

A PROPOSAL FOR A MARS ANALOG MICROBIAL OBSERVATORY

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INTRODUCTION

In the late 1990's, the National Science Foundation (NSF) recognized the value of studying microbial ecology in order to better understand ecological systems and instituted a program devoted to the development of microbial observatories. The program's purpose was to establish a network of sites where scientists could focus on the discovery of unique microorganisms and the study of the microbial diversity and ecological processes in various ecosystems. Currently, there are eight Microbial Observatories (MO) funded in the United States under this program, which awarded approximately 2.5 million dollars per year between 2002-2004. The average award varied from one-half million to one million dollars over five years. The major goals of a microbial observatory are to identify unknown microbes, characterize the properties and activities of newly discovered and poorly understood microorganisms and their communities, provide educational and outreach activities and to disseminate these findings using an established internet-accessible knowledge network^{1, 2}. In addition, microbial observatories each follow an established long-term ecological research (LTER) program, while allowing for additional environment specific research³. The LTER program was established in 1980 by the NSF to investigate ecological processes over long temporal and broad spatial scales and to promote synthesis and comparative research across varying sites and differing ecosystems. There are currently twenty-four LTER sites in the United States and a network office in New Mexico. The program has an annual budget of 17.8 million dollars and supports 1100 scientists and students with an additional 44 million dollars in funding. Twenty countries other than the United States now have LTER programs of their own, including Canada and Australia⁴.

The goals of these two programs are similar to many of the goals identified as being important at Mars Analog sites: to discover new microorganisms and learn more about those we have already identified, to use databases and the internet to communicate and disseminate the results of scientific research being done at a specific location, and to educate the general public and to support student researchers. For these reasons, the Mars Analog Research Station Program would benefit from adopting the procedures and protocols developed by the scientific community for the study of microbes and their ecology at microbial observatories.

What is the applicability of a Microbial Observatory to Mars? It is true that we do not know if there is or has been life on Mars. However, if there is life on Mars, studies here on Earth can focus on developing methods that will best allow us to discover it. One example is to use NASA's "Follow the Water" Hypotheses for the Mars Exploration Rovers Spirit and Opportunity and apply it to Mars analogs on Earth. Another example is to take our knowledge of latent microbial cultures and develop techniques here on Earth that will address the challenge of delayed growth in any possible Martian microbes.

A commitment to long-term research and a database that demonstrates that commitment is necessary in order to qualify for funding as a LTER/ MO site. MDRS already has three years of data applicable to the development of a Microbial Observatory. However, some LTER sites with many years of data still do not receive funding. We need an ongoing directed research program, a consistently updated online database and website, and collaborators from other institutions and universities. To do that, we need to demonstrate to these institutions that we can support their research over the long term. Once we have developed a program that has the rigor, depth and breadth of current LTER and MO sites, we can apply for NSF funding. If we were to receive NSF funding, most of the funds would need to be used for projects in the United States. As a result, MDRS would be the analog station that would benefit most. Some funding could be used to support microbial observatory programs at the remaining analog stations; however, those stations would also have to apply for additional funding from other sources, including the governments of Australia, Canada and Iceland, for their major support. Figure 1 shows a possible organizational chart for a Microbial Observatory at all four analog sites. While this chart would be most applicable once major funding has been granted, it can also serve as a model for how we should currently structure any collaborative efforts between scientists at the different stations.

Possible studies which could be used to develop a microbial observatory include, but are not limited to, phylogenetics, physiology, metabolism, genomics, growth, adaptation, survival, interactions, ecosystem processes, novel properties and modeling of systems. For example, terrestrial ecosystems can provide models for possible extinct or extant Martian ecosystems. Since development of methodologies on Mars will not be easy, it is best to develop methodologies for life detection here on Earth. Prior to our exploration of Mars, there are many things on Earth that can teach us about possible life on Mars. Therefore, hypotheses based on Earth analogs are valuable. The Mars Desert Research Station (MDRS) in the United States, Flashline Mars Arctic Research Station (FMARS) in Canada, MARS-OZ in Australia and the future EuroMARS in Iceland are promising sites for development of a worldwide microbial observatory. We could use the same research criteria as other established microbial observatories for our research, within the framework of Mars analog environments. This would also give us the opportunity to explore more varied science questions than at most Microbial Observatories.

Most established microbial observatories are site specific. Proposing a microbial observatory based on a set of Mars analog sites has never been done. However, a recently added component to NSF's Microbial Observatory Program, new in 2004, would allow for smaller, shorter-termed projects that need not be based at a single site. This part of the program, called Microbial Interactions and Processes (MIP), will accept funding proposals through 2007, and would be ideal for Mars analog microbial ecology⁵. At up to \$500,000, these awards are smaller than those awarded for Microbial Observatories, which are expected to range from one-half million to two million dollars in this funding round. Combined, the MO and MIP have a current budget of 6.5 million dollars per year⁵. This increase in funding over the last few years demonstrates the importance of microbial ecology to the scientific community at large. The flexibility of the new MIP programs points to a recognized need for examining how microbial processes differ and remain the same in varying locations.

MARS ANALOG SITES

The Mars Society initiated the Mars Analog Research Station Project in 1998. One of its goals was long duration geological and biological field exploration conducted in the same style and under the same constraints that will be encountered when humans first travel to Mars. The rationale for the selection of the four proposed sites for the stations, Devon Island in Nunavut Territory, Canada (where FMARS is located), Wayne County, Utah, U.S.A. (MDRS), the Australian Outback (near Arkaroola in the North Flinders Ranges of South Australia) (MARS-OZ) and Iceland (the future EuroMARS), were that each provided excellent geological and operational analogs. The Canadian site was chosen because it has at its center an ancient impact crater and is a polar desert. Australia was chosen because it has fossil-containing deserts that date from the time when we believe the surface of Mars held water. Iceland was chosen because its basaltic and geothermal areas most closely resemble where we believe extant life may be found on Mars. Interestingly, the Utah site was selected for its ease of access and physical resemblance to Mars, and was originally slated as a test bed for equipment and isolation experiments⁶. As mentioned before, none of the four, with the possible exception of Iceland, was selected based on biological characteristics. However there are common biological links at each site that are of great research value. Cryptobiotic crusts, found worldwide, are abundant at all sites and constitute the majority of ground cover in some areas associated with each site. Biologically, up to 80% of the living ground cover is cryptobiotic crust in nature, which consists of cyanobacteria and its associated green algae, moss and lichen. The cyanobacteria help maintain soil stability and moisture and assist in the germination and growth of the area's native and non-native plant species. These crusts are very fragile and are easily damaged by human and livestock intrusion, both of which are a problem at MDRS and possibly MARS-OZ. In addition to these cryptobiotic crusts, there are non-organic structures called desert varnish that are of great interest biologically, as they are believed to be fossilized forms created due to biological activity. Finally, all four sites can be categorized as extreme in terms of their environmental conditions. Scientific interest in extremophiles is on the rise and these sites undoubtedly contain microorganisms with unique and/or evolutionarily similar adaptations to their harsh environments.

PREVIOUS RESEARCH

Microbial studies related to the proposed project were started three years ago. During the 2002 field season at MDRS, a project studying the distribution of microbial communities based on water availability was instituted. Soil samples were classified as either wet, meaning they were collected from places where water persists (washes, run-off channels and ephemeral basins), or dry, meaning where water does not persist (escarpments and sloped terrain). Incubation of samples using soda lime as a measure of microbial respiration demonstrated a significant difference in carbon dioxide output between treatments. Wet samples appeared to contain more microbial life than dry samples, based on this measure. This suggests that it is possible to quantify microbial richness across treatments, and that more microorganisms persist during the dry season in areas where water lingers longest⁷. We applied the requirements used to assess long-term distribution patterns of microbial life at established microbial observatories to our study. This work was continued and expanded in 2003 by the science teams of Expedition One. The resulting data represent the equivalent of a four-month intensive field study. The results of

this work are still being analyzed. Preliminary analyses suggest that microbial richness is dependent on water and water persistence is dependent on soil type and not microhabitat, as was the assumption in the prior year. In addition, delayed growth of up to one month in cultured samples in several microbial groups suggests that some microbes have adapted to these environmental constraints^{8, 9}. Due to the success of these studies we suggest that they be continued at all Mars analog stations. However, in order to use the same methodology and apply the same assumptions across sites, baseline measures of biological diversity, both spatial and temporal, must be calculated.

CURRENT RESEARCH

Objectives of this study were to conduct baseline surveys that include transect monitoring of terrestrial plant communities, macroinvertebrate identification counts, and water quality measurements. Having quantifiable measures of biodiversity at each site will give us an indication of how similar and/or different each site is from the others, and we can design experiments accordingly. Consequently, this would allow for collaborative biological projects, such as microbial taxonomy, ecological investigations, and LTER studies at all sites. Because the locations for MDRS, FMARS, MARS -OZ and EuroMARS were selected based on their geological, and not biological, analogous characteristics, a baseline biodiversity study was needed to provide researchers information on biological richness and equitability at the macroscale level, which could then be applied to processes at the microscale level.

The development of universal methodologies for all sites must be based on measures of biological similarity between sites. Biodiversity indices developed from plant and macroinvertebrate communities, two main biological ecosystem components, are valid for the determination of biodiversity. In order to better determine the feasibility of linking all Mars analog sites into a single unified Microbial Observatory for study, we proposed that a baseline biological survey and calculation of biodiversity indices at each site be undertaken. Surveys, following the below methodology, were conducted at MDRS in May 2004, at FMARS in July 2004, and at MARS-OZ in August 2004. Research dates for EuroMARS are still to be determined.

BIODIVERSITY STUDY SITES FOR MDRS, FMARS, AND MARS-OZ

Based on each Mars analog's geomorphology, stream order, elevation and environmental conditions, various watercourses were selected for our study. Plant survey sites were determined based on location of freshwater sampling sites.

At MDRS, we completed our surveys along three permanent lotic systems: Muddy Creek, Salt Creek and the Fremont River. Muddy Creek, a third order stream, was surveyed in two places: below the confluence with Salt Creek and above the confluence with the Fremont River. Salt Creek, a first order stream, was surveyed almost at its spring source in Salt Wash, and again just above the confluence with Muddy Creek. The Fremont River, also a third order stream, was sampled just east of the turnoff to MDRS at Highway 24 and again directly south of Factory Butte. Fifty-six aquatic invertebrate samples were collected from the above streams. Initial

assessment indicates low benthic invertebrate diversity as well as low abundance. Taxonomic identifications of the organisms have not been completed.

At FMARS, nine streams were sampled: FMARS River (second and third orders), HMP Creek (first and second orders), Snowy Creek (first order), Hinsa River (second order), No Man River (third order), Little Comet Creek (first order) and Seven of Nine Creek (first order)¹⁰. A total of 75 aquatic invertebrate samples were collected. Taxonomic identification of the organisms has not been started. A preliminary assessment of the macroinvertebrate samples indicates a low diversity. Very few organisms were found and it is believed to be due to the limited amount of carbon falling (organic matter such as leaves etc.) into the stream however, further assessment is needed. Plant diversity was also low; a total of nine different species were found and documented. This is consistent with other studies of plant diversity in the area¹¹.

At MARS-OZ, our goal was to sample at least three creeks from their headwaters to the lowlands. By doing this, we would have sampled first through third order streams at various elevations. This would have allowed us to compare first order streams (highland to highland) and third order streams (lowland to lowland) and the surrounding vegetation. However, the study area was experiencing an extended drought, and no streams were flowing. Study sites consisted of various natural springs and waterholes. Waterholes were located where the groundwater table had reached the surface, while the springs were pressurized systems. Most of the springs were slightly radioactive and had warm water while the waterholes were not radioactive and had cold water. Site names did not always reflect this distinction. Survey sites included Paralana Hot Springs, Arkaroola Springs, Noodulanoodula Waterhole, Munyallana Spring, Nepouie Spring, Black Spring, Old Paralana Homestead Spring, Bolla Bollana Spring, Arkaroola Waterhole, Echo Camp Waterhole, and two waterholes in Barrarna Gorge. These sites were between 100 and 500 meters in elevation. We conducted plant and macroinvertebrate studies at all sites except Old Paralana Homestead, where no plant survey was conducted because most of the vegetation surrounding the spring had been planted there by homesteaders. A low diversity is expected from these lentic sources¹².

MATERIALS AND METHODS

Preliminary identification of sampling locations was conducted using topographic maps. Once actual sampling locations were established, GPS coordinates and site photo-documents were taken. In addition, water quality measurements were recorded for each sampling location prior to macroinvertebrate sample collection, and a visible physical habitat assessment was conducted.

- 1) Using a water quality data logger and/or water quality meters we recorded the dissolved oxygen concentration, pH, temperature and conductivity of each riffle habitat or pool. These measurements were taken at the lowest riffle sampled at each reach or, in the case of the pools, at 0.5 – 1 meter from the water's edge.
- 2) Aquatic macroinvertebrate collection and identification was conducted using the following method when sampling locations consisted of riffle habitats.

- a) A 100-meter reach of the stream was identified and three riffle habitats were randomly selected for each sampling location. The reach could be of greater length if riffle conditions dictate.
 - b) From the randomly selected riffles up to 3 sub samples of aquatic invertebrates, depending on the width of the stream, were collected across the stream channel¹³.
 - c) Samples were collected using a Surber-square stream sampler.
 - d) Sample was transferred to a labeled sampling bottle and preserved with 95% ethanol.
 - e) Steps 1-5 were repeated for each riffle habitat at each sampling location.
 - f) Sorting and identification of aquatic macroinvertebrates will be conducted in the laboratory using a dissection microscope and the identification key of Merritt and Cummins¹⁴.
 - g) In Australia, this methodology was slightly altered to allow for better collection of macroinvertebrates in pools. A 30 cm square area of sediment was disturbed to collect a single sample in both springs and waterholes. All other methods were unchanged.
- 3) Plant distribution and identification was conducted using the following method and was completed within a 50 m radius of each aquatic sampling location at the same time, or within the same week, that the stream was sampled. The exception to this was MDRS, where plant surveys conducted in May 2004 were incomplete.
- a) From the middle of the lowest sampling riffle at each reach or from the single sample collection point in lentic waters, a random point, generated from 50 m radius/360 degree random numbers tables, was identified as the starting point for the transect.
 - b) Plant counts and identification were completed along transect lines run for 25 m out from each randomly selected point in the cardinal directions (N, S, E, W).
 - c) Unidentifiable plants were photographed for later identification in the lab. None were collected.
 - d) A plant checklist was developed for all plants within the area. All species were photographed for reference.
- 4) The Shannon Wiener index and Simpson's index are used to calculate biodiversity indices that are relative measures of richness and equitability for both plants and aquatic macroinvertebrates¹⁵.

- 5) The % similarity between sampling locations is calculated following a protocol whereby the family biotic index (FBI) is first calculated for each sampling location and used to calculate % similarity as follows in this example: % similarity = (FBI of first order site A/FBI of first order site B) x 100₁₆. Significant differences between sites is determined by calculating a T-test at $\alpha = 0.05$ using the FBI₁₇.

EXPECTED OUTCOMES AND SIGNIFICANCE

Although we are still at the preliminary stages in the analysis of our collected data, we expect to find similar biodiversity indices for MDRS and MARS-OZ. We do not expect FMARS to be as similar to either MDRS or MARS-OZ as they are to each other. It may be that we use universal methodologies at both MDRS and MARS-OZ, but alter them slightly to reflect the biological differences at FMARS, when the same studies are conducted there. The ultimate goal of our study is to develop universal methodologies for biological research, and more specifically microbial ecology, conducted at Mars analogs worldwide. Having a baseline measure of each site's macroscale biodiversity allows us to determine whether or not we can do this with some degree of confidence. The more biologically similar two sites are, the more confidence we can have that assumptions and experiments created for one Mars analog are appropriate for another. The benefits of conducting this study, therefore, beyond creating a baseline biological survey at each site, were three fold: (1) To develop the methodology and conduct the surveys to determine the indices at these three sites, (2) the developed methodology can then be applied to other Mars analog sites and (3) if we determine that these sites (MDRS, FMARS, MARS-OZ and EuroMARS) are biologically similar, a wide range of scientific assumptions and methodologies can be applied to all three Mars analog sites. For example, in an earlier study we determined that the soil moisture content at MDRS correlated to soil composition and not to the proximity of a water source. If our determined indices are statistically similar we can then assume with some degree of confidence that this will also be the case at MARS-OZ, FMARS and EuroMARS.

The current Mars analog sites were chosen based on their geology and extreme environments. We recognize that human activities, water quality, climate, and evolutionary adaptations of the biota at these various sites may influence differences in biodiversity. Therefore, in a scenario opposite to the one outlined above, our determined diversity indices may indicate that the sites are not biologically similar. Consequently, the methodologies developed by the proposed study may not be used as indicated in (3) above. However, if this is the case, we still believe that data provided by this study are valuable in the sense that we will have determined that one or more stations are site specific. Therefore, each site may require its own methodologies, which may also be the case on Mars. Additionally, the development of these site-specific methodologies can be used as alternatives to the proposed universal methodologies. Fortunately, the scientific community has recognized the value of looking at microbial ecology across time and space, and, just recently, in applying the same questions to more than a single physical site. Either way, studies of microbial ecology at Mars analogs should be a priority and development of a fundable Mars Analog Microbial Observatory is recommended.

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FIGURE

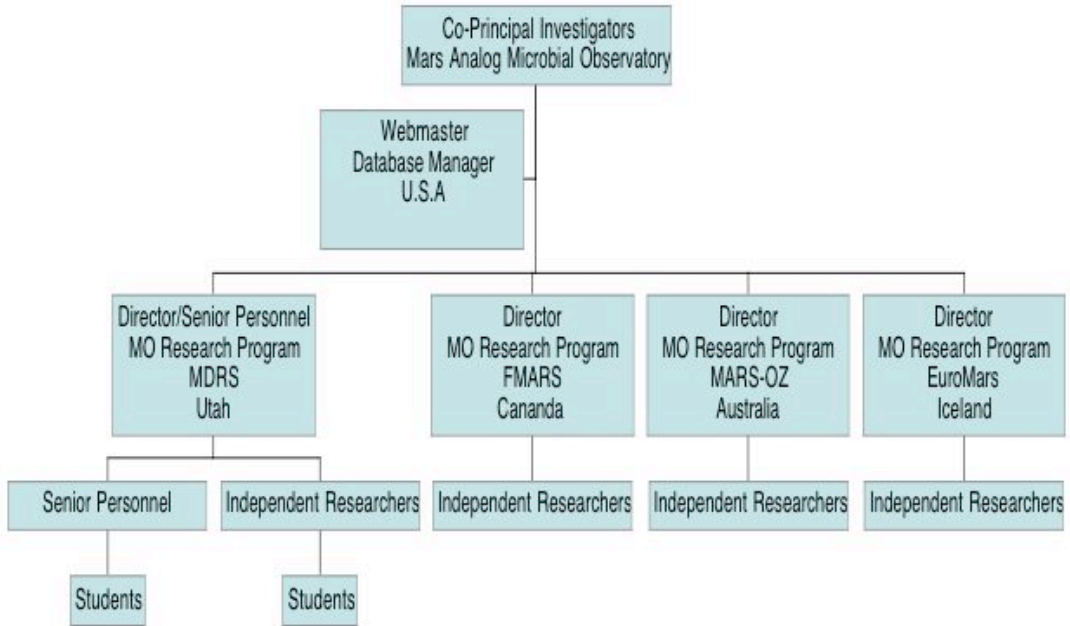


Figure 1. Proposed Organization of a Mars Analog Microbial Observatory (MO). Because NSF funding rules require that most funds for a Microbial Observatory must be used in the United States, MDRS would begin the focus of much of the research and would receive much of the funding. A small percentage may be used to fund liaisons for the other Mars analog stations but most funding for those stations would have to come from additional sources.