Space Biology Lunar Surface Science Workshop
Virtual Session 7
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Report on the Break-Out Sessions Comments and Answers to the Key Space Biology Program Questions

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Summary: The NASA Science Mission Directorate's Biological and Physical Sciences Division (BPS) and its Space Biology Program hosted a workshop on space biosciences on the lunar surface science with the objectives of 1) informing and education the life sciences academia, engineering, and commercial space communities about NASA's goal for the scientific utilization of the Moon, and 2) engage these communities via break-out sessions to obtain their input for space biology fundamental and applied research for lunar and deep space mission. The scope of the workshop focused on lunar surface science; cis-lunar orbit science; integrated ground, low-Earth orbit (LEO), and lunar investigation; and preparation for future Mars science and deep space exploration mission. The workshop included plenary talks on space biology research beyond LEO, human lunar science research, planetary protection, Astrobiology, Artemis and Human Exploration and Operations Directorate capabilities for science and technology utilization, commercial lunar payload services and landers, Artemis III Science Definition Team Report briefing, and space radiation at the Moon and its implications for space biology. Also, abstract and poster presentations were held describing community ideas for lunar sciences in several different biological disciplines and model systems. The workshop culminated in a series of break-out sessions to obtain community comment and input on key questions pertaining to space biology and deep space exploration concerning life science research and enabling technology and engineering applications. The workshop was held with organizational and implementation support from the Lunar and Planetary Institute (USRA, Houston, TX), Solar System Exploration Research Virtual Institute (NASA, Ames Research Center, CA), and the NASA Science Mission Directorate's Lunar Surface Science Workshop Working Group. Additional information about BPS, the Space Biology Program, and the Space Biology Lunar Surface Science Workshop may be found at the following websites:

- For the Space Biology Lunar Surface Sciences Workshop posted presentations and posters: https://lunarscience.arc.nasa.gov/lssw/events

- For information about the Biological and Physical Sciences Division and its Space Biology Program: https://science.nasa.gov/biological-physical

A. Space Biology LSSW Break-Out Sessions

The specific aim of the break-out sessions was to obtain community input on key questions pertinent to deep space exploration concerning life science research and enabling technology and engineering applications in support of space biological research. The primary focus on was on fundamental and applied lunar research for space biology. The Space Biology Program identified three key questions to been considered and commented on by the break-out session attendees:

Question 1: What are the top priority space biology research topics that must be conducted on the Moon or in cis-lunar orbit (in order of priority) to facilitate sustained lunar habitation and future Mars missions? Which need to be initiated in the near term (5 to 10 years in order of priority)?

Question 2: How can we begin to separate the biological effects of multiple spaceflight stressors (e.g., ionizing radiation, partial gravity, social isolation, altered gas compositions, etc.) that will enable countermeasure development?

Question 3: What are key technologies, technical capabilities, and resources needed to conduct biological research on the Gateway and Moon (e.g., CLPS landers, Artemis vehicles, lunar base camp)?

An individual break-out session addressed each of the key questions by focusing on one of the model organisms or system that is used by the Space Biology Program to study fundamental biology and translation to human physiology and biomedicine: Vertebrates, Plants, Invertebrates, Microbiology, and Cell Biology Systems (e.g. tissue-on-a-chip and multi-physiological systems). Two break-out sessions were held per model organism or system, which enabled attendees to participate in different break-out session for the different model organisms or system.

A.1. Discussion Points from the Vertebrate Break-Out Sessions

Discussions were primarily focused on the use of rodent research.

Question 1: Priorities

- Space Biology can augment human research by conducting and expanding radiation research on vertebrates, since radiation research is not feasible with humans.
- Focus should be on outcomes such as SANS, thrombosis, immune dysfunction, vascular changes, brain function/cognitive deficits, sex-specific differences.
- Additional focus on sleep deprivation and. circadian rhythm studies should be included.

Question 2: Separating the Biological Effects of Multiple Spaceflight Stressors

- Studies involving radiation shielding (inactive vs active) on the moon or in cis-lunar orbit.
- Ground studies that separate deep space radiation into its different components.
- Parallel studies from lunar and ISS missions to tease out effects of differing radiation levels; can use centrifugation on ISS to simulate partial gravity vs microgravity.
- Studies of lunar dust exposure on animals, either on the surface or bringing lunar dust back to Earth for study (chronic vs acute effects).

Question 3: Technologies, Capabilities and Resources

- Mouse habitat/breeding facility on the Moon.

- Live animal return from the lunar environment as a biological analog to the re-adaptation/return phase that humans go through.
- Advanced imaging capabilities (CT, DTT), video capabilities, ultrasound, etc. for in-situ analyses.

A.2. Discussion Points from the Invertebrate Break-Out Sessions

Question 1: Priorities

- Organisms suggested: Tardigrades (resistance to extreme environments), *Drosophila, C.elegans* (model organisms), vermiculture/regolith conversion with simple organisms, host-microbe interactions, etc.
- Studies should begin with simple experiments using well-vetted spaceflight models.
- Initially short-term experiments leading to future multigenerational studies in the lunar environment, measuring the environmental effects on mechanotransduction.
- Compost/soil studies assessing conversion of regolith to soil, pollination studies.

Question 2: Separating the Biological Effects of Multiple Spaceflight Stressors

- Parallel experiments should be run on ISS/Gateway/Lunar surface and include using centrifugation to similar microgravity to parse out the effects of radiation.
- Suggested use of genetic tools/specific strains of organisms that are more sensitive to one stressor vs another to parse out different stressor effects, use these lines to test countermeasures.
- Additional radiation studies on ground should be conducted in parallel with ISS/lunar studies.

Question 3: Technologies, Capabilities and Resources

- Build on known habitats for invertebrates used on ISS, test effectiveness of radiation shielding.
- Video capabilities, simple radiation dosimetry, and data loggers for temp/humidity/pressure etc.
- Centrifuge for $1xg_e$ control in each environment.
- Sample return - with simple preservation – either frozen or fixed.

A.3. Discussion Points from Cell Biology Systems Break-Out Sessions

Question 1: Priorities

- Establishment of Tissue on a Chip and 3D printed/culture systems, while understanding limitations and caveats of these systems.
- Cell systems should be used to conduct drug therapy studies, assess DNA damage and repair from radiation, and assess the effects of radiation on proteins and other cellular components.
- Cell differentiation studies in the lunar environment.
- Cell model/tissue type studies: nervous, cardiovascular systems, and others.

Question 2: Separating the Biological Effects of Multiple Spaceflight Stressors

- Conduct cross-platform experiments (Gateway, Moon, ISS) to isolate/characterize the effects of radiation and microgravity across them.

- Examine probiotics (microbes)/nutraceuticals (plants) as countermeasures for cellular health.
- Conduct radiation/countermeasure studies on the ground and look at production and effects of reactive oxygen species/free radicals.
- Conduct high throughput studies (omics) and compare with spaceflight models.
- Examine the effects of lunar dust on cell models.

Question 3: Technologies, Capabilities and Resources

- Imagery, telemetry, dosimetry; temperature control (refrigeration/heating); real time sampling (material extraction for omics analysis); real time measurement collection.
- Sample recovery; automated preservation methods; remote data recovery.
- Partial gravity on flight platforms(centrifugation).

A.4. Discussion Points from Microbiology Break-Out Sessions

Question 1: Priorities

- Increase our microbial monitoring capabilities of both the habitat and of its inhabitants (Microbiology of the Built Environment; MoBE). Develop better sterilization techniques. Characterize antimicrobial resistance.
- Gain better understandings of plant/microbe and animal (human)/microbe interactions in space and lunar environments (look at bioregenerative life support potentials).
- Look at biofilm formation in lunar/space environment.
- Examine interactions of microbes with regolith, characterize their ability to convert regolith to soil.
- Expand studies to extremophiles and radiotrophic fungi (potential use as organic shielding?).

Question 2: Separating the Biological Effects of Multiple Spaceflight Stressors

- Develop a system of standard measures for microbial assays and monitoring systems that can be used across platforms and species, to biomap environmental/biological conditions.
- Use centrifugation to conduct fractional gravity studies, in parallel with radiation studies, or with other stressors (nutrient availability/deficiency, absence/presence of lunar dust). Examine effects on microbes when exposed to individual stressors, or in combination.

Question 3: Technologies, Capabilities and Resources

- Autonomous microbiology monitoring systems; real time monitoring; remote data collection; microscopy and mass spectrometry for solids and solutions; sample to sequencing techniques.
- Micromanipulation techniques (to deal with micro amounts of regolith).

A.5. Discussion Points from Plant Biology Break-Out Sessions

Question 1: Priorities

- Work should begin by expanding LEO studies with current model plants (e.g., *Arabidopsis*) to lunar environment but should also be extended to crops. Seed to seed, lifecycle experiments must be conducted. The objectives of crop research should focus more on increasing nutrient density rather than caloric density.

- Little work has been done examining the effects of radiation on plants (especially crops). The mutation rate of plants/crops under radiation should be examined, as well as effects of radiation & 1/6 g's on germination, development, and gene expression.
- Understand how lunar gravity effects plants differently from microgravity. Past work suggests they have similarities. Gain a better understanding of the relationship between gravitropism and phototropism.
- Examine ability of plants to grow on regolith/converted regolith (some feel that this is a lower immediate priority, but important for future sustained plant growth on moon). Particle size of regolith matters, because reduced gravity impacts water flow through it.

<u>Question 2: Separating the Biological Effects of Multiple Spaceflight Stressors</u>

- Prioritize integrated studies (rather than reductionist studies) to understand the synergistic effects of a multi-stressor environment. It may not be easy to tease all variables apart and get meaningful data.
- Conduct studies on multiple platforms (ISS, Earth, Gateway, lunar surface) to tease apart effects of microgravity and radiation (centrifuge studies, BNL radiation).

<u>Question 3: Technologies, Capabilities and Resources</u>

- Modular systems for different roots or plant types; incubator system/atmospheric control; automated systems for sample collection, preservation and analysis (omics); imaging capabilities, telemetry; sample return.
- Consider using genetic strains resistant to radiation, drought stress, hypoxia, etc.

A.6. Summary of the Collectives Comments and Findings from the Break-Out Sessions

In the course of the brainstorm sessions, a set of common scientific points were identified by each of the individual break-out session participants:

a) Space biosciences ground-based and LEO research are a complement to deep space research.
   - These platforms are important when answering Question # 2 (separating the biological effects of multiple spaceflight stressors), as both Earth and the ISS have different conditions and capacities than Gateway or even the lunar surface.
     - Centrifugation studies on the ISS can recapitulate the fractional gravity of the lunar environment but with less radiation.
     - The radiation levels between the ISS and cis-lunar orbit are different, the biological effects of which can be studied.
     - Ground based-radiation studies are still needed.
     - The ISS can still serve as a proving ground for testing new technology and hardware to be used in cis-lunar orbit, or even on the lunar surface

b) Sample preservation and return on/from the moon is a concern and a priority.
   - How can we extract and store nucleic acids? Can samples be properly frozen? Can we lyophilize proteins in the lunar environment?

c) Lunar dust is a major concern across disciplines regarding lunar surface work.
   - Lunar dust is abrasive, potentially toxic, and easily tracks everywhere. There is little work that has been done looking at the consequences of breathing in lunar dust with

effects on lungs or other systems in animals. Also how does exposure to lunar dust effect plants, microbial growth, or even microbial communities?

d) Bioregenerative Life Support is on everyone's mind, but the priorities and timelines are still open points of discussion.
   - Discussions have been held, from many outside the plant community, in converting lunar regolith into useful soil, which led to discussion among members within the plant community regarding the practicality of the approach and consideration of the short-term vs long-term needs for sustainability.

e) Low-Earth Orbit platforms, like the ISS, provide valuable information on the effects of the spaceflight environment on living systems and can serve as an important frame of reference for future Beyond LEO work.
   - Many break-out session participants supported the idea of beginning lunar and cis-lunar work using familiar model organisms and systems of the Space Biology Program and its community, while also expanding that work to using systems that have translational value for future exploration work
   - Continue to use LEO platforms as "controls" in conjunction with lunar/deep space experiments to separate out the effects of partial gravity (e.g., ISS studies with centrifuges) and differing exposures to high LET radiation

f) Future work needs to be a balance of basic science to gain knowledge of how BLEO environments impact biological systems and applied science with a focus on crew health, safety, countermeasures, and the creation of a sustainable exploration environment.
   - Many recommendations on the radiation and gravity of the lunar environment with a focus on future animal work on understanding how human health is impacted and developing countermeasures.
   - Working with plant model organisms is a good place to start but work also needs to be done to produce edible plants with high nutritional value for astronauts.
   - Microbial work should be a balance of developing techniques to monitor and control the built environment microbiomes in these new locations, while also working to understand how microbes evolve in these environments, and if they can be manipulated for translational applications including bioregenerative life support.