C-DEBI “Limits to Life” Theme Team Workshop

May 17-18, 2011

Redondo Beach, California

OVERVIEW

This report provides a brief summary of a 1.5 day workshop on the subject of the limits to life in the deep subsurface beneath the ocean floor.

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PURPOSE

The Center for Dark Energy Biosphere Investigations (C-DEBI) is an NSF-funded Science and Technology Center established in 2010 and dedicated to the study of the biosphere that exists at and beneath the ocean floor. C-DEBI has three specific objectives: 1) to coordinate, integrate, support and extend the science associated with seafloor drilling projects, 2) to foster and educate an interdisciplinary community of researchers in deep sub-seafloor biosphere research, and 3) to educate, inform, and translate knowledge of the deep sub-seafloor biosphere to the broader community. To facilitate these objectives, C-DEBI provides funding for graduate and post-doctoral fellowships, a research-related travel exchange program, and support of scientific research projects related to investigation of subsurface life.

Scientific research within C-DEBI is focused around four major themes: 1) the metabolic and biogeochemical activity of life in the deep sub-seafloor biosphere, 2) the biogeographical distribution and phylogenetic relationships of lifeforms within the deep subsurface, 3) environmental and nutritional factors that limit the distribution and activity of subsurface organisms, and 4) evolution and adaptation of organisms in subsurface environments. Progress in these focus areas within C-DEBI is being facilitated by the organization of four “Theme Teams,” whose primary functions are to identify key scientific issues within each theme, to promote collaborative research to address relevant issues, and to stimulate new research initiatives when critical information is needed to address a particular issue.

The objectives of this inaugural workshop of the "Limits to Life" Theme Team (theme #3) were to assess the current state of knowledge of the factors that might limit the distribution and activity of life in subsurface environments, and to identify critical areas where focused research is needed to improve our knowledge of limitations to life. The outcome of the workshop is intended to help guide future research efforts and the development of new scientific investigations, both within C-DEBI and beyond, and to identify areas where scientists who have not currently been involved in study of deep subsurface life might contribute additional expertise. The workshop results will also be used as a basis to help formulate future calls for proposals for funding of students, post-docs, and scientific research under the auspices of C-DEBI.

PREAMBLE: DEFINING "LIMITS TO LIFE"

Uninhibited growth of microbial populations in nature is probably something that occurs somewhat rarely and intermittently in a few environmental settings. Instead, it is likely that most microbial populations are subject to some factor that limits their growth and metabolic activities to some degree. These factors may include physical parameters such as temperature, pressure, and salinity, or chemical parameters such as the abundance of essential elemental nutrients, availability of substrates for metabolic energy, and pH. In many cases, multiple parameters may interact to define the limiting factor. For instance, the amount of metabolic energy required for cellular maintenance and growth appears to increase with increasing temperature, requiring a greater availability of energy sources in warmer environments.

The physical and chemical parameters that potentially limit microbial populations vary spatially and temporally within a particular environment. In this respect, limits to microbial life can probably best be viewed as a continuum rather than a singular value (Fig. 1). At one end of the continuum, microbial growth is allowed but is limited by some environmental factor, such as temperature or a key elemental nutrient, which restricts the amount of growth that may occur. When this factor becomes sufficiently limiting, growth of the population may no longer be possible, but cells can still persist, possible indefinitely, in a state in which they are able to maintain their central metabolic functions and biochemical structure. Further restriction of the limiting factor may require organisms to switch into a "survival state", in which only the most essential structures and metabolic functions are maintained, but where the organism can return to full functionality if conditions change. In environments where a factor is sufficiently limiting that an organism is not able to maintain its structure and metabolic capabilities over time, the organism can no longer survive in that environment, and the environment becomes uninhabitable for that particular organism (although, of course, it may be perfectly habitable for other organisms with other physiologic requirements).

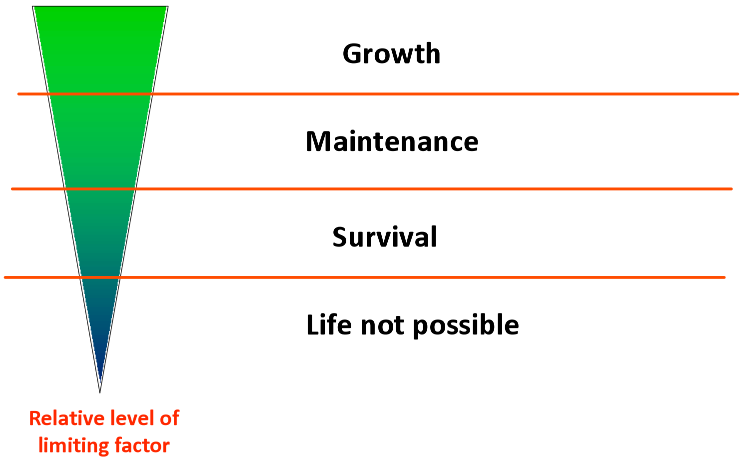

Figure 1. Conceptual diagram of varying levels of a generic limiting factor for microbial growth and metabolism.

A useful perspective for conceptualizing and evaluating limits to life is illustrated in Fig. 2 (after Shock and Holland, 2007, and Hoehler, 2007). In this figure, the metabolic requirement for a generic potential limiting nutrient is plotted against the supply of that nutrient by the environment. Every organism requires some minimal level of substrates to maintain its metabolic functions, although the requirement for a particular nutrient may vary from one organism to another. At the same time, different environments vary in their capacity to supply required nutrients to organisms. Physiochemical environmental factors such as temperature, pressure, and pH contribute to the picture by effecting the minimal requirements needed to maintain metabolic functions.

Growth is possible whenever the level of the potentially limiting nutrient exceeds that required for cellular maintenance (the area above the line in Fig. 2). Conversely, if the environmental supply fails to meet the organism's requirement, the environment is uninhabitable. If the supply just meets the metabolic requirement, an organism would be able to maintain itself in the environment, but not grow. When the level of the potential limiting nutrient falls just below that required for long-term maintenance, the organism may be able to exist in a survival state for some amount of time.

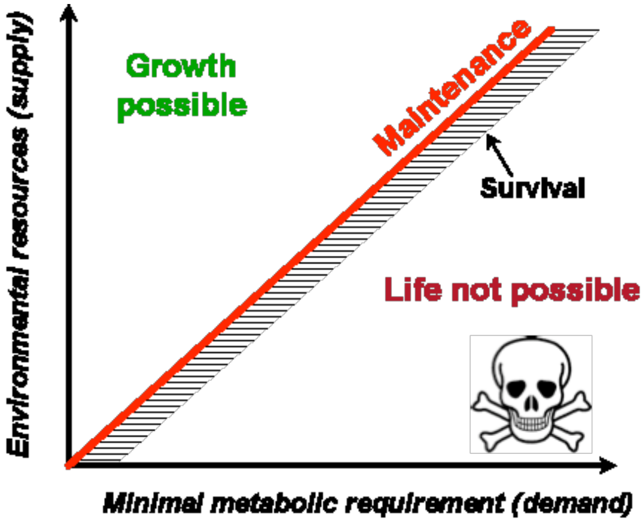

Figure 2. Conceptual diagram of of the relationship between the metabolic demands of an organism for potentially limiting factor and the environmental supply of that factor.

Within this framework, evaluation of potential limiting factors to life can be readily facilitated by comparing the metabolic requirement of an organism for a particular factor with the supply of that factor by the environment. Of course, the challenge in understanding environmental limits to life lies in clearly defining what constitutes the basic metabolic requirements for various different kinds of organisms and for the various different environmental conditions that might be encountered, and in defining the extent to which the environment is capable of supplying that factor.

MEETING SUMMARY

Key issues

There were several topics which arose repeatedly during the discussions that clearly represent key issues that will need greater attention in order to understand limits to life in the deep subsurface. These key issues include:

- There is a clear need to better define the minimal metabolic requirements for slowly metabolizing cells to maintain themselves in subsurface environments. Most organisms in the deep subsurface appear to metabolize at very slow rate where they may only be maintaining themselves with little or no growth, but there appears to a lack of knowledge of the metabolic inputs required to persist under appropriate conditions. More information is needed on the levels of metabolic energy and nutrients required to maintain cells under appropriate conditions, how these requirements vary with environmental conditions and organism, the basic physiological pathways involved, and the consequences for existence at maintenance levels for extended periods (potentially millions of years).

- More extensive culturing efforts are likely to yield valuable new insights that will be useful in investigation of the limits to life in the subsurface. Cultures allow the physiological capabilities of organisms to be characterized and their response to environmental variables tested. Once in culture, "push-pull" tests can be used to observe the physiological response of an organism to environmental stress factors and limitations, and how they recover from when the stress is relieved. Such studies can potentially be used to develop genetic and biochemical markers to assess physiological states and define limiting factors. The value of such studies will be enhanced if they are done with organisms known to be present in the subsurface environment rather than typical "lab rat' organisms (e.g., *E. coli*).

- More information is needed concerning the availability of organic substrates in deep sedimentary environments and the metabolic pathways organisms employ to utilize these substrates. Many, if not most, organisms in deep sediments presumably utilize organic compounds derived from recalcitrant, complex organic matter in the sediments; however, there appears to be few studies to characterize the organic matter, and limited knowledge of metabolic pathways involved in the degradation and utilization of recalcitrant organic matter.

Other C-DEBI specific recommendations that arose during the workshop:

- There was strong feeling expressed at the workshop that there should be community meetings to discuss what experiments should be deployed in CORKs rather than relying on single-investigators.
- It would be worthwhile to either establish or provide links to a list of cultured organisms from deep subseafloor environments (e.g. Cypionka website). This would facilitate experimental & characterization studies with these organisms by letting the community know what is available.

Summary of Discussion Topics

Discussions during the workshop encompassed a wide range of topics related to the limits to life in general and to the specific applicability of these limitations to life in deep subsurface environments. The following is a brief summary of some of the principal topics discussed during the workshop, presented in no particular order:

Temperature as a limiting factor

The increase temperature with depth in the subsurface and near hydrothermal systems makes it inevitable that at some point the microbial community will undoubtedly encounter environments where temperature becomes a limiting factor to life, both as a factor limiting the extent of growth of individual species or completely precluding life altogether. Determination of the response of organisms to elevated temperature will therefore be a critical element of defining the limits of life in the subsurface. However, it appears that the ultimate temperature limit to life as well as the underlying physiological processes that limit organisms from growing at temperatures above their "comfort zone" remain highly uncertain. In laboratory experiments, organisms have been cultured at temperatures as high as 122 ºC, but there is no fundamental reason to believe that this represents a definitive upper temperature limit.

Many physiological processes have been suggested as the ultimate factor that limits growth at elevated temperature. Enzymes are often discussed as a factor that limits growth at elevated temperature since they have a tendency to degrade from thermal stress, but structural modifications have been found to stabilize many enzymes to some degree and a number of enzymes have been found to be stable temperatures well above the upper survival temperature limit of the organism the enzyme is derived from. Another possibility mentioned at the workshop is that superoxide dismutase becomes inactive at elevated temperatures, leading to the potential for harmful effects of reactive oxygen species to increase. Other possibilities include the thermal instability of molecules such as ATP and NADH.

It also appears likely that temperatures limits respond to environmental conditions, but the extent to which environmental factors effect the limit are unclear. For instance, it seems likely that organisms might be able to tolerate higher temperatures in environments where energy and nutrients are abundant relative to environments where these requirements are in limited supply. Since laboratory experiments tend to be performed at conditions where metabolic requirements are supplied in abundant amounts, it is uncertain how temperature growth limits observed in the lab might apply to natural environments.

Consideration of temperature as a limiting factor stimulated discussion about how well the effects of high temperature in the environment are known. For instance, there appears to be some anecdotal evidence for significant drop off in productivity at temperatures above 80 ºC relative to lower temperatures, but these observations have not been rigorously tested.

Discussion on this topic also lead to consideration of the possibility that there might be characteristic changes in gene expression as organisms approach their upper temperature limit. If such an effect could be identified, it would provide a tool for use in the environment to determine which organisms might be stressed by environmental temperatures.

The supply side: energy supply in sediments

It is generally presumed that the main sources metabolic energy in deep marine sediments are heterotrophic utilization of organic matter and radiolysis. What is the metabolic driver for these communities? It is not clear whether kinetic or thermodynamic factors dominate in substrate utilization.

Although recalcitrant particulate organic matter is thought to be the main substrate of energy supply for deep sedimentary communities over a significant portion of the seafloor, there appear to be significant gaps in knowledge of how this occurs. For instance, do microbes promote the breakdown of the organic matter, or utilize compounds released from the organic matter by thermal processes? In either case, what compounds are the important intermediates between complex organic matter and microbial uptake?. There have been few studies that have characterized the recalcitrant organic matter in drill core samples, and limited knowledge of how the organic carbon evolves with time and depth. Although the rate of utilization of recalcitrant organic matter may be the limiting factor in the heterotrophic microbial community, there seems to be a limited knowledge of the mechanisms through which utilization, including whether thermodynamic or kinetic factors dominate utilization and the relative roles of abiotic and biotically promoted processes.

Life in the slow lane

It is widely presumed that the majority of microbial life in the deep subsurface metabolizes at a slow rate, and growth occurs only gradually, if at all. There are some techniques presently available to study slow growth in lab (such as the use of isotopically labeled substrates that can be monitored at very low concentrations), but there appears to be considerable room for innovative approaches in this area to expand knowledge of the metabolic activity of slow metabolism. There may some significant challenges to these studies, since slow growing organisms may not grow fast even if fed a rich broth.

Metabolic status

A critical issue in assessing the distribution and metabolic activity of subsurface populations is the ability to distinguish active cells from those that might be dead, or surviving but not currently metabolically active. Tests have been applied to samples from subseafloor and elsewhere to distinguish between live and dead organisms, and these appear to be more or less robust (although further confirmation of these methods would be worthwhile). However, it is not clear that there are robust methods for distinguishing between cells that are inactive and those that are active but metabolizing at an extremely slow rate. In surficial environments, ADP/ATP ratios have been used as an indication of maintenance/survival, but this appears to have limited applicability to subsurface environments.

The possibility that some organisms in the seep subsurface might be in an inactive state raises the question of how long an organism can survive in such a state. There does not appear to be a good understanding of the physiological requirements for long term survival, or even a good definition for what constitutes a survival state.

The cultural imperative

Many concepts related to understanding limits to life will require study of organisms in culture. Examples discussed included examination of the physiological response to limiting factors and examination of metabolic requirements of slow growing organisms. There do appear to be some ongoing efforts to obtain novel cultures from deep subseafloor environments (e.g., Cypionka and colleagues), but it is not clear how representative these cultures are of the in situ community, and there has apparently been limited effort to thoroughly characterize the physiological capabilities of these organisms under subseafloor conditions. Further efforts to culture representative organisms may provide new insights into their in situ metabolic activity and how organisms adapt to limiting conditions, and may reveal biomarkers of slow or low-energy metabolism that could then be searched for in natural system.

Relieving the stress

One traditional approach for testing conjectures about the limiting factor for a given organism or community is to “relieve the stress” by providing the organisms with an abundance of the potentially limiting factor and observing its response. For example, if a population of plants is thought to be nitrogen limited, the community can be fertilized with nitrogen to see if the plants begin to grow better. This approach can likely be applied to test proposed limiting factors in subsurface microbial populations. For instance, a potentially limiting nutrient could be fed into a borehole to see if the community responds, or materials retrieved from the subsurface can be augmented with the nutrient to see if it stimulates activity.

There do appear, however, to be some potential caveats to this approach. First, many subsurface populations may be adapted to slow metabolism under nutrient limiting conditions, and might not show a clear growth response to increased nutrient levels. Laboratory experiments performed with organisms from surface environments have shown that some slow growers exhibit no response, or even a negative response, when fed enriched media. Also, in natural communities, some organisms may be better adapted to take advantage of nutrient enrichments, so that members of the community might not show a response when fed a limiting nutrient because they are out-competed for obtaining the nutrient.

There are also potential logistical difficulties. Nutrient amendments need to be done with either fresh samples or by deployments in borehole observatories (CORKs) rather than archived samples, or samples removed from the environment. Consequently, this approach might be useful only during drilling when fresh samples are obtained, and not on archived samples. Another potential difficulty is that there also may be multiple limiting factors for some organisms, requiring multiple tests to be conducted with limited samples.

Modeling metabolic requirements

Participants noted that there are several existing numerical models of microbial metabolic energy flow that could be adapted to the study of energetic requirements of subsurface microorganism. For instance, one model uses a network of reactions to evaluate ATP costs of growth for utilization of different energy substrates by *E. coli* under varying conditions (so-called metabolic flux analysis). Another modeling approach makes energetic cost/benefit analyses of metabolic pathways reconstructed from genomic data. Although these models are designed for assessing the costs of growth rather than maintenance, and are optimized for organisms not found in subsurface environments, they could potentially be adapted to study of slow growing or maintenance-level metabolism in subsurface communities.

The importance of flux

There is a time component is inherent in both the biological community's metabolic requirements and the supply of those requirements by the environment. Conversely, study of the deep subsurface relies on "snapshots" of particular points in time. Study of seep subsurface life can be enhanced by greater consideration of the time element, such as comparison of the fluxes of supply and demand of key nutrients over time. However, it is not clear that we currently have the data to calculate fluxes. For example, heterotrophs in the subsurface must rely on a continuous source of organic compounds they can metabolize, but the organic compounds organisms in the community utilize and the rate at which they become available are not yet known.

Pressure as a limiting factor

Pressure invariably increases with depth in the subsurface, which brings up the question of what impact pressure may have in limiting growth. The effect of pressure on membrane composition and function have been examined, and other metabolic processes such as DNA replication are also known to be pressure sensitive but appear to be less well understood. To some degree, increased temperature may counteract some of the negative physiologic effects of pressure, so the impact of pressure with depth may be ameliorated to some degree. Organisms from relatively shallow depths can be grown and studied at laboratory pressures, but microorganisms from depths of 5 km and below appear to require pressure to grow, meaning that study of these organism require special culture apparatus.

Radiation as a limiting factor?

Although radiolysis products has been identified as a source of chemical energy supporting life in subsurface environments and are particularly significant in low organic carbon sediments such as the South Pacific Gyre (D'Hondt et al., 2009), it does not appear that radiation fluxes are sufficient to produce radiation damage that would be restrictive to subsurface life in most settings.

Detection of upper temperature limit to life

Increasing temperature with depth in the subsurface and near hydrothermal zones will inevitably restrict the distribution of life at some point. Discussion of the upper temperature limit of life and the difficulties involved in detecting very low density microbial population brought up the issue that it may be difficult to detect the upper temperature limit of life in the deep subsurface. As temperatures increase, population densities will decrease, but if detection limits are high, how will we know when we cross over the transition from habitable to uninhabitable temperatures?

Are there uninhabited habitable niches in the deep subsurface?

The question arose at the workshop as to whether there might be environmental niches in the deep subsurface that could potentially be inhabited by organisms, yet that niche remained unoccupied. One suggested scenario for how such a situation might arise is that evolving conditions within the subsurface might create a novel niche over time, but the proper type of organism capable of occupying that niche might not be available in the local population, and organisms from elsewhere that are capable may not be able to access the environment. Alfred Spoorman provided the analogy that a banana tree might colonize a new tropical island, but if no monkeys are able to get to the island, those bananas would go uneaten.

This discussion brought up the possibility that physical inaccessibility of an environment to appropriate organisms might represent a limit to life. For instance, if a fracture opened up in the deep subsurface that was not in direct communication with the outside world, it might have all the ingredients suitable for life and yet no organisms would be capable of living in that environment. This possibility raises a distinction between definitions of habitable environments and limits to life.

Is there a "biological evolution" limit to life?

A related topic was brought up by Everett Shock, who suggested the possibility that there may be habitable niches that remain uninhabited solely because biology has never evolved a suitable organism to inhabit that niche. If this occurs, there is the possibility that there may be a limit to life based on life's capacity to adapt and evolve. A cited example is that of aluminum oxidation: although humans have made metallic aluminum commonplace on the surface of the planet where it is out of equilibrium with O_2 in the atmosphere, no known organism is capable of utilizing this abundant energy source. Perhaps a less exotic example is that of oxidation of molecular nitrogen (N_2). While N_2 and O_2 in the atmosphere are in disequilibrium, no known organism takes advantage of the abundant and widespread metabolic energy that is be available from reaction of these compounds together.

Technology

Emerging technologies that could support research to identify limits to life were discussed throughout the workshop. Because characterization of organisms in laboratory culture is essential to establishing metabolic requirements and might be useful in identifying potential biomarkers of physiological response to low energy metabolism, research on limits to life would benefit from improved methodologies and techniques for culturing organisms found in the deep subsurface. The ability to culture organisms at in situ conditions (pressure, temperature, substrates, etc.) could enhance these efforts. High-throughout techniques to rapidly screen environmentally relevant genes (e.g., geochips) will be useful to assess the diversity and metabolic activity of the in situ microbial community, and would be provide information useful in defining the factors that might be limiting to life. Adaptation of these techniques to sea-going conditions would allow for analysis of fresh samples, and provide an opportunity for real-time assessments that could guide further research in the field. Because organisms inhabiting severely limited subsurface environments are present in low numbers, continued development and application of techniques to extract and manipulate single cells will be beneficial.

Notable quotes from the workshop

Jason Raymond on why *E. Coli* is not found in Yellowstone hot springs: "We're blaming Yellowstone for not looking like pooh."

Alfred Spoorman on the possibility of uninhabited habitable niches: "On this island there were no monkeys, even though there were bananas on it."

Acknowledgements

The success of the meeting was largely attributable to the extraordinary and excellent efforts of Ann Close to organize and coordinate every aspect of the meeting. Matt Janicak also did a great job in providing support for travel arrangements and other aspects. Funding for the workshop was provided by C-DEBI (Katrina Edwards, PI).