterize stable isotopic variability in these samples on scales down to ~ 1 – 50 μm (i.e., a scale relevant to individual microorganisms and their immediate environments). Multiple, adjacent measurements can be strung together to construct 2D isotopic datasets that document vertical isotope gradients as well as lateral heterogeneity [Fike et al. 2008]. Initial results include identification of unsuspected isotopic variability (~20‰ over distances as small as 1 mm) at depth – far from near-surface redox gradients – suggesting our understanding of the spatial controls on metabolic activity remain poorly understood. In addition, isotopic variability is observed to scale with ambient sulfate concentrations, which can not only help reconstruct the evolution of the marine sulfate reservoir, but also serve to distinguish different generations of sulfide formation (e.g., syndepositional vs. formation by ongoing metabolic in the deep biosphere. The isotopic variability on these fine spatial scales provides unique insights into spatial, temporal, and thermodynamic controls of microbial activity in these complex systems [Fike et al. 2009].

A Natural Subsurface Laboratory in the NW Bohemian/Vogtland Area (Czech Republic/Germany)
Jens Kallmeyer (unable to attend)

The Vogtland/NW Bohemia area in the western part of the Eger rift system on both sides of the Czech-German border is known as one of the most interesting European earthquake swarm regions, characterized by the repeated occurrence of seismically active periods with thousands of small and intermediate magnitude earthquakes (ML < 5). Earthquake swarms have been interpreted as a common feature of continental volcanic regions and mid-ocean rifts- However, they are unusual in intraplate regions without active volcanism. The Vogtland/NW-Bohemia area represents such a region with an anomalously high swarm activity. Since 1985/86 most swarms have been located near Novy´ Kostel (50.24°N, 12.44°E). Parotidis et al. [2003] hypothesize that ascending magmatic fluids trigger the earthquakes by causing pore pressure perturbations, which change the effective stress regime causing small and intermediate magnitude swarm earthquakes.

The area also hosts a number of mineral springs, among them the Wettinquelle, one of the most radioactive natural springs on Earth (27 kBq L^{-1}), owing to the high content of ^{222}Rn. Most of the dissolved gasses are mantle derived, according to their isotopic signature. The active wells are commercially used and allow easy access for scientific sampling. There is some inflow of shallow groundwater into the springs and therefore only a mixed signal of shallow and deep organisms can be obtained. Still, over the last decade, several studies showed the close relationship between the subsurface microbial community and tectonically driven processes.

Due to increased exploration of the mineral springs, a number of deep (>1 km) wells are being drilled with several of them not being economically feasible due to limited flow rates. Abandoned holes may become available for science at no cost. They therefore represent a wonderful opportunity to use them as deep observatories. Access to these holes would be extremely cheap compared to subseafloor observatories and should therefore be considered a viable potential alternative.

Functional Gene and ^{34}S-Isotopic Evidence of Microbial Methane and Sulphur Cycling in 3.5 Million-Year-Old Buried Ridge Flank Basalt
Mark A. Lever, Olivier Rouxel, Jeff Alt, Nobu Shimizu, Shuhei Ono, Fumio Inagaki, and Andreas P. Teske

The community composition and metabolism of microbes inhabiting subseafloor basalt ecosystems remains largely unknown. We detect functional genes (mcrA) diagnostic of methane-producing and methane-oxidizing Archaea in anoxic veins of deep subseafloor basalt on the eastern flank of the Juan de Fuca Ridge. The methane cycling gene signatures co-occur with strong isotopic depletions in δ34S of pyrite grains (to -72‰) that indicate microbial sulfate reduction and sulfur disproportionation and suggest that microbial methane and sulfur cycling are coupled by sulfate-dependent methane oxidation. Our findings demonstrate that microbial carbon and sulfur cycling is sustained in buried subseafloor basalt to millions of years after its formation.

Microbes Produce Thermogenesis-Like Methane in Deep Subsurface
Li-Hung Lin, Yu-Chen Ling, Pei-Ling Wang, Chih-Hsien Sun, and Yue-Gau Chen

Determination of the origin of gaseous hydrocarbons is essential to assess the migratory pathway and reserve capacity during hydrocarbon exploration. The discrimination against each mechanism stems from numerous observations that thermal maturation would produce isotopically heavier hydrocarbons

progressively depleted in ^{13}C with decreasing carbon number and low C_1/C_2+ ratios, whereas microbial processes would yield isotopically lighter methane and high C_1/C_2+ ratios. To seek whether microbial methane is significant, and to identify the exact pathway catalyzing its generation in deep reservoirs, we started with examining previously published $\delta^{13}C$ values of hydrocarbons (C_1-nC_5) retrieved from gas seepages and mud volcanoes in southwestern Taiwan where thermal maturation has been considered as the source mechanism for hydrocarbon generation. We also amended sediments retrieved from a hot (63.8°C) mud pool with a synthetic solution (without substrate) adjusted to *in-situ* salinity and pH, and incubated sediment slurries anaerobically at temperatures from room temperature (RT) to 90°C for a prolonged period of time. Our analyses revealed that thermophilic, acetoclastic methanogens affiliated with *Methanosaeta thermophila* produced methane with carbon isotopic compositions resembling those by thermal maturation. When considering that fermentative acetate production fractionates carbon isotopes at a negligible magnitude and the obtained α values are small, acetoclastic methanogenesis would contribute more than 50% of the observed methane. Such microbial methane produced at the upper crustal level mixes with thermogenic hydrocarbons ascending along deeply-rooted faults in an unnegligible proportion.

An *In-Situ Electrochemical Analyzer* for Studying Redox Disequilibria in Subseafloor Basement Fluids at IODP CORK Observatories

Michael T. Matzinger, Brian T. Glazer, James P. Cowen, and Michael S. Rappe

An upgraded *in-situ* electrochemical analyzer (ISEA) was deployed at CORK (Circulation Obviation Retrofit Kit) observatories affixed to Integrated Ocean Drilling Program (IODP) boreholes in Cascadia Basin on the Juan de Fuca Ridge Flanks. The ISEA was configured for either mobile real-time measurements on a submersible, or as part of an instrument sled package for multi-day time-series sampling at a selected CORK. Voltammetric electrodes, in both deployment scenarios, were mounted into a flow cell to allow for simultaneous detection of redox species (O_2, H_2O_2, HS^-, S(0), S^{2-}, $S_2O_3^{2-}$, $S_4O_6^{2-}$, Fe(II), Fe(III), $FeS_{(aq)}$) concurrent to sample filtering or fluid collection. During real-time voltammetric scanning, qualitative assessment of the relative integrity of fluids delivered through the Fluid Delivery Lines could be made, thus guiding sample collection 'on the fly'. Here, we report results of *in situ* electrochemical deployments at multiple borehole observatories, corroborating data from wet chemistry analyses on discrete samples, and present & planned improvements for the ISEA.

Microbial Communities in Serpentinizing and Ultramafic Environments

Jennifer Kepp, **D'Arcy Meyer-Dombard**, and Dawn Cardace

Serpentinizing seeps may be considered the surface expression of percolating fluids in the deep biosphere, or perhaps the interface between the deep subsurface and surface environments. As such, they represent an opportunity to investigate near-surface habitats that may reflect microbial activity in the deeper subsurface on Earth, with implications for similar processes on other silicate planetary bodies. This study focuses on serpentinizing seeps in the Poison Bay and Transit River region of New Zealand's South Island, in the vicinity of Milford Sound. Fluids and solids [biofilms] of varying degrees of ultramafic and serpentinizing input were collected from surface seeps, some of which likely communicate with fluids traveling along the Alpine Fault at depth. Efforts are underway to culture unique members of these communities, using geochemical data as a guideline for appropriate growth media chemistry. Metabolic options that will be explored will focus on heterotrophy, sulfate reduction,

ferric iron reduction, hydrogen oxidation, and methanogenesis. Results of these culturing efforts and evaluation of microbial community composition will enable comparison of essentially pristine and deeply sourced serpentinizing fluid environments vs. weathered ultramafic environments experiencing ongoing meteoric inputs in the near subsurface.

Seeking Common Ground: Deep Continental and Marine Subsurface Habitats
Duane P. Moser

In recent decades, deep biosphere research has proceeded in both marine and continental settings, but rarely by the same workers or in a coordinated manner. Given the very different natures of these habitats, this may not be surprising. Conversely, many of the same questions and challenges face those who seek to explore both environments. As ultimately marine and terrestrial subsurface microorganisms must utilize a shared biochemistry and are constrained by the same rules of thermodynamics, it seems likely that communities from both will circumvent universal challenges of isolation and substrate limitation in similar ways. As major strategies for deep biosphere energetics and dominant microorganisms from both habitats become better understood, it will be increasingly informative to extrapolate discoveries and technical advances from one realm to the other. It is already evident that the deep continental lithosphere and ocean basement/sediment contact may share certain aspects (e.g., the availability of hydrogen and oxidants). Further similarities may include the relaxation of sulfate reducer domination over methanogens in habitats with high hydrogen concentrations and the abundance of archaea in some marine sediments related to candidate phylum (SAGMEG-1 (1)), first detected in the South African gold mines. These observations suggest that subsurface life in marine vs. continental subsurface biomes may have much in common and correspondingly, that the sharing of insights and resources by workers in both fields will be productive.

Mariana Forearc Serpentine Mud Volcanoes Harbor Novel Communities of Extremophilic *Archaea*
Andrea C. Curtis, C. Geoffrey Wheat, Patricia Fryer, and **Craig L. Moyer**

Extremophilic archaeal communities living in serpentinized muds influenced by pH 12.5 deep-slab derived fluids were detected and their richness and relatedness assessed from across seven mud volcanoes located along the Mariana forearc. In addition, samples from two near surface core sections (Holes D and E) at ODP Site 1200 from South Chamorro were subjected to SSU rDNA clone library and phylogenetic analysis resulting in the discovery of several novel operational taxonomic units (OTUs). Five dominant OTUs of *Archaea* from Hole 1200D (DSA) and six dominant OTUs of *Archaea* from Hole 1200E (ESA) were determined by groups having three or more clones. A representative clone was chosen from each group and fully sequenced. T-RFLP analysis revealed all of the dominant OTUs were detected within both clone libraries. Cluster analysis of the T-RFLP data revealed additional archaeal communities from sites on Big Blue and Blue Moon were analogous to the South Chamorro Hole 1200E site. This study examined seven different mud volcanoes along the entire Mariana forearc system. The discovery and detection of novel Euryarchaeota as well as Marine Benthic Group B *Crenarcheaota* phylotypes were determined as efficacious indicators possibly involved with anaerobic methane oxidation and sulfate reduction fueled by deep subsurface serpentinization reactions.

Colonization of Subsurface Microbial Observatories Deployed in Young Ocean Crust
Beth Orcutt, Wolfgang Bach, Keir Becker, Andrew Fisher, Brandy Toner, Geoff Wheat, and Katrina Edwards

Oceanic crust comprises a massive, hydrologically active environment on Earth, but our understanding of life in this realm is in its infancy. Considering that geologically young, chemically reduced basaltic crust is not in thermodynamic equilibrium with seawater or hydrothermal fluids, life could exist in this environment by taking advantage of the disequilibrium. Current methods for drilling in oceanic crust tend to return microbiologically compromised samples, hampering efforts to resolve the extent and functioning of a subsurface biosphere in oceanic crust. An alternative method for exploring the crust biosphere is deployment of microbial observatories inside boreholes drilled into the crust. We report the design of innovative microbial observatories that can be deployed in the sub-seafloor, highlighting field trials in CORKed boreholes on the Juan de Fuca ridge flank and at the seafloor at hydrothermal vents at Loihi Seamount. Our results demonstrate that *in situ* microorganisms colonize the observatories, confirming that subsurface observatory experiments will be a useful mechanism for exploring the subsurface ocean crust biosphere. We also evaluate the suitability of borehole construction materials in order to inform the development of the next generation of observatories for microbial studies, with attention towards minimizing *in situ* leaching of (potentially) growth-inducing substrates (i.e. carbon, nitrogen, iron).

Is Pressure a Variable that Should Considered in the Design of Long-Term Colonization Experiments for the Subseafloor Biosphere?
Matt Schrenk

There are numerous physical and chemical parameters that are commonly considered and must accounted for when considering the habitability and ecology of deep subseafloor environments (e.g. nutrient concentrations, permeability, and temperature). These environments also exist at elevated pressures relative to the Earth's surface- the average depth of the world's oceans is 3800 m (~38 MPa) and the habitable biosphere exists to some, unconstrained depth beneath this. In the limited number of detailed studies of piezophilic microorganisms, pressure has been shown to have profound effects upon cell physiology including membrane biochemistry, ribosomal structure and diversity, and internal vesicle formation. Furthermore, pressure has been shown to impact the survival and activity limits of pure cultures, and recent studies from our lab has shown that *in situ* pressures also influence the outcome of enrichment cultures inoculated with deep-sea samples. Due to the substantial challenges associated with obtaining laboratory cultures of microorganisms from most natural environments (including the subseafloor biosphere) efforts are being made to promote the *in situ* colonization and enrichment of organisms native to these habitats. This presentation will discuss whether pressure should be considered as an important variable in the design of these experiments and outline some of the technical considerations associated with the design of such experiments.

Bacterial Community Composition in Hydrothermal Plume Environments is Heterogeneous and Distinct

Jason B. Sylvan and Katrina J. Edwards

Hydrothermal plumes are an important link between the microbiology of the marine subsurface and the deep ocean. Here we identified the dominant bacterial populations within the hydrothermal vent plumes of the East Pacific Rise to determine if an endemic population of plume microbes exists, and to establish ecological relationships between microbial populations and vent chemistry. We collected time series samples from two hydrothermal vents at 9°N on the East Pacific Rise (EPR) using sediment traps deployed adjacent to each vent. The collected samples were assessed for differences in bacterial community using automated rRNA intergenic spacer analysis (ARISA) to identify samples from which we then constructed six clone libraries of the 16S rRNA gene. ARISA indicated that there are separate communities at the two different vents, as well as temporal community differences at each vent. 16S rRNA clone libraries of the EPR plume sediment trap samples revealed the presence of epsilon-proteobacteria that are indicative of sulfur cycling and evidence for iron oxidizing gamma-proteobacteria. We conclude by showing that the plume environment is genetically distinct from the surrounding seawater, acting as an incubator for microbes that can thrive there. Little previous work specifically assessed plume microbiology with molecular tools; we begin here.

***In Situ* Colonization Systems in Serpentinizing Microbial Habitats**

Quinn Woodruff and Matt Schrenk

Serpentinization occurs when iron-rich minerals in ultramafic rocks (peridotites) are exposed to water and chemically oxidized; yielding altered mineral compositions referred to as serpentinites. Serpentinites make up a large portion of the seafloor in slow-spreading mid ocean ridge environments, and also occur as exposures in terrestrial ophiolite settings. Progressive serpentinization of these rocks yields highly reducing, high pH fluids rich in hydrogen and in many cases methane and small organic molecules, which can be abundant fuels to support microbial growth in such ecosystems. To this point it has been challenging to obtain laboratory cultures of anaerobic, alkaliphilic microorganisms from serpentinites. As yet, the only reported cultures obtained directly from the serpentinite subsurface yielded abundant neutrophilic heterotrophs and no true alkaliphiles. Furthermore, most alkaliphilic organisms that we have in pure culture are aerobic, and thus not in line with the characteristics of these ultramafic habitats. Verifying the occurrence of a native subsurface biosphere in serpentinized peridotites remains enigmatic. For these reasons we sought to develop experimental devices to promote the *in situ* enrichment of organisms in their environment, referred to as *In Situ* Colonization Systems (ISCS's). We have deployed such systems in both marine (Lost City) and terrestrial (Tablelands serpentinite) settings to some success, and will present preliminary data on these experiments. The ISCS's contain substrates to facilitate attachment and colonization by microorganisms. For instance, the ISCS's deployed in Tablelands contain crushed olivine, which provided a naturally-occurring substratum upon which microbial communities can develop. Some of the issues and challenges with the use of ISCS's in serpentinized peridotite will be discussed including choice of substrata, length of incubation, materials, and ease of analysis. We hope to stimulate discussion of this technology and its adaptation to the unique demands of serpentinizing environments.

Appendix D: List of References Related to the Deep Biosphere

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Final Report

11/12/2009

Please take a moment to answer the following questions regarding the recent workshop on the Big Island. As you know there will be at least 4 more RCN DEBI workshops, and we want to make sure that each subsequent workshop benefits from what we learn from the earlier ones.

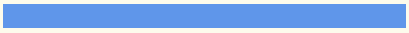
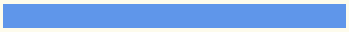
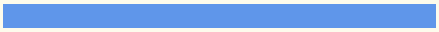
I. These first questions relate to the program, which consisted of lectures, breakout discussion groups, breakout hands-on demonstrations and plenary reports and discussion time.

1. Regarding balance between lectures and breakout groups (choose as many as are appropriate):

#	Answer		Response	%
1	There was appropriate balance		22	73%
2	There should have been more lectures		6	20%
3	There should have been fewer lectures		0	0%
4	There should have been more breakout group time		1	3%
5	There should have been less breakout group time		7	23%

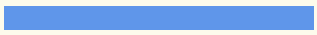
Statistic	Value
Total Responses	30

2. Regarding breakout discussion groups (choose as many as are appropriate):

#	Answer		Response	%
1	There was adequate time for group discussion		27	90%
2	There was inadequate time for group discussion		2	7%
3	The suggested questions were helpful to focus group discussions		23	77%
4	The suggested questions were NOT helpful in focusing group discussions		5	17%
5	The size of the discussion groups was about right		29	97%
6	The size of the discussion groups was too large		0	0%
7	The size of the discussion groups was too small		0	0%

Statistic	Value
Total Responses	30

3. Regarding the plenary report/discussion sessions (choose as many as are appropriate):

#	Answer		Response	%
1	There was adequate time for reports and discussion		20	69%
2	There should have been more time for reports and discussion		4	14%
3	There should have been less time for reports and discussion		4	14%
4	The atmosphere of the workshop invited open discussion		26	90%
5	The atmosphere of the workshop inhibited open discussion		1	3%

Statistic	Value
Total Responses	29

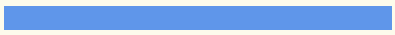
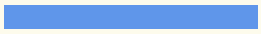
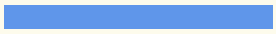
4. How could the atmosphere of the breakout sessions and plenary sessions have been improved to invite open and productive discussions?

Text Response
i think there could have been alittle less time devoted to breakouts and breakout reports. the lectures i think were perfect length and breadth
1) it would have been really helpful if all of the meeting participants had received the breakout group questions ahead of time, so that we knew what we would be discussing. 2) some of the breakout group sessions were too long 3) I didn't like the ranking aspect requested in some of the discussion groups - I feel that this should be a more organic process amongst the community
In general, I thought there was a good balance between the two. THe breakout sessions started to get a little bit long by the second day, and having a lecture or something in the middle would be good to break up the breakouts [!] Also, I loved the hands on aspect of the one breakout session. More please!
I would have appreciated more time for "unstructured" discussion, that just basically follows a train of thought rather than focused discussions on a particular topic.

I'm not sure how this could be improved. There are some rather contrived devices that allow such discussions to proceed effectively. I think that the most important thing is that the leaders are sensitive to having anyone monopolize the discussion and also that they are fast to call on people who have not expressed an opinion. Maybe such an announcement at the beginning of each session is useful" "You will be asked, so think about the question!"
they were fine
The lectures were very helpful in providing background information. I feel that another lecture or two may have been helpful in providing a better foundation for group discussions. In one of the break out groups, for example, we spent about 15 minutes discussing a topic that we then learned was based on inaccurate information.
there was sometimes little summary by the group leaders, it would be better if there was a summary slide or two as opposed to just reading the scribes' notes
My only concern was the very limited time between breakout sessions and the 'reports and discussion' sessions. The reporters should have more time to organize their report, thus making a more efficient contribution to the discussion and be more easily able to initiate a debate.
All things considering it was set up quite well with regards to the atmosphere. Somewhat unrelated to this though, there were times when it was noticeable that there was not enough time to distill everything from the breakout discussions into a presentation for the rest of the group. Some of the ideas and their importance relative to other topics discussed in the sessions may have been obfuscated due to the time constraints imposed between discussions and their subsequent presentations.
The questions were a little redundant.
Arrange a more even-distributed participants in each break out group. Make sure each group is composed of experts in several fields (biologist, geologist, drilling expert, CORK expert etc.) Select group leaders who know the group members and able to encourage every "expert" to speak up.
Most (all?) of the talks were on day 1; perhaps there should have been some on day 1 and some on day 2. As a result there was perhaps too much discussion time on day 2 -- people got a bit burned out.
I thought they were quite good and ran for an appropriate amount of time. A round table format vs. classroom-like setting might have helped encourage discussion, but this wasn't a big deal.
I felt it was great and very inviting. Please keep up the great work.

Statistic	Value
Total Responses	15

5. Regarding the hands-on demonstration sessions (choose as many as are appropriate):

#	Answer		Response	%
1	These 'demo sessions' were very useful		26	87%
2	These 'demo sessions' were fairly useful		4	13%
3	These 'demo sessions' were not useful		0	0%
4	There was adequate time for 'demo sessions'		14	47%
5	There was inadequate time for 'demo sessions'		17	57%
6	The size of the 'demo sessions' was about right		28	93%
7	The size of the 'demo sessions' was too large		1	3%
8	The size of the 'demo sessions' was too small		1	3%
9	There was the right number of 'demo sessions'		18	60%
10	There should have been more 'demo sessions'		11	37%
11	There should have been fewer 'demo sessions'		1	3%

Statistic	Value
Total Responses	30

6. How could the 'demo sessions' have been improved?

Text Response
i think there could have been up to one more demo but we wouldn't want more than that. what would have been helpful is to have a follow-up demo breakout discussions. everyone was so enthused, and i think felt that they barely got enough - were hungry for more!
from the demo session leader side, it would have been nice to organize a 'trial run' amongst the demo leaders, so we could also have a chance to learn what was going on in the sessions we weren't leading (and it would also provide an opportunity for the demo leaders to figure out what works/doesn't work)
I really like all the "hands-on" demo's and I wish there was more time. Perhaps .75 to 1 hour would have been ideal.
They were just a bit too short! We were just starting to get into them, and then had to change rooms. 5-10 mintues longer would be good.
I found the short presentations on selected topics as well as the hands-on demonstrations of tools and technologies very informative. Depending on the tools or instrumentation that are being presented it would be nice to have more time available if needed.
I thought these were great; it changed my impressions of how things were being done. Could even have been useful to see a field demonstration of deployment/measurement.
These were fantastic and a unique attribute of these meetings. At the next site review (whenever that might be) such demo sessions should include the reviewers... being taught by students. I did not attend the site review, and these may have to conform to some pattern, but the demos are super effective at being inclusive and dispelling myths. It's an excellent way to bring researchers together.
A little more time might have been helpful.
there could have been a bit more time for these, they were great as is but i wouldn't have minded 45 minutes or an hour per session.
The demo sessions were fantastic. I would like to see more of them. And perhaps earlier in the workshop to better help give everyone a basic understanding of some of the techniques before discussion.
Demo sessions were a little bit to short. Five to ten minutes more per session would have been useful. However, note that these sessions were perfectly organized and at the right wavelength. Congratulations.
There should've been more time for the demo sessions so that people to talk amongst themselves as well as those organizing the demo sessions.
more demo and less breakout.
A longer time at each station would have been helpful to gain a better understanding of each part.
The demo sessions were really good. If possible, more sessions would be helpful
1. Give us more time to practice and ask questions. Do not let hands-on sessions become lectures. 2.It is good to have more than one person to lead the demo sessions. The Glazer group did a great job. 3. Dry run before the workshop to estimate the time and people's

response to each hands-on demo sessions.
This was a great idea and worked very well. Only thing to improve is to have more of them.
More time was needed to have a real chance to play with the items being presented.
Might have been ok for people to pick and choose their demo session and spend more time at one or two? On the other hand, it is possible that some sessions would have been better attended than others. Just something to think about.
more time
These were awesome and I strongly encourage doing at the next meetings as well.

Statistic	Value
Total Responses	21

II. This part of the questionnaire deals with the venue: The Fairmont Orchid Hotel.

1. Please rate the venue in terms of (choose best answer):

#	Question	Great	Adequate	Could be better	Responses	Mean
1	Quality of sleeping accommodations	30	0	0	30	1.00
2	Quality of plenary room	26	3	0	29	1.10
3	Quality of breakout rooms	26	3	0	29	1.10

Statistic	Quality of sleeping accommodations	Quality of plenary room	Quality of breakout rooms
Mean	1.00	1.10	1.10
Variance	0.00	0.10	0.10
Standard Deviation	0.00	0.31	0.31
Total Responses	30	29	29

2. Was the venue conducive to casual chats/discussions outside of formal agenda times?

#	Answer		Response	%
1	Very much so		25	83%
2	Adequate		5	17%
3	Could be better		0	0%
	Total		30	100%

Statistic	Value
Mean	1.17
Variance	0.14
Standard Deviation	0.38
Total Responses	30

3. Food provided was:

#	Answer		Response	%
1	Great		21	70%
2	Fine		8	27%
3	OK		1	3%
4	Could be better		0	0%
	Total		30	100%

Statistic	Value
Mean	1.33
Variance	0.30
Standard Deviation	0.55
Total Responses	30

Specific comments on food:

Text Response
Awesome
preferred the buffet options to the served meal at lunch-
Hard to beat ;-)
great veggetarian options!
More variety of food would be nice for such a high price
Since I'm not usually on the coast, I would've appreciated the opportunity to enjoy more seafood from that region.
Great options for vegetarians!
For the cost the breakfasts were sub par. Too many of the meals were buffet style.
Too expensive.
It was excellent, but too pricey for guests.
more coffee!
Entertainment was pretty good too.

Statistic	Value
Total Responses	12

4. Considering your NET out of pocket costs, the venue (including travel time and costs) was:

#	Answer		Response	%
1	Way too expensive		4	13%
2	A bit too expensive		19	63%
3	Not bad		6	20%
4	What a deal!		1	3%
	Total		30	100%

Statistic	Value
Mean	2.13
Variance	0.46
Standard Deviation	0.68
Total Responses	30

5. How could the venue have been improved?

Text Response
but hey, it was hawaii!
cheaper alcohol :)
Nothing. It was the best venue I have EVER been to for a science meeting. Lets have all meetings there...;-)
thought it was great, but pricey.
The venue was excellent although a bit too expensive
These days, I really value my time, and having a venue that required two days of travel time off my schedule was a real negative. I would not travel that far ouot of the way for another DEBI workshop.
??? are you kidding? How, indeed? This venue lands near the top of any venue at which I have attended a meeting. Glorious... just needed more time to enjoy it.
The hotel was very nice, almost too nice.
it was a great way to kick off the RCN workshops, but a bit expensive. plus, we were in a fantastic location, but in meetings all day and could only enjoy the place in the evening. price aside, however, the location was fantastic.
Don't have the conference at such a nice hotel. Complaining about a lack of funding in science while having conferences at the Fairmont is as hippocritical as you can get.

It was a very good venue, but flying to Hawaii and lodging was expensive even at negotiated price.
As usual one would say "closer and cheaper", but there is always a hard balance to reach between the two; especially with such a diverse crowd. I enjoyed it very much and would not turn down revisiting that venue in the future.
I think it was perfect.
It was a very nice venue for Hawaii.

Statistic	Value
Total Responses	14