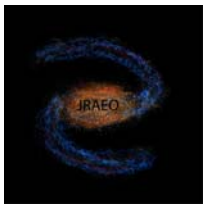

Planetary Biosphere Analogs with Extremophiles; Informal Science Education and Inquiry by Undergraduates Attending a Two-Year College

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Exploring exoplanets *in situ* isn't feasible now, but that doesn't stop student interest in simulating it with extreme environment exploration on Earth.

Keywords: students - undergraduate-science majors - astrobiology - learning theory and science teaching - informal science education

There is a paucity of research involving students at the two-year college doing independent inquiry research coupled with Informal Science Education (ISE) presentation of findings to the general public. We undertook to fill this gap with students at a two-year college and a Nevada NASA Space Grant Consortium ISE Program course that related astronomy and extreme climate biology. The goals of this project were to design a stimulating science, technology, engineering and math experience outside of the formal classroom environment in the targeted field of astrobiology. Students participating in this project conducted library research to understand the nature and distribution of habitable environments in the universe. They determined the characteristics of potential habitable planets beyond the Solar System, and then drew analogs to these planets from their findings for culturing extremophiles found in the field.

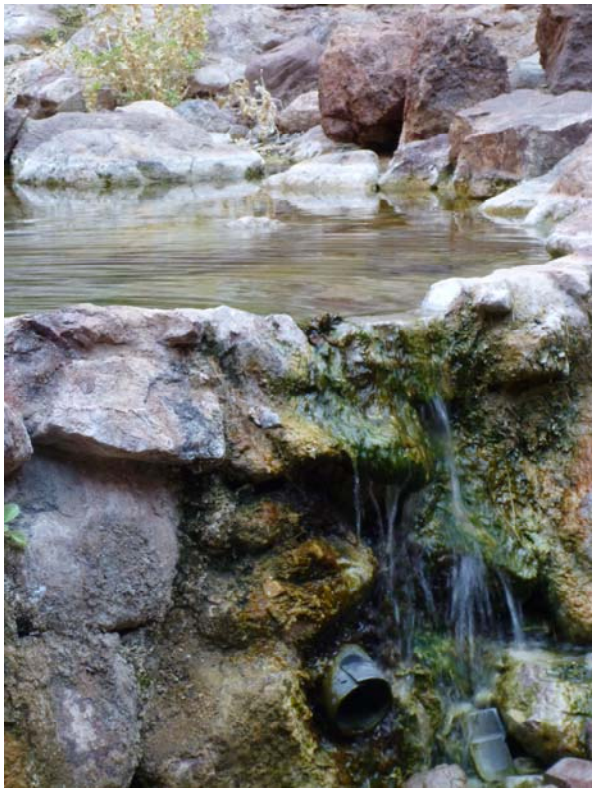

Figure 1. Pond 2, where biofilm samples were collected.

Twenty undergraduate students self-selected from a BIOL 251: General Microbiology class participated in the voluntary research. The project was outside of their regular course curriculum and was a free choice investigation. The purpose was to collect and study extremophiles (microbiological organisms that live in extreme conditions – in this case the ponds of hot springs, see Figure 1) and their potential relationship to life forms that could exist on planets within our solar system and on exoplanets. Collected samples were taken to the college's biology laboratory and cultured. This laboratory work took place on Saturdays and Sundays to allow the project to conform around the academic course lab schedule. The project time frame was one year in duration and consisted of two cohorts with 10 students in each cohort. At the end of each six-month cohort, students presented their research in a poster presentation during an astrobiology evening at the college planetarium.

Course Timeline

The general project timeline began with students first undertaking inquiry investigations to understand how life emerges from cosmic and planetary precursors. In the field and lab component' research they performed observational, experimental, and theoretical investigations to understand the general physical and chemical

principles underlying the origins of life. Students also participated in free choice instructional sessions led by two of the college's Ph.D. microbiology professors. Instruction included PowerPoint lecture discussions and academic journal discussions as a basis for learning about extremophiles. Sessions focused on the evolutionary mechanisms and environmental limits, particularly some of the molecular, genetic, and biochemical mechanisms that control and limit evolution, metabolic diversity, and acclimatization of life. These would be used as a template for real world experiments, through the collection and culturing of extremophiles.

Figure 2. A biofilm in pond 2.

Finally, students were given presentation context and background skill help through NASA materials and resources, to help students become ISE educators. Students delivered knowledge to the general public on the recognition of signatures of life on other worlds and the early Earth, including identifying possible biosignatures which are usable to reveal and characterize past or present life in ancient samples from Earth, or extraterrestrial samples measured *in situ* or returned to Earth. A biomarker or biosignature is something that signifies that life was/is present. They can come in a few broad categories: a chemical compound that is produced by only living things, a type of visible-to-the-eye structure (see Figure 2), structures like stromatolites, or liquids with turbid conditions or colors not found in the rocks around it.

The project focused on providing an opportunity for ISE to take place on varied levels of inquiry depending on the background and course experience each student brought to the project. In this way the students were able to work in collaborative groups to collect and culture two types of extremophilic organisms. Participants collected halophiles and thermophiles in two permitted collection trips within an hour's drive from the college. Halophiles are found in the salt abundant areas around dry lakebeds. Thermophiles live in hot springs that occur naturally in the region and use of these was the ultimate tool for completing the research.

Student Findings

Samples showed growth characteristics of temperature-dependent extremophiles, with distinct growth curves (Figure 3). Optimum growth temperatures varied between samples but remained in the expected range of 40 to 50^{0}C. Data collected in experiments of the pond 2 samples (collection areas were identified by pond and number) were determined to be thermophiles. When these cultures were incubated at temperatures higher than the above maximum temperature, all but two cultures died and no further growth was established. (See Sidebar for lab procedures.) The two cultures that survived provide interesting possibilities for future research; although these two cultures did not grow when placed at temperatures higher than their maximum, the organisms in them survived and grew again when the cultures were incubated at the appropriate temperature. This indicates that there may be places both on Earth and on other planets where microbes are not actively growing but do exist and it

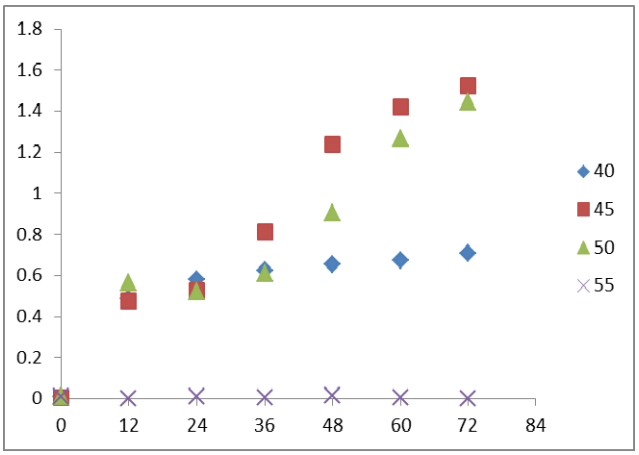

Figure 3. Growth curves of extremophile samples, absorbance at 550 nm versus hours.

would be worth sampling different regions that may appear to be void of life and attempting to grow any microbes that might be there under different conditions. This possibility further expands options for finding life in the universe.

Students also suggested additional studies to determine how these organisms exist in biofilms, as opposed to individual cultures. Biofilms are complex communities in which, sometimes, organisms will not grow without other organisms around them, or in which, sometimes, organisms take on very different characteristics than they normally have. Biofilms have relevance in not only identifying life on other planets but also understanding how it works on that planet. In our students' cases, they were detecting thermophiles by looking for its biomarker, formations indicating water flow, flexibility, and slimy or shiny appearances. These were identified, in our case, as the thermophile biofilms/mats that were seen when we were sampling.

Figure 4. A poster session for the college community.

Students served as conduits of ISE when they presented their material in poster form. The poster session was an open invitation to the college community and the general public to view student research and ask questions (Figure 4). During the poster presentation students demonstrated their emergent understandings by explaining how they did their background research, how they did sample collection in the field, and how they conducted their laboratory experiments. The student poster session exposed to the general public to the role of astrobiology in science.

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The Lab Procedure

A project like this encourages interdisciplinary instruction, such as an astronomy course with biology department participation. Collection procedures (for those who wish to duplicate this) are described here. The student participants, with National Park Service collection permits, took water samples from some of the numerous hot springs found in the Las Vegas area. The collection area along Gold Strike Canyon trail in Lake Mead National Recreation Area was chosen for abundant saline formations and thermal springs.

Samples were inoculated onto M17 (Difco) media. Once inoculated the cultures were maintained at 45^0C in lighted conditions for 18 hours prior to being transferred into four different types of growth media. The growth media used included Nutrient Broth (NB), Lysogeny Broth (LB), Tryptic Soy Broth (TSB), and Lysogeny Broth; deMann, Rogosa, and Sharpe (LBMRS) growth medias. Their initial growth was established. All samples were transplanted to TSB growth media. Isolation of the organisms was performed with Chromagar orientation on plates. The isolated samples were placed in TSB media at fifty degrees Celsius (C).

Unique microorganisms were isolated and classified as individual samples. The ideal growth temperatures of these organisms were determined and these were maintained for an incubation period of 72 hours. Culture density was measured with a spectrometer (550 nm wavelength) to obtain growth curves. Broth culture incubates were streaked onto Tryptic Soy agar plates and incubated to determine whether the cells remained viable at 55^0 C. Staining was done and cell shapes and arrangements were examined at 100x power. Cultures were analyzed by means of a disc diffusion method. Each organism was grown in 15 mL of TSB for 48 hours and then filtered using a Nalgene filter unit with a 0.45 micron filter. The resulting fluid was used to soak Whatman antibiotic assay discs (2017). The discs were then placed onto bacterial lawns grown on TSA and incubated at 50^0C for 24 hours. The plates were then checked for zones of inhibition to determine whether there was antibiotic activity.