

# Exobiological Protocol and Laboratory for the Human Exploration of Mars – Lessons from a Polar Impact Crater

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The search for life (or the examination of the reasons for its absence) is one of the most compelling scientific activities on Mars. We describe the study of the microbiology of the Haughton impact crater in the Canadian Arctic, from a simulated Mars lander (the

FMARS). Impacts events have had a profound influence on Mars, and thus on any putative microbial habitats that future explorers might seek. The study of microbial habitats was accomplished under simulated EVA time constraints and with simulated Mars communications. The work was catalogued to develop a computer model for Mars mission planning – ‘Brahms’. We implemented a program of cosmic ray dosimeter deployment and we describe how sampling of paleolake deposits might be accomplished from a lander. We suggest that science on the surface of Mars can be accomplished from the testing of hypotheses through to the preparation of peer-reviewed manuscripts during a long-duration stay, a significant difference to merely sampling as on the Apollo expeditions. The design of a Martian surface exobiology laboratory is described.

Keywords : exobiology, Mars, human missions, lander, EVA, communication, paleolake, impact crater, Haughton

## **1. Introduction**

Unlike the Apollo missions, human expeditions to Mars will involve long duration stays (between 40 and 400 days depending upon mission configuration [1]).

Thus, scientific activity on Mars is not limited to sample collection, but could involve a program of great scientific complexity, from the testing of new hypotheses through to the submission of reports and scientific manuscripts prior to return to the Earth. To successfully plan protocols for the human exobiological exploration of Mars and to design a laboratory that is sufficiently equipped to answer the questions anticipated on Mars requires substantial pre-planning on Earth.

One way to plan for human missions to Mars is to carry out a program of research in a Mars analog environment on Earth. From these experiences it is possible to formulate a plan for a laboratory with the required instruments to answer the expected diversity of scientific questions. Furthermore, communications protocols and human factors scenarios can be tested within the framework of this analog activity. An important Mars analog environment is the Haughton impact structure on Devon Island, Nunavut, Canadian High Arctic, a 24-km diameter impact crater formed from the collision of an asteroid or comet with the Earth ~ 23 million years ago.

In this paper we describe how the exobiological research being undertaken at Haughton can provide lessons for the human exploration of Mars, and specifically, how these lessons have been learned from the Flashline Mars Arctic Research Station (FMARS, Figure 1), a simulated mars lander.

## **2. Location of the FMARS and characteristics**

The Haughton impact structure is a well-preserved complex impact crater [2]. The ~24 km diameter structure was formed  $23.4 \pm 1.0$  Ma [3] in target rocks made up of a

~1,750 m thick series of lower Paleozoic rocks overlaying the Precambrian metamorphic basement of the Canadian Shield. Many of these metamorphic basement rocks were shocked and excavated to the surface of the crater where they can be found today. Because of its high latitude polar desert location and impact-altered characteristics, Haughton offers an important geological and exobiological analogy to Mars [4] The FMARS is a simulated Mars lander that was constructed in the Haughton impact structure in July 2000 by the Mars Society and the NASA Haughton-Mars Project. The FMARS is situated on the north-west rim of the crater at location 75°25'52.39" N, 89°49'28.05"W at 267.7 m elevation. The FMARS is a two-deck structure with a diameter of 8 m and a height of 7.8 m. The first simulated EVA's from this facility occurred during July and August 2001.

During EVAs a suit developed by the Mars Society was worn as shown in Figures 3 and 4. This is not a prototype Mars exploration suit. The canvas suit and transparent helmet connected to a backpack air supply has two functions. Firstly, the suit and helmet introduce restrictions into field science. These restrictions take a number of forms including; restrictions in mobility and dexterity, a mass burden imposed on the individual and restrictions in the field of view from within the helmet. The suit introduced a greater fidelity into the simulation by slowing the team down in the same manner in which they would be on Mars, particularly in the suit-up and de-suiting procedures required before the team could undertake fieldwork. This improves accuracy of time stamping when developing EVA protocols. The restrictions helped considerations on the design of certain instruments for scientific activity on Mars, e.g., the design of rocking collecting tools or digging implements.

Secondly, the suits provide a public outreach function. Discovery channel and other media outlets filmed the simulations during 2001. The suits provide a visually important context to the field research in Haughton that can serve to illustrate to the public the likely experience of human Mars exploration and provide a way of demonstrating how human scientific activity on Mars might be influenced by the need to wear EVA suits. The suits inspire public interest in the possibility of human Mars missions.

### **3. Microbiological Investigations in Haughton – Relevance to Mars**

#### **3.1 Mission objective 1 – study of shocked microbial habitats**

During the asteroid or comet impact many of the target materials were subjected to high levels of shock and high temperatures that altered their density, porosity and translucence and thus altered their suitability as sites for microbial colonization. Because the surface of Mars is essentially an impact processed surface exposed to polar-like physical conditions, the way in which impact-processed rocks are colonized by microbial populations is of substantial exobiological interest. The microbiological investigations in Haughton accomplished during the simulations in 2001 were focused on the collection and study of microbial populations associated with shocked gneisses that are found in outcrops of impact melt rocks in the crater. The nature of these microbial habitats and the protection they afford to microorganisms that inhabit them has been described previously [5].

Cyanobacteria are found to colonize Precambrian basement gneiss shocked to pressures of 20 gigapascals (20 GPa) or greater in much higher abundance than unshocked or low shocked ( $< 20$  GPa) gneiss. The cyanobacteria found in these habitats are of the genus *Chroococidiopsis*, a desiccation, radiation resistant cyanobacterium (Figure 2). Although these organisms can also be found in the sublithic environment (on the underside of rocks) and in macroscopic cracks (as chasmoliths), their association with the interior of the shocked rocks, made possible by the increase in porosity and translucence caused by shock processing, illustrates a rare instance in which the shocking of metamorphic rocks by an impact event can improve the availability of habitats for photosynthetic microorganisms. This finding is of relevance to Mars because although Mars may well have sedimentary rocks, it is primarily a surface of impact shocked non-sedimentary rocks. By characterising the way in which impact-shocked rocks provide a habitat for life in an extreme polar environment we are able to derive insights into the types of micro-habitats that might be examined on Mars by human explorers.

We have also found that heterotrophic microorganisms (organisms that feed off organics) inhabit the interior of the rocks [6] and so it is clear that shocked rocks, as well as offering habitats to the more evolutionary advanced photosynthetic branches of the eubacterial line, may also offer habitats for heterotrophs and probably chemolithotrophs (organisms that derive their energy from chemical transformations) as well, which may be of greater relevance to any search for putative microbial activity or biomarkers on Mars.

The study of these shocked habitats, as well as being of obvious exobiological and terrestrial microbiological interest in its own right, provides an outstanding context in

which to implement analogue studies of EVA communication protocols, determine laboratory procedures, determine laboratory equipment requirements and develop predictive models of human factors in the Mars environment such as the ‘Brahm’s’ computer simulation.

### **3.2 Mission objective 2 – deployment of cosmic radiation dosimeters**

Our secondary mission objective was the deployment of cosmic radiation dosimeters in five locations in the crater. Andrew Karam at the University of Rochester, USA provided these dosimeters, designed for measuring neutrons impinging on the microbiota in our location close to the magnetic north pole. The cosmic radiation dosimeters were deployed 1 m above the ground, on the ground and 30 cm under the ground in five different locations. Control dosimeters were held inside the habitat and in a lead box outside the habitat. Three dosimeters were deployed in each position.

The deployment of cosmic radiation dosimeters has relevance to Mars exploration because the lack of a magnetic field on present-day Mars makes the surface one that is bombarded by cosmic rays and high-energy particles during Solar Particle Events (SPE). The deployment of micrometeorology and radiation dosimetry stations around the habitat and in areas under intense scientific investigation will therefore be a high priority task during a human surface stay. Incorporation of this activity into EVAs provided information on the time taken to deploy instrumentation around a Mars base as described for the EVA of July 14.

### **3.3 Mission Objective 3 – retrieval of lake cores**

The sampling of paleolakes was not accomplished from the FMARS during 2001, but from the NASA Base camp. However, because this is likely to be an important exobiology activity on Mars we use the EVA and laboratory lessons learned from the FMARS to provide some observations on how lake coring might be incorporated into scientific activity on Mars.

Sediment that accumulates at the bottom of water bodies such as lakes and ponds can be recovered and investigated as a means of gathering historical climate data. Lake cores provide a comprehensive historical overview of the past environmental conditions not only found within the aquatic environment, but also from the airshed and surrounding basin. Life that may have flourished in these water bodies such as bacteria, algae, and larger multi-celled organisms can also leave their physical and chemical signatures behind in these lacustrine deposits, providing a glimpse into the aquatic ecology of the lake's past.

In remote areas such as the Canadian High Arctic where there is a paucity of both baseline and historical limnic data, the recovery and analysis of lake sediments using paleolimnological techniques has been shown to be an effective tool for acquiring the necessary information to investigate and monitor climate and environmental change [7,8].

Among other characteristics, the remoteness of the High Arctic makes it a good analog for Martian investigations. In the case of High Arctic, paleolimnological studies may act as an analog to martian climate investigations since paleolake sediment on Mars may prove to be the key to uncovering the secrets of its climate history. More specifically, observational data from Mars indicate that water may have pooled into lakes in the natural depressions provided by impact structures, and that these ancient crater

lakes may have subsequently left behind lacustrine deposits that are still visible today. In the future these sediment remains may prove to be a prime region in which to probe in our quest to understand and document: (a) martian climate history, (b) how the climate may have changed over time, and (b) the possibility of life having taken hold during the time that these ancient lakes (paleolakes) were present.

In the Haughton region paleolimnological investigations were carried out on both extant and extinct water bodies. With respect to the first category, a small pond (depth < 2m) at the periphery of the crater was investigated. This included recovering 10 cm of sediment from the deepest point in the pond using a Glew maxi gravity corer, extruding the sediment from the core tube at 0.5cm intervals, and archiving samples in sterile plastic bags labeled with the core code, date, and sample interval. With respect to the latter category, Miocene lacustrine remains were recovered from the lacustrine sequence laid down in the post-impact lake within the Haughton structure using U. S. Army Corps of Engineers Cold Regions Research and Engineering Laboratory (CRREL) coring systems and samplers, and several other pieces of equipment constructed by Salisbury & Associates, Inc. The use of drilling fluids was eliminated from this sediment recovery exercise in an effort to simulate potential coring conditions on Mars. Upon retrieval, core sections were individually wrapped in plastic, appropriately labeled for core number and depth, and kept frozen while in the field. These samples were subsequently shipped to NASA Ames Research Center and stored in a -20°C cold room. Although the coring activities conducted on the Miocene post-impact lakebeds may have more direct pertinence to future Mars paleolake investigations, both coring activities provide a working context from which to base potential Mars exploration protocols.

#### **4. Implementation of Communication with Remote Scientists**

Using analogue locations as a model for Mars exploration, in particular human missions to Mars, must be done with care if we hope to gain results that are truly a property of the Mars exploration environment, and not one of “simulation” aspects of the research. Research on Devon Island has demonstrated that we can expect continuous audio and video communications from scientists in the field during extravehicular activity (EVA) as long as Earth is visible from the landing site. This is, indeed, the case for a large fraction of the daylight period on Mars, for the average sol, and communications will only be lost for a period when Mars passes behind the Sun, viewed from Earth.

In reality, we can therefore expect that Earth-based scientists will have a very important role in the exploration of Mars, as they will have the resources to analyse the constant stream of data coming, primarily automatically, from the team on the planetary surface. They will have access to a large amount of data that may never be seen by the Mars-based crew, such as automated multi-spectral imaging of the traverse route, precise object field positioning of millions of targets per EVA through automated LIDAR, and more. Many of these functions may be performed by robotic helpers that may be totally controlled from the Earth-based Mission Control facilities, without an EVA crew even being involved in the data collection process.

The technologies for such a pervasive telepresence by Earth-based scientists on a human Mars mission are being tested and developed on Devon Island inside the NASA Haughton-Mars and Canadian Space Agency (CSA) MarsCanada projects. However, it is

not appropriate to test all these technologies, at this point, in the low-fidelity FMARS environment. Thus there is a certain fidelity loss, in the sense that the scientists remote from the Haughton-Mars field site have significantly less situational awareness and control of the scientific activities that occur in the field than they can be expected to have in an actual planetary surface exploration mission.

With the above caveat, satellite communication tools have been placed in FMARS, during the 2001 Field Season, that provide data rates comparable to the minimum speeds expected on a real mission, at similar packet loss rates. Data rates were approximately 512 Kbps symmetric (uplink and downlink). A parallel activity tested Internet protocols and techniques being developed inside the CSA MarsCanada framework for interplanetary links using Simon Fraser University's Deep Space Planning, Operations, and Communications (DeepSPOCC). Testbed architecture. The methods for data exchange used in FMARS were then kept compatible with those in the DeepSPOCC experiments, without using the systems for that research. In practice, this involved E-mail of smaller files, and delivery of larger files to a Chief Flight Engineer in the field for appropriate data transfer to specially designed server systems located in the simulated "Earth" environment.

The resulting infrastructure thus supported FMARS experiments concentrated on the collaborative exchange of theories and results in the exploration field environment as we describe below for the July 14 activity, but it will less concentration on situational awareness and Mission Control needs. However, this provided an appropriate decoupling of various parts of the experiment and allowed us to concentrate on the "traditional"

aspects of scientific collaborative networking, namely conjecture building and write-up of the exobiology work in the distributed scientific environment.

### **3.4 Modeling life and work in the habitat - a laboratory design tool**

In parallel with the exobiological activity, we were comprehensively recording other activities in the FMARS habitat in order to create a computer simulation of how people used available space, what activities they did when and where, and how the various tools, facilities, and schedules affect the quality of scientific work. This computer simulation of FMARS activities is implemented in a tool called Brahms [9-11]. A Brahms model is a way of formalizing—stating, collecting, and organizing—observations about a work system, so information can be correlated, shared, and applied in improving or redesigning the work process. Running the model in various "what if" simulations may have many scientific and engineering purposes, varying from instruction to crew scheduling and software design.

With respect to design of an exobiological laboratory, the Brahms model provides a way of formalizing the biology work in the habitat, using mutual observations and objective recordings (e.g., time lapse photography). For example, using the time stamps recorded on photographs, we were able to create a precise timeline of the EVA to Trinity that occurred on July 14, 2001 and the sample analysis activities that occurred afterwards. This information is now being used to develop models for human Mars mission.

During the FMARS simulated mission, the exobiologist and computer scientist discussed the work underway, for example, to contrast the work practices on an EVA day with a sample analysis day (see below). Subjective impressions of activity frequency

(e.g., shuttling between the decks; performing a procedure 100 times in two weeks) provide data for the computer model, which are correlated with time lapse photography and field notes.

Many activities are invisible even to other crew members, such as sending images to colleagues via email, and can only be revealed by repeated reviewing of the events in subsequent interviews and by analyzing videos together. For example, on learning that "during the science activity it is necessary for the scientist to be concentrating but aware of other activities...having an EVA radio close-by", as described below, the computer scientist creating a Brahm's model asks whether it was a practice to always carry a radio inside the habitat to monitor an EVA. On this basis, we generate ideas for redesigning the work practice. For example, would broadcasting EVA reports throughout the habitat using an intercom system be a better way for scientists to undertake their work in the laboratory, but be able to respond to EVA situations?

More specifically, we represent how protocols and EVA planning influence microbiological investigations, to determine what correlations may exist and derive statistics. How did the biologist coordinate his individual work with the demands of group activities? Are the periods of uninterrupted work in the laboratory sufficient or so fragmented that the quality of the work is impaired? This information will suggest causal relations between habitat roles (e.g., the person monitoring an EVA), operating procedures, schedules, tools, and layout. Using the simulation, we are experimenting with alternative organizations, plans, and designs, to be investigated in subsequent analog studies.

## **5. Example microbiological sampling and study protocols**

### **5.1. A typical protocol**

Science, communications and human factors protocols were consolidated during simulated EVAs from the FMARS. A typical exobiology template protocol over two days involving an EVA, sample collection and analysis would proceed as follows.

#### **Day 1 EVA day**

9:00-10:00	Wake-up and breakfast
10:00-11:00	Briefing and preparation for EVA
11:00-15:00	EVA. Sampling of shocked rocks in target location / deployment of cosmic radiation dosimeters
15:00-17:00	Ingress and debrief
17:00 – 19:00	Dinner
19:00-22:00	Initial sorting of samples and note taking
22:00-24:00	Leisure activity (music, reading)

#### **Day 2 Sample analysis**

9:00 –10:00	Wake-up and breakfast
10:00-11:00	Assist with EVA suit up and prep. After EVA egress enter laboratory. Sort samples and prepare samples for study. Cut rocks open if necessary
11:00-14:00	Lunch / Microscopic analysis of samples
14:00-16:00	Preparation of more samples based on microscopy
16:00-18:00	More microscopy and note taking

18:00-21:00            Dinner / Write-up notes and prepare mission control  
science report for day

21:00-23:00            Examine data, plan next stage of studies and send images to  
specialists on ‘Earth’

## **5.2. A specific EVA example.**

### **5.2.1. EVA Schedule**

A more specific example is provided by the EVA on July 14, 2002. On this day a sample collection and dosimeter deployment EVA was undertaken with the time stamps shown below. These time stamps were collected by B. Clancey for input into the Brahms model. Meal time are not included.

9:15	Pre-EVA brief and discussion
10:10	Suit up
11:19	Sitting on ATV waiting to leave FMARS area
13:06	Arrived at Breccia hill near Trinity Pond
13:09-13:18	Burying cosmic radiation dosimeters
13:23	Examining east side of Breccia hill
13:31	Looking at a rocks at Trinity Pond/ Begin collection of shocked rocks
13:56	Still at Trinity Pond
14:23	On the way back to habitat
15:04	Arrival back at habitat and de-suit
15:30	EVA debrief

16:30	End of debrief
17:00	Sample analysis
19:00	End of laboratory work. Begin e-mail communication
23:00	End of work schedule

### 5.2.2. Description of EVA

During the EVA, samples of shocked gneiss were collected at 75°24.53'N, 89°49.76'W at 'Breccia hill' near Trinity Pond at an approximate distance of 2.5 km from the habitat. At this location there is an isolated outcrop of melt-rocks shown in Figure 2 that contain within them (exposed on the surface) fragments of gneiss shocked at varying levels (20-60 GPa). The EVA was conducted on all-terrain vehicles (ATV's) to the site (Figure 4). Rocks were collected using a remote handling device depicted in Figure 5. This device allowed us to selected and grab rocks in suits that otherwise provide restriction for bending down to the ground. Rocks were transferred into plastic sterile collection bags and placed into a sample bag on the back of one of the ATV's. A problem in opening sterile bags was encountered in that EVA gloves have little dexterity. Bags needed to have pull-wires to enable them to be easily opened, as is the case for Whirlpak<sup>®</sup> bags (Nasco, Pittsburgh, USA).

Because rocks were of approximate dimensions 15 x 15 x 15 cm, no dexterity problems were encountered during sample collection. We note, however, that most of our analysis was accomplished back in the laboratory, but the deployment of assay systems and biomarker searches *in situ*, might suffer greater encumbrance from gloves will low

dexterity. Fifteen samples of gneiss were collected and returned to the habitat for analysis.

During this EVA cosmic radiation dosimeters were deployed (Figure 6). Because this involved digging the dosimeters into the ground, tools such as that depicted in Figure 5 could not be used. A trowel implement was used to dig a hole after two of the EVA personnel, V. Pletser and C. Cockell, had lowered themselves onto the ground in the suits. On Mars a digging apparatus that can be used in the upright position, as for rock collecting in Figure 5, might be developed. Most of the time spent during this EVA was in sample collection. Because we had a specific location in which to deploy the cosmic radiation dosimeters, this took only took ~10 minutes to do.

After return to the habitat, samples of gneiss were transferred into the laboratory through the main EVA hatch by the EVA team. In a real Mars habitat samples might be brought directly into a sealed laboratory area through a sample hatch that leads directly into a flow cabinet. This would provide stringent back contamination protocol.

After sample transfer the team desuited and carried out a debriefing session in the top deck to discuss the conduct and protocols of the EVA. Unlike the typical protocol described in section 5.1, sample analysis was undertaken after 16.30 on the same day as the EVA.

### **5.2.3. Sample analysis**

On July 14, as with typical sample analysis on other days, a protocol was followed:

1. The shocked rocks were first examined with the naked eye for visible exterior microbial growth, primarily manifested as biofilms of photosynthetic microorganisms on the surface or subsurface of the rock. Any evidence of this growth was recorded.
2. Rocks were cut open using a rock saw (Gryphon Corporation, CA) and the presence of any endolithic growth was recorded.
3. In the case of epilithic (surface) or sublithic (underside) growth a small sample (<1 x 1 mm) was removed and placed on a standard glass microscopy slide. ~20  $\mu$ l of water was placed on the sample and a coverslip was placed over the sample. The sample was examined by visible light microscopy on a BX-51 Olympus microscope and under UV epifluorescence with an excitation filter (<550 nm) and an emission filter (>590 nm) to detect for the presence of photosynthetic accessory pigments.
4. Images were electronically recorded on an E-10 Olympus microscope and transferred to the exobiologist's laptop computer. This procedure was repeated at least 100 times during the course of a two-week stay in the habitat for a range of samples, which also included some incidental analysis of soil and water samples. During a typical day 10-20 images were collected from samples of interest. Images were catalogued on the laptop and some samples were placed in bags and labelled for future analysis.

#### 5.2.4. Use of communication system for collaboration

We undertook identification of some of the cyanobacteria ourselves, but we also involved Dr. Paul Broady at the University of Canterbury in New Zealand, who provided identification during the mission from 'Earth' through the simulated CSA Mars communications network described in Section 4. He is a specialist in polar cyanobacteria and had undertaken much of the work on chasmolithic and sublithic cyanobacteria in Antarctic polar deserts [12, 13]. Images from the microscope were sent to Paul Broady on July 15 by e-mail with a description of the location and specific habitat in which they were found (epilithic or sublithic). Usually by the next day we had a response with tentative identification. Because of the similarity of many cyanobacterial forms (for example different species of *Gloeocapsa* are extremely difficult to tell apart), it was later necessary to send Broady samples from the field to be able to examine directly, hence the importance of gathering catalogued samples as well as carrying out *in situ* examination.

In the case of Mars exploration, samples may be subject to stringent quarantine before return to Earth and cannot be returned before the end of the mission. This underlines the importance of excellent imaging systems on Mars that reduce the need to actually return samples to researchers on Earth, thus reducing the delay in research during multi-months stays on Mars.

Based on identification by Broady we could go back and improve our image capture. One specific example of this protocol on July 15 was the study of a filamentous cyanobacterium, which was later identified as a *Calothrix*. On inspection, Broady informed the team that it would be necessary to study the branch points of the cyanobacterial filaments better as the existing image was not acceptable for

identification. On July 16 further images were obtained of the branch points of the cyanobacterium and transmitted to New Zealand, which allowed a better identification. This experience demonstrates the importance of collaboration with terrestrial experts and the way in which feedback can be used to improve the next round of analysis from the surface of Mars directly if the imaging systems are of sufficient quality.

After a period of several days our collaborations with 'Earth' scientists even developed into discussions on peer-reviewed manuscripts. Images were obtained that were sufficient for publication and discussions on the required levels of identification with respect to peer-review were initiated. Approximately half of the manuscript, Cockell et al., 2002, was written inside the habitat during the first two phases of the simulation, illustrating that after several months on Mars, scientists can submit papers to journals from Mars itself. The responses from anonymous peer-review will be used to improve analysis and data collection during the mission. Thus, our experiences show that the duration of surface operations is sufficient that the entire scientific process can be implemented on Mars.

E-mail communication with 'Earth-based scientists' was a common evening activity. The time delay was not really a constraint, because e-mails could be picked up later in the evening or the next day. Because individuals often do not reply to e-mails immediately because they are away, eating etc., a 20 minute time delay was not even noticeable in these non-emergency scientific protocols.

### **5.3. Coring lakes on Mars from a habitat – lessons from the High Arctic EVAs.**

The retrieval of lake sediments from the surface of Mars will require a well orchestrated plan of execution as activities such as these may require several EVAs and numerous contingency plans. From coring activities in extant high arctic lakes it has been shown that a substantial amount of pre-planning is required in order to ensure that once the target site is reached coring can proceed smoothly. In the High Arctic, once the crew leaves the field station or habitat and proceeds to the remote coring site, returning for additional equipment or information is no longer time or energy efficient. This will also be the case on Mars where any deviation from a set coring EVA as a result of improper planning may jeopardize a full day's activity.

Aside from sediment retrieval, three other components to any coring activity are subsampling, archiving and sample preparation for future analysis. In the High Arctic short cores taken by way of Glew gravity corers, for example, can be subsectioned or extruded immediately in the field. Each interval of sediment is then stored separately in sterile bags and kept cool for the duration of the field season. Sediment samples are then shipped south to relevant labs for preparation and analysis. Longer cores taken by means of piston core, for example, are not typically extruded in the field but rather shipped to labs in core tubes. Lake sediment retrieved from paleolakes on Mars may be of substantial length depending upon the maximum depth attained during coring. Total lengths retrieved will significantly impact how the cores are stored, archived and shipped.

Given the brevity of the high arctic field season and the difficulties involved with shipping, storing, utilizing and disposing of acids necessary for sample preparation, this step is typically carried out once the research crews have returned to their respective

institutions. In areas such as the Antarctic, however, where field stations are better equipped, sample preparation activities are readily performed prior to the termination of the season. On Mars, where crew members may spend up to 400 days on the surface of the planet prior to returning to Earth, being able to move seamlessly from sediment retrieval to sample preparation and analysis while in the field would not only provide distraction to the confined crew, but also potential results to send back to an anxious earthbound audience. This will require careful and meticulous planning to ensure that (a) core structure is maintained at time of retrieval in order to ensure chronological integrity; (b) sample contamination is minimized at the time of retrieval and subsampling; (c) at least \_ of the core is archived and not subsampled; and (d) during sample preparation and analysis all chemicals and by-products generated are properly stored and disposed.

#### **5.4 Integration with other activities / Effects of Mars simulation on science**

The details of the daily science routine were strongly influenced by the need to help other segments of the team prepare for EVA. So, for example a three-person EVA, leaving three people inside the habitat, could involve two people on EVA support in the habitat and the third (a scientist) to do further microscopy during a day off.

The exobiologist carrying out research work, as with all crew members, had to be flexible with sample analysis and science. If an EVA was aborted early (as happened on one occasion because of malfunctioning equipment) then the scientist needed to be ready to terminate scientific analysis to provide mission support. The situation is different from a normal laboratory environment where the scientist often has the opportunity to work

uninterrupted. Because surface support on Mars can, in some instances, be a matter of life and death, the scientist cannot operate without restriction.

It was found that flexibility in the science laboratory program could be implemented by segmentation. Each task was heavily segmented so that it could be terminated at short notice without prejudicing the science. Examples included; studying one sample intensively and completing this before moving onto the next one (i.e. not lining up a series a samples to be examined with the assumption that the time will exist to study them all), preparing small quantities of stock solutions such as dyes at each stage under the assumption that work will have to be terminated at some point, keeping a tidy work surface at all times with the perspective that if work has be terminated suddenly the laboratory space will be clean.

At all times during science activity, it is necessary for the scientist to be concentrating, but aware of other activities. Having an EVA radio close-by to the microscope and listening in on surface activities at all times was found to be important.

## **6. Comments on the design of a laboratory**

The FMARS geo/biological laboratory was situated on the lower deck of the FMARS facility. The equipment was spaced on the workbench in a sequential order of sample processing. From left to right : rock cutter, rock polisher, preparation and cleaning area, slide preparation area, microscope, centrifuge and pigment/biomolecule extraction area (Figure 7).

The placement of the laboratory on the lower deck has two disadvantages associated with it. Firstly, it was found that during intensive scientific study it was

necessary to be shuttling back and forwards from the lower to the top deck to carry out briefings, mission support, meals etc. In a more cramped Mars habitat, this may be problematic for science operations. Secondly, dust often collected in the laboratory from the EVA room, which was next to the laboratory. EVA activities were sometimes detrimental to science operations because dusty equipment was often left in the laboratory area. This problem can be dealt with by better isolation of the laboratory from the ingress area to the EVA room, perhaps by physical separation of the laboratory area from the rest of the habitat.

The advantage of the laboratory location is that dust from rocks is kept isolated to the lower deck and away from eating and sleeping quarters on the upper deck. In view of the fact that Martian soils may be toxic because of high concentrations of superoxides, this is currently a good decision, but even if this is not the case, the planetary protection concerns and concern about false positive identification of life would suggest that humans still should be kept isolated as much as possible from the laboratory facilities. Thus the need for a scientist to move up and down a deck to reach the operations area may be a necessary inconvenience of biological containment, both forwards and back.

## **7. Equipment required in the laboratory**

The microbiological investigations of the shocked lithic microorganisms were accomplished with the following equipment:

BX-51 visible/epifluorescence Olympus microscope with electronic E-10 camera

1 laptop computer for image download and analysis

Gryphon rock saw

1 eppendorf centrifuge

Microscope slides and cover slips

Sterile plastic bags (for field collection)

Pipettes (20\_μL, 200\_μL, 1 mL) and pipette tips

Distilled water bottle and double distilled (sterile) water

Sterile blades for scraping rocks

Oil for oil immersion microscopy

Dyes for microbial study (e.g. DAPI, Acridine orange)

Small sterile 10 mL tubes for sample collection

As an example of some items that may potentially be included in the establishment of a fully operational paleolaboratory inside of a Mars habitat, the following is a list of equipment that is typically found in paleoenvironmental assessment laboratories such as those located at the University of Toronto (Toronto, Canada) and Queen's University (Kingston, Canada) focused specifically on siliceous microfossil analysis of lake sediment cores:

Vented fumehood

Distilled water source

Borosilicate scintillation vials

Hot water bath

Benchtop Centrifuge and Centrifuge tubes

Microscope slides and cover slips

Slide warmer

Slide storage boxes

Chemicals for sediment digestion (e.g. HCl (10%), 1:1 H<sub>2</sub>SO<sub>4</sub>:HNO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub> (30%),

Ethanol (40%))

DIC (differential interference contrast) light microscope (e.g. Leica DMLB, Tri-ocular)

Immersion Oil, Lens Paper

Digital Frame-Grabber (e.g. COOLSNAP RS Photometrics)

Image manipulation and storage software

The above is an equipment list focused on the analysis of biotic remains in sediment cores. On Mars, the most immediate analyses conducted on paleolake remains may instead be centered on abiotic components to, for example, describe the chemical and physical properties of the cores. As an analog to what may be performed on sediment cores from Mars, the following analyses have been conducted on the Miocene paleolacustrine remains from Haughton Crater:

X-ray and grain size analysis to discern diagenetic characteristics

Magnetic susceptibility to describe mineralogical changes

Stable isotopic ratio analyses to identify potential biogenic signatures

Other analyses that could potentially be conducted on Mars paleolake cores include paleomagnetism and/or radiogenic isotopic analyses to estimate core age, mass spectrometry for the detection, identification and quantification of specific organic

compounds, and scanning electron microscopy to examine rock textures and mineral structures.

## **8. Conclusions**

The FMARS station provides an important opportunity to see how protocols and EVA planning influence exobiological investigations on Mars.

It was possible, within the space of one month and averaging one EVA every three days, to accomplish scientific examinations that went from initial sampling collections to collaboration with 'Earth' scientists and initial write-up of manuscripts.

The primary constraints on microbiological investigations were; the need to break off concentrated activity to be involved in other mission objectives (EVA support, briefings etc), the limited EVA time, and the restrictions of accomplishing science in an EVA suit, in that order of importance. The first constraint could be dealt with by segmenting work such as microscopy in such a way as to be ready to break off for other activities without warning. The second constraint could be alleviated by good planning in advance of the activity to be undertaken during the EVA (rather than a more random opportunistic sampling regimen, which is often the case for field biologists) and the third constraint of the EVA suit was a necessary encumbrance. However, after a time the effects of the EVA suit (reducing field work speed) could be dealt with by taking this into account during EVA planning.

We conclude that over a long-term Martian surface stay (many months) even with these encumbrances, a single microbiologist could develop a significant program of study with a well-planned and simple laboratory.

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## Figure Legends

### Figure 1.

The Flashline Mars Arctic Research Station (FMARS) was the first simulated Mars habitat to be established in a Mars analog environment. Using the characteristics of a polar impact crater as a biological and geological analog for science operations on the surface of Mars, it offers an important benchmark for how future explorers might conduct science on the impact-influenced surface of Mars.

### Figure 2

Micro-organisms associated with the impact-shocked gneiss at the Haughton impact crater. These types of microhabitats serve as analogs for the types of microhabitats explorers would search for on Mars. 1a. A piece of unshocked gneiss, also seen under Scanning Electron Microscopy (SEM) in (1b). Note that it contains few access points for microbial colonization. 1c shows a sample of shocked gneiss containing a coherent band of cyanobacterial endolithic growth (arrow). The organism inhabiting these rocks is the desiccation and radiation resistant cyanobacterium, *Chroococcidiopsis* sp. 1 d shows a typical field of view in shocked rocks under SEM illustrating the presence of micro-factures and entry points for microbial colonization of the shocked micro-habitats. 1e shows an SEM of a single *Chroococcidiopsis* cell occupying an impact-induced micro-fracture. 1f shows a colony of cells inside their sheath under light microscopy. SEM images were obtained back in the UK. The visible micrograph was obtained in the habitat after the EVA.

Figure 3.

Preparation for an EVA. ESA astronaut Vladimir Pletser prepares for an EVA to search for shocked rocks and deploy cosmic radiation dosimeters.

Figure 4.

The team reached their sampling sites all all-terrain vehicles (ATV's). These four-wheeled vehicles provide access to distant sampling sites and can be used as sample carriers.

Figure 5.

Rocks can be collected from the ground without bending down in cumbersome suits by using a grappling tool.

Figure 6.

Cosmic radiation dosimeters required Vladimir Pletser (left) and Charles Cockell (right) to dig a hole in the ground. Here it was necessary to bend down. The dosimeters are being deployed in the impact melt rocks. Bending down in this way may not be possible in real Mars suits. Thus, this activity provided insights into how tools might be developed to allow for upright digging.

Figure 7.

Microscopy was accomplished in the lower deck laboratory (7a). In 7b the configuration of the laboratory on the lower deck of the habitat is shown with the locations of some instruments. The bold arrow indicates the direction of the view in Figure 7a.