National Aeronautics and
Space Administration

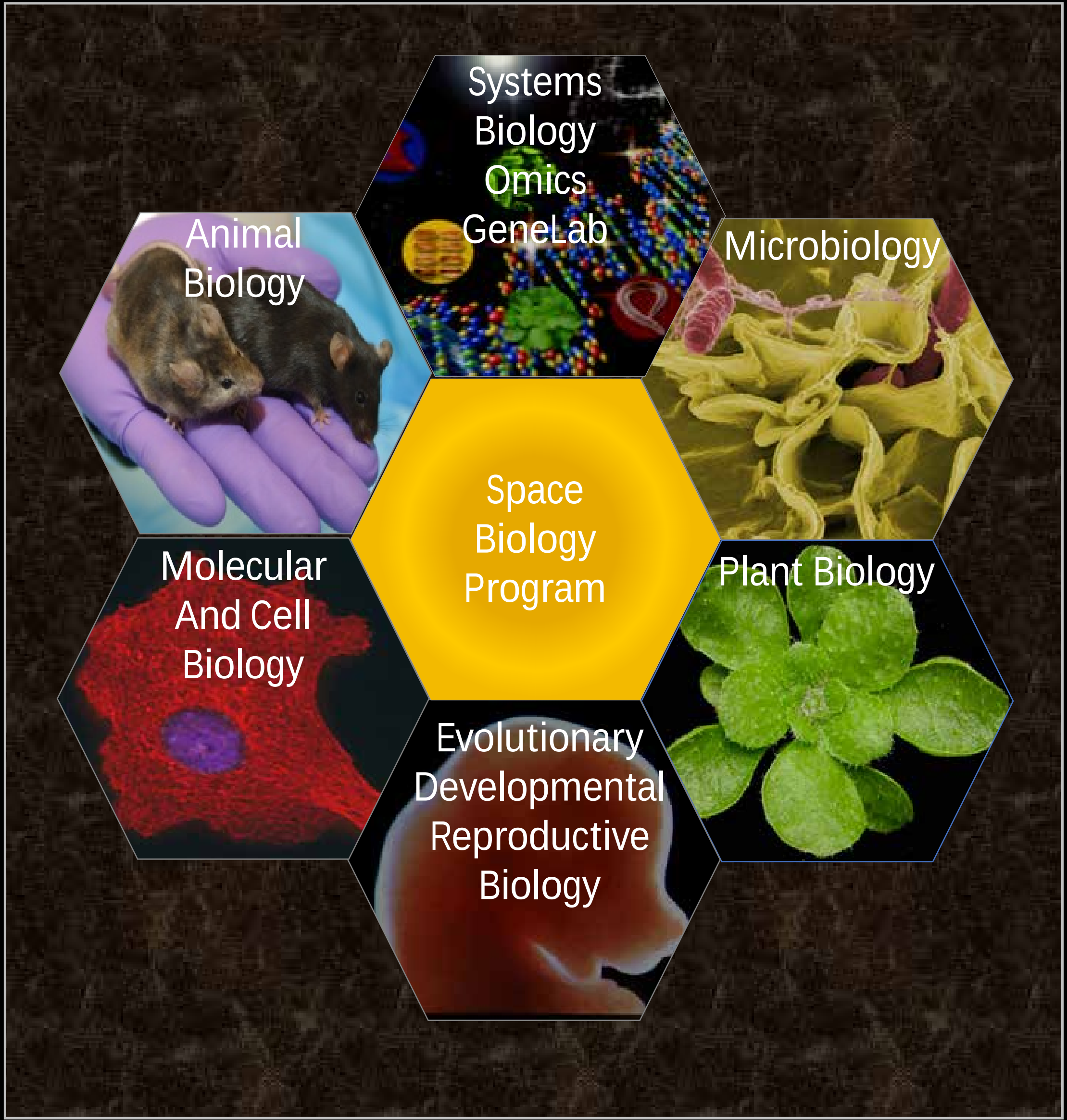
EXPLORE SCIENCE

Space Biology Program

Sharmila Bhattacharya, Ph.D.
Program Scientist for Space Biology
October 27, 2020

Agenda

- Space Biology program objectives
- Status, Planning, Research Accomplishments
  - Recent grant selections
  - Upcoming solicitations
  - Research highlights
    - GeneLab
    - Animal Research
    - Plant Research
    - Microbiology Research
- Forecasting Science Directions in the Community
- Challenges of Interest for the future



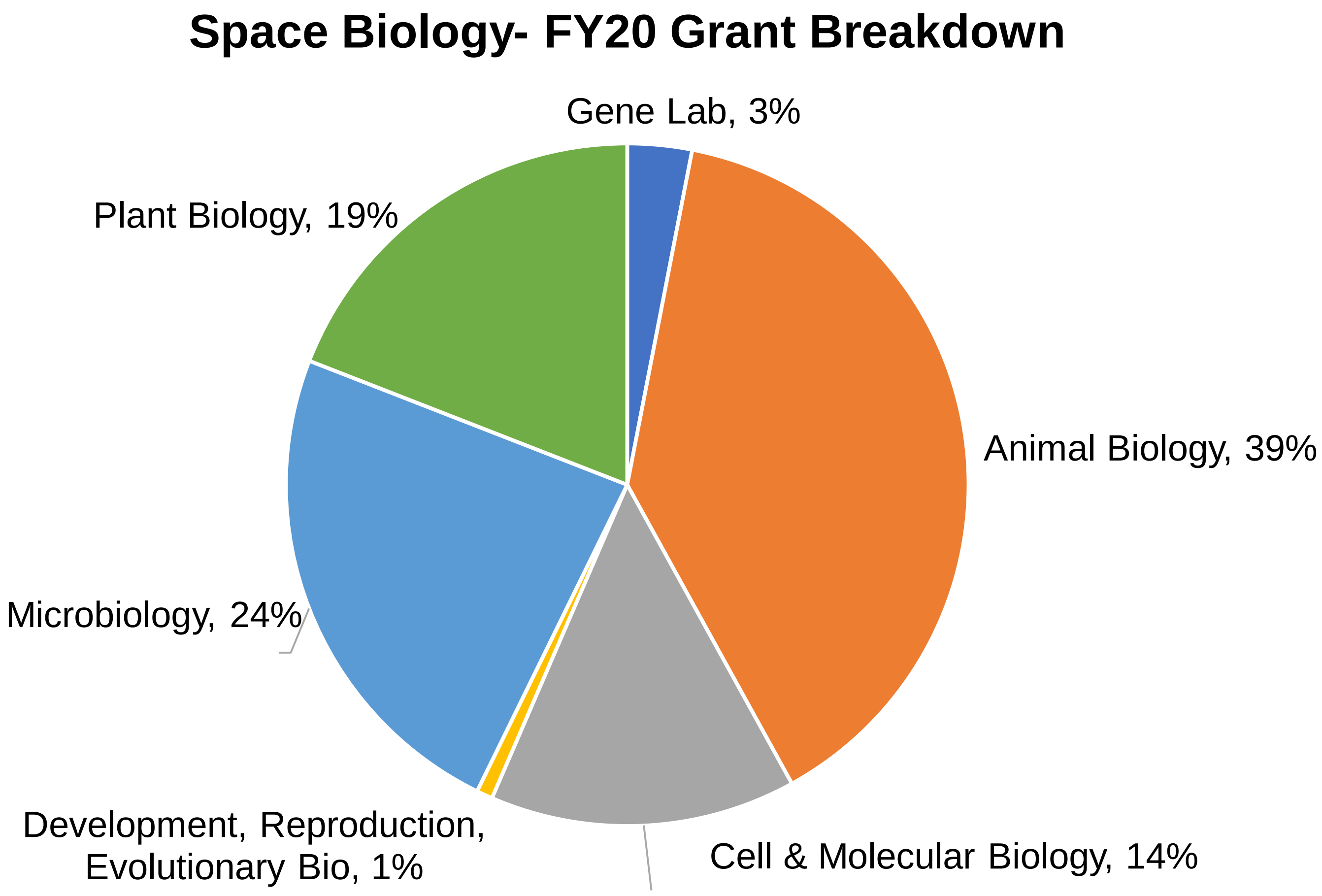
Space Biology's overall objectives:

- Discover how biological systems **respond** to the space environment
- Identify the fundamental **mechanisms** and develop physiological models for biological systems in space
- Promote **open science** through the GeneLab Data System and Life Science Data Archive
- Conduct world-class research and develop cutting-edge biological **technologies**
- Provide mechanistic understanding to support human **health in space**
- Support the transfer of knowledge of space-based research to **benefit life on Earth**

Databases and Biospecimen Sharing

- *GeneLab (genelab.nasa.gov)*
- *Life Sciences Database Archive (lsda.nasa.gov)*

Types of Grants in Space Biology



Total SB FY20 Grants	131
Flight	67
Ground	64

Number Directed vs Competed	
Directed	2
Competed	129

Transformative Research in the Context of SMD

Transformative research involves **ideas, discoveries, or tools that radically change our understanding of an important** scientific or engineering **concept** or educational practice or leads to the creation of a new paradigm or field of science, engineering, or education. Such research challenges the current understanding or provides pathways to new frontiers.

(National Science Foundation)

Think Hubble Space Telescope or Mars 2020 Perseverance Rover in the context of SMD!

What this could mean for Space Biology:

- Studies may be a part of a large research campaign
- Larger grants awarded
- With a fixed budget that could mean fewer grants awarded per year. However, we would like to expand our small Space Biology science community in the future.

Status, Planning, Research Accomplishments

Recent Solicitation Activity

Annual Space Biology Solicitation NRA NNH18ZTT001N-FG2 Appendix D: Solicitation of Proposals for Flight and Ground Space Biology Research: Released: October 30, 2019

Research Topics:

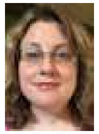
- **Microbiology** studies**:** how microbiological organisms and/or communities acclimate to, evolve, and/or behave in the spaceflight environment.
- **Plant Biology** studies: how plants adapt to spaceflight and provide new understanding of how to grow plants in space to effectively enable human space exploration.
- **Animal Biology (vertebrate and invertebrate)** studies: how do animals respond to the space environment, including the characterization of organ systems, behavioral adaptations, and the underlying cellular and molecular mechanisms of changes within tissues and between physiological systems.

Awards were made to 16 investigators + 2 postdoctoral fellows from 16 different institutions to conduct flight and/or ground-based research projects.

Total value of awards = $8.3M

16 (+ 2 postdoc) selected out of the 176 submitted Step-2 proposals

Selections for NRA NNH16ZTT001N-FG2 Appendix: “Proposals for Flight and Ground Space Biology Research: Microbiology Selections



Wayne Nicholson, Ph.D. University of Florida, Gainesville
Alteration of *Bacillus subtilis* DNA Architecture in Space: Global Effects on DNA Supercoiling, Methylation, and the Transcriptome.

Kelly Rice, Ph.D. University of Florida, Gainesville
Assessing the impact of Agr Quorum Sensing on *Staphylococcus aureus* Physiology in the Space Flight Environment

Sergio Santa Maria, Ph.D. NASA Ames Research Center
Acquisition of Beneficial Mutations through Adaptive Evolution Under Simulated Microgravity

Andrew Settles, Ph.D. University of Florida, Gainesville **(New to Space Biology)**
Understanding Genome-Wide Mutation Load in Spaceflight Culture of Cyanobacteria

Camilla Urbaniak, Ph.D. ZIN Technologies, Inc,
Microgravity Analogs as Proxies for Spaceflight to Validate Biofilm Formation and the Exchange of Genes Between Organisms

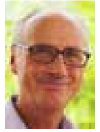
Zheng Wang, Ph.D. Naval Research Lab
Harnessing Extremophilic Black Yeasts to Understand the Adaptive Strategies of Eukaryotes to Mars-Like Conditions

Selections for NRA NNH16ZTT001N-FG2 Appendix: “Proposals for Flight and Ground Space Biology Research: Animal Biology Selections

Xiaohong Lu, Ph.D. Louisiana State University System **(New to Space Biology)**
Develop a Novel Single-Cell Biodosimetry for Brain Genomic Instability and Neurodegeneration to Predict Clinical Health Outcomes in Human Spaceflight Crews

Meghan McGee-Lawrence, Ph.D. Augusta University Research Institute, Inc, **(New to Space Biology)**
Osteocyte Plasma Membrane Disruptions in Skeletal Adaptation to Loading and Unloading

Anand Ramasubramanian, Ph.D. San Jose State University Research Foundation **(New to Space Biology)**
Thrombosis in microgravity

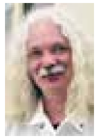


Donato Romagnolo, Ph.D. University of Arizona **(New to Space Biology)**
Space Environment and Epigenetics of Endocrine Regulation of DNA Repair and Cell Cycle in Mammary Epithelial Cells

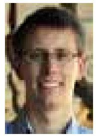
Jeffrey Willey, Ph.D. Wake Forest University
A Technology To Measure Gait, Egress, and Locomotor Performance in Perturbed Environmental Conditions After Simulated Spaceflight

Abba Zubair, Ph.D. Mayo Clinic, Jacksonville (**New to Space Biology)**
Role of Mesenchymal Stem Cells in Microgravity Induced Bone Loss

Selections for NRA NNH16ZTT001N-FG2 Appendix: “Proposals for Flight and Ground Space Biology Research: Plant Biology Selections



Simon Gilroy, Ph.D. University of Wisconsin, Madison
Spaceflight Effects on Plant-Microbe Interactions



Robert Jinkerson, Ph.D. University of California, Riverside (**New to Space Biology)**
Evaluation of Small Plants for Agriculture in Confined Environments (SPACE) Tomatoes for Space Flight Applications

Christina Khodadad, Ph.D. AECOM Management Services, Inc.
Microbe-Plant interactions with International Space Station microbial communities in Veggie flight crops

Scot Wolverton, Ph.D. Ohio Wesleyan University
RNA-Seq Guided Mutant Analysis to Discover New Components of Gravity Signaling in Plants

Selections for NRA NNH16ZTT001N-FG2 Appendix: “Proposals for Flight and Ground Space Biology Research: Space Biology Postdoctoral Fellowships

Eliah Overbey (PI: Christopher Mason, Ph.D.) Weill Medical College of Cornell University
High-resolution, murine spatial transcriptome mapping of the impact of spaceflight

Megan Rosa-Caldwell (PI: Seward Rutkove, Ph.D.) Beth Israel Deaconess Medical Center, Inc,
Influence of sex hormones on nervous system and musculoskeletal health in micro- and Martian fractional gravity in rat analogues

Upcoming Space Biology Solicitation Activities

Annual Space Biology Solicitation to be released as <u>Program Element E.10 in ROSES 2020</u> on NSPIRES:
Planned Released: ~ No earlier than November 16, 2020

Research Topics:

<u>Integrated physiology (Animal Biology – Vertebrate and Invertebrates):</u>

Studies that will address fundamental questions to advance the understanding of how animals adapt to spaceflight (alterations in gravity, radiation, stress, etc.).

- Projects are to utilize an integrated physiological approach, from omics to individual tissue organs and across multiple physiological systems and the whole organism, or with comparisons across organisms.

<u>Plant Biology:</u>

Studies that will address fundamental questions that will advance the understanding of how plants accommodate the spaceflight environment (e.g. alterations in gravity, radiation, stress, etc.).

- Studies should characterize environmental impacts on plant physiology, biochemistry, cellular, and molecular biology during development, growth, and/or propagation.

<u>Microbial Ecosystem:</u>

Studies that will address fundamental questions to advance the understanding of the microbial ecosystem in the closed environment of ISS.

- Studies should characterize the dynamics of microbial communities within built environments, among different microbial species, or between microbes and plants and/or humans, and utilize integrated microbiology approaches. Subtopics relating to prevention, monitoring, intervention of potentially harmful organisms promote partnership with NASA's Planetary Protection group.

Other Upcoming Solicitation Activities

Established Program to Stimulate Competitive Research (NASA-EPSCoR)

(https://www.nasa.gov/stem/epscor/home/index.html)

Topics of Interest to Space Biology:

EPSCoR Rapid Research Response (NNH21ZHA002C): Crop Plant Stress.

- Release date no earlier than Oct 30, 2020
- 1-year ground-based study.
- Studies will be of predefined crop plants and should be designed to gain new understanding of **crop plant environmental stress tolerance** (e.g. water and nutrient delivery challenges, low relative humidity, high CO2 levels, etc.) and its impacts on plant yields, nutritional composition, and secondary metabolism.
- Data gained from study should provide the scientific knowledge necessary for guiding approaches, such as horticulture methods, environmental control, and crop plant breeding, which will result in practical methods and technologies to produce healthy, hearty, and nutrition crop plants in any exploration environment and return Earth benefits.

EPSCoR CAN (NNH21ZHA003C): Organismal Biology – responses of whole organisms and their systems to ionizing radiation and/or other spaceflight-relevant stressors such as altered gravity simulators.

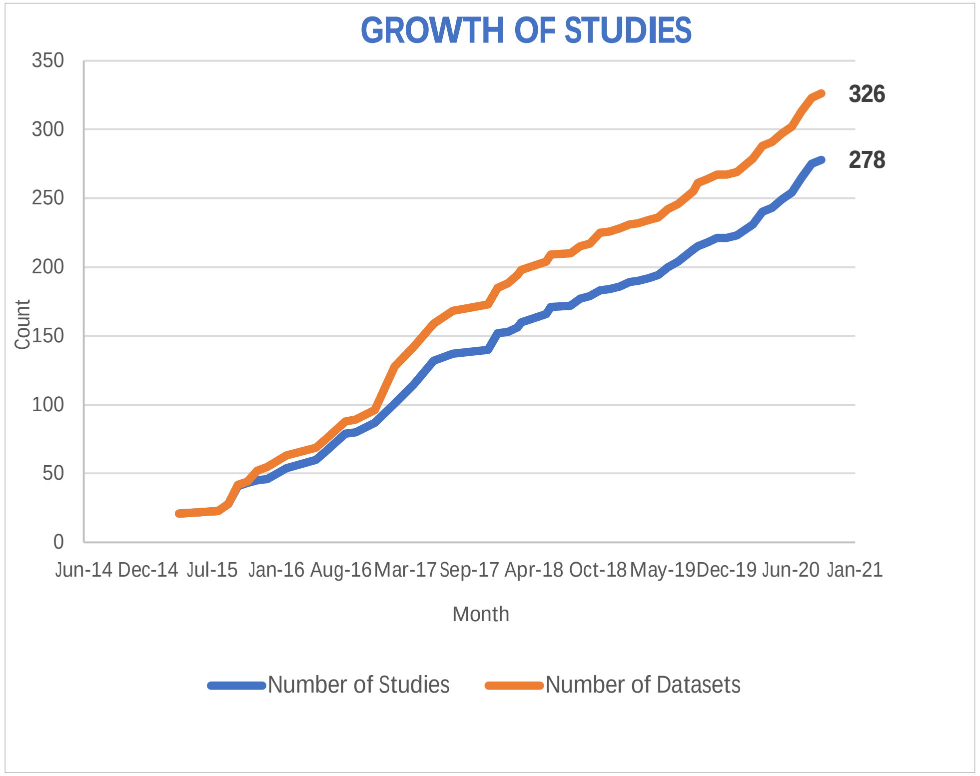
- Release date no earlier than Dec 4, 2020
- 3-year ground-based study.
- Studies should effectively delineate the biological effects of ionizing radiation and altered gravity regimes (partial gravity and microgravity separately and/or in combination where possible.
- Investigators encouraged to use a systems biology approach that could include genetic, cellular, or molecular effects.

Research Highlights: GENELAB

GeneLab

https://genelab.nasa.gov/

- GeneLab is an effort to maximize the value of spaceflight experiments through meticulous curation, augmentation with additional data, and widespread distribution to a motivated community.
- GeneLab houses data from ~280 studies representing investigations of many kinds of spaceflight experimental factors.



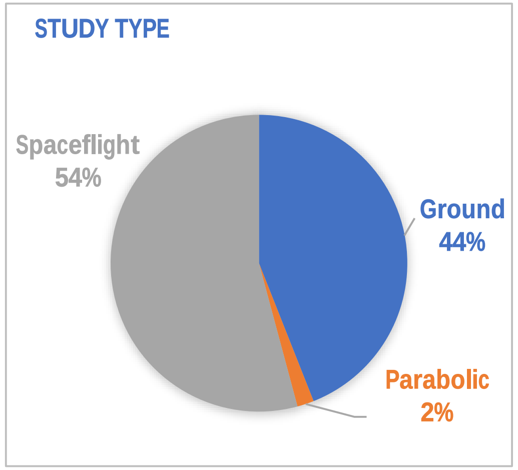
278
Studies

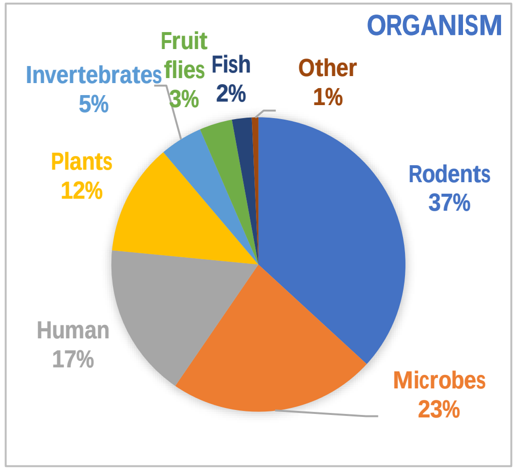
326
Datasets

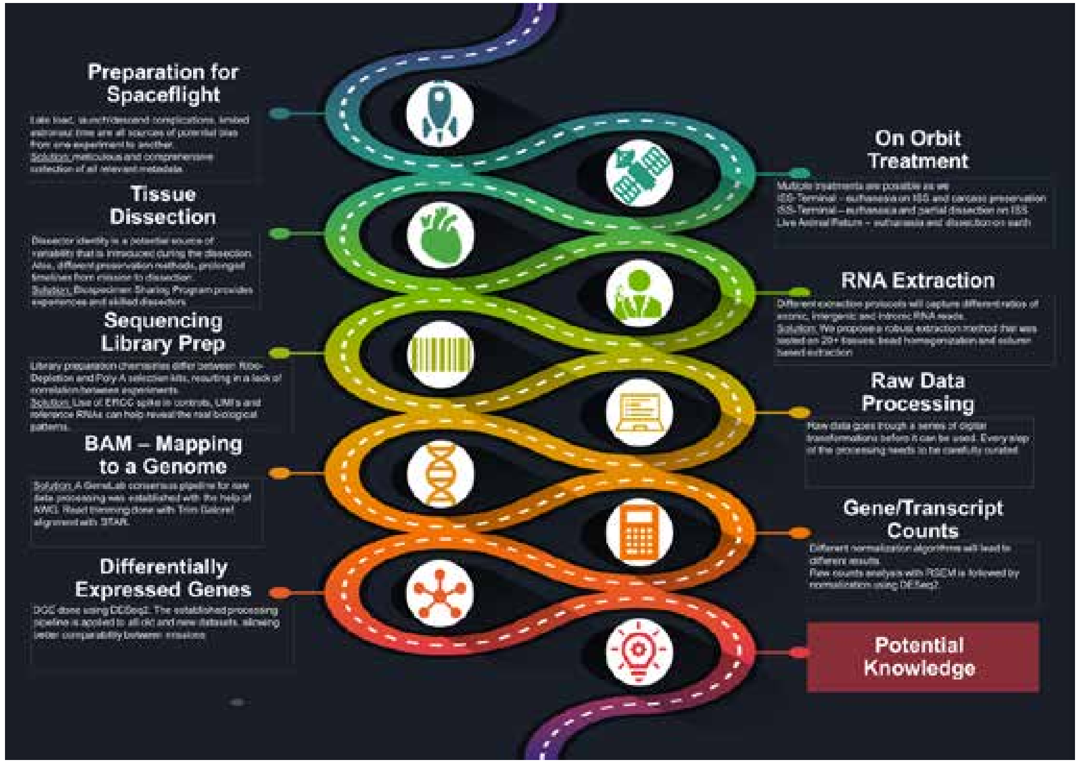
45
Species

>10
Assays

>130TB
Data



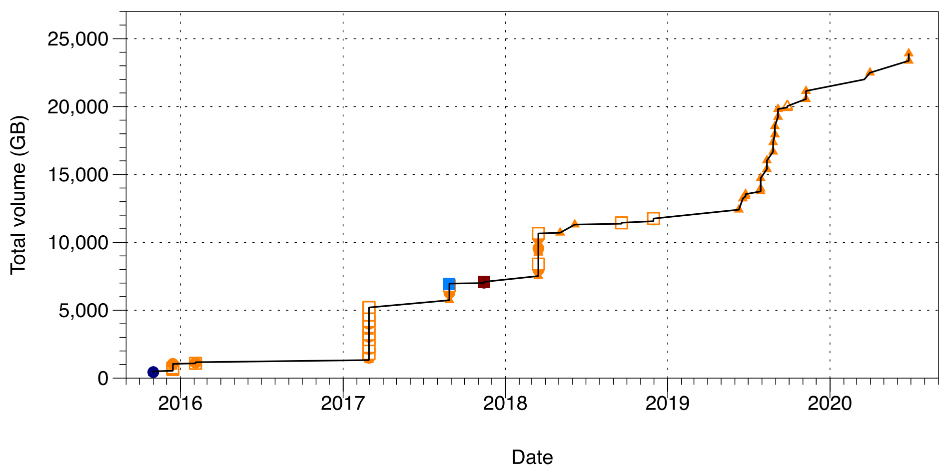




GeneLab: Open Science Data Generation

GeneLab has generated >20 TB of open science data from unused spaceflight tissues.

GeneLab generated datasets (up to Nov. 2019)



Shapes: Assay type
Triangle: Transcriptional profiling
Open square: DNA methylation profiling
Closed square: Metabolite profiling
Circle: Protein expression profiling
Diamond: RNA methylation profiling

Color: Species
Dark blue: *Arabidopsis thaliana*
Light blue: *Bacillus subtilis*
Green: *Drosophila melanogaster*
Orange: *Mus musculus*
Red: *Staphylococcus aureus*

In-house omics data generation.

Data has been used to generate new knowledge:

- *Overbey EG, Paul AM, da Silveira WA, et al. Mice Exposed to Combined Chronic Low-Dose Irradiation and Modeled Microgravity Develop Long-Term Neurological Sequelae. Int J Mol Sci. 2019.*
- *Morrison MD, Fajardo-Cavazos P, Nicholson. Comparison of Bacillus subtilis transcriptome profiles from two separate missions to the International Space Station. NPJ Microgravity. 2019.*
- *Beheshti A, Chakravarty K, Fogle H, et al. Multi-omics analysis of multiple missions to space reveal a theme of lipid dysregulation in mouse liver [published correction appears in Sci Rep. 2020.*

GeneLab: Analysis Working Groups and Training

GeneLab Analysis Working Groups (AWGs) *consist of 120+ scientists from multiple space agencies, international institutions, and industry. Scientists meet monthly with each group to analyze data in the GeneLab repository. Majority of members are non-NASA PI's – many have applied for NASA funding following AWG interactions.*

https://genelab.nasa.gov/awg/join

Educational Working Work (EWG) *consists of educators and scientists spanning across high school and college, focusing on developing content to learn about spaceflight OMICS, promoting the use of GeneLab's database via the repository, and the visualization and analytical portals.*

Annual AWG Workshop

GeneLab Interns
Undergrads, Grads, Postdo

Total AWG Members: ~120

Members are now group leads

AWG Members Per Group:

Animal	30
Multi-Omics/System Biology	45
Plants	15
Microbes	17

**Some members are in multiple groups*

SJSU SAN JOSÉ STATE UNIVERSITY

GeneLab included in Bioinformatics curriculum of degree granting university

GeneLab for High School (Tutorial + Tools)

AWG Members represent:

- 48 US Universities
- 4 NASA Centers
- 4 Other Government-funded Organizations
- 3 Institutes or Private Industry
- 3 International Universities

GeneLab: Publications (AWG and GeneLab)

46 original Publications and 19 Derived publications (13 original and 7 derived publications in the past year).

SCIENTIFIC REPORTS
natureresearch

Corrected: Author Correction

OPEN

Multi-omics analysis of multiple missions to space reveal a theme of lipid dysregulation in mouse liver

Afshin Beheshti[1,8*], Kaushik Chakravarty[2,8], Homer Fogle[1], Hossein Fazelinia[3], Willian A. da Silveira[4], Valery Boyko[1], San-Huei Lai Polo[1], Amanda M. Saravia-Butler[5], Gary Hardiman[4], Deanne Taylor[6], Jonathan M. Galazka[7] & Sylvain V. Costes[7*]

SCIENTIFIC REPORTS
natureresearch

Check for updates

OPEN

Sierra Nevada sweep: metagenomic measurements of bioaerosols vertically distributed across the troposphere

Crystal Jaing[1], James Thissen[1], Michael Morrison[1], Michael B. Dillon[1], Samantha M. Waters[2,7], Garrett T. Graham[3], Nicholas A. Be[1], Patrick Nicoll[4], Sonali Verma[5], Tristan Caro[6] & David J. Smith[7]

RNAseq analysis of rodent spaceflight experiments is confounded by sample collection techniques

San-Huei Lai Polo, Amanda M. Saravia-Butler, Valery Boyko, Marie T. Dinh, Yi-Chun Chen, Homer Fogle, Sigrid S. Reinsch, Shayoni Ray, Kaushik Chakravarty, Oana Marcu, Rick B. Chen, Sylvain V. Costes, Jonathan M. Galazka

doi: https://doi.org/10.1101/2020.07.18.209775

CellPress Sneak Peek
A PREVIEW OF PAPERS UNDER REVIEW

Multi-Omics Analysis Reveals Mitochondrial Stress as a Central Hub for Spaceflight Biological Impact

Cell

High impact research publications in review

- A New Era for Space Life Science: International Standards for Space Omics Processing (ISSOP) **(Accepted – Patterns, Cell Press)**
- Multi-Omics Analysis Reveals Mitochondrial Stress as a Central Hub for Spaceflight Biological Impact
- Rad-Bio-App: a discovery environment for biologists to explore spaceflight-related radiation exposures **(Accepted – Nature Microgravity)**
- NASA GeneLab RNA-Seq Consensus Pipeline: Standardized Processing of Short-Read RNA-Seq Data
- NASA GeneLab: Interfaces for the Exploration of Space Omics Data **(Anccepted – Nucleic Acid Research).**

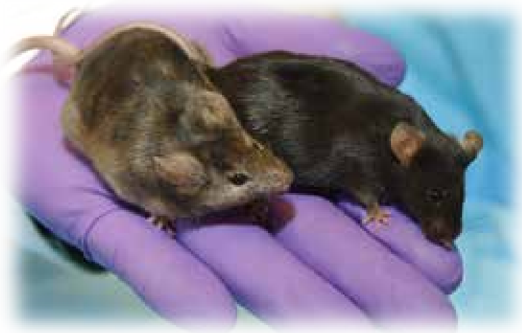
Research Highlights: ANIMALS

24 New Publications

Validation of a New Rodent Experimental System to Investigate Consequences of Long Duration Space Habitation

Choi, Saravia-Butler, Shirazi-Fard, Leveson-Gower, Stodieck, Cadena, Beegle, Solis, Ronca, and Globus. **Sci Rep,** February 2020:10(1):2336. doi: 10.1038/s41598-020-58898-4

- **The main objective of Rodent Research 1 (RR-1) was to develop new capabilities for conducting reliable, reproducible, long-duration experiments with mice with on-orbit sample collection**.
- Objectives also included monitoring animal health and welfare, and recovering samples of sufficient quality for global gene expression analysis.
- This system establishes a new capability for conducting long-duration rodent experiments in space, enables sample recovery on-orbit, and avoids triggering standard indices of chronic stress.



Dr Ruth Globus

Altered Rodent Gait Characteristics After ~35 Days in Orbit Aboard the International Space Station

Kwok, Rosas, Bateman, Livingston, Smith, Moore, Zawieja, Hampton, Mao, Delp, Willey. **Life Sci Space Res,** February: 24:9-17. doi: 10.1016/j.lssr.2019.10.010

- The objective of the study (Rodent Research-9 flight experiment) was to evaluate changes to gait as they occur in relation to spaceflight and after a brief period of recovery.
- Gait metrics were altered for 12/18 measures, gait patterns in the hindlimbs also changed significantly for 11/18 measures, and gait characteristics in the forelimbs were also altered.
- **Application of the modified gait measurement protocol used in the current study may provide an opportunity for a non-invasive analysis of countermeasure efficacy**.

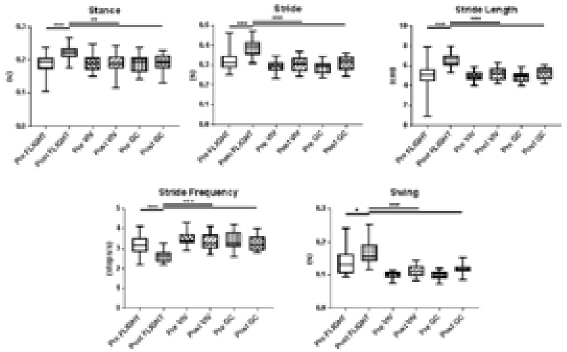


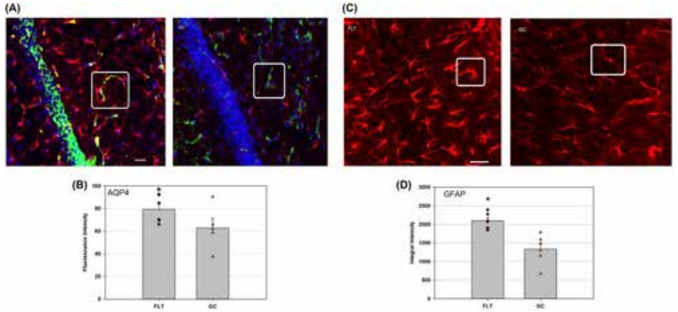
Fig. 4. Longitudinal effects of spaceflight on the gait kinematics of the left forelimbs of mice. Within and between group differences were tested using repeated measures ANOVA and Holm-Šídák post-hoc ($\alpha=0.05$; $*p < 0.05$, $**p<0.01$, $***p < 0.001$).

Life Sci Space Res February: 24:9-17. doi: 10.1016/j.lssr.2019.10.010

Spaceflight induces oxidative damage to blood-brain barrier integrity in a mouse model.

Mao, Nishiyama, Byrum, Stanbouly, Jones, Holley, Sridharan, Boerma, Tackett, Willey, Pecaut, and Delp. **FASEB J**, September 2020: doi: 10.1096/fj.202001754R

- Spaceflight-induced neuronal damage and potential adverse neurovascular effects constitute a significant health risk for astronauts
- Dr. Mao and her team studied spaceflight's effects on oxidative damage in the mouse brain and its impact on the blood-brain barrier (BBB) integrity.
    - Samples were collected from Rodent Research-9 male mice launched on SpaceX-12 and maintained onboard the ISS for 35 days.
- **The results showed increased oxidative damage and disruption in BBB integrity**, as evidenced by changes in the expression of BBB-related proteins, **changes in proteomic profiles and pathways, cell cycle progression, apoptosis, mitochondrial function, metabolism, and behavior**

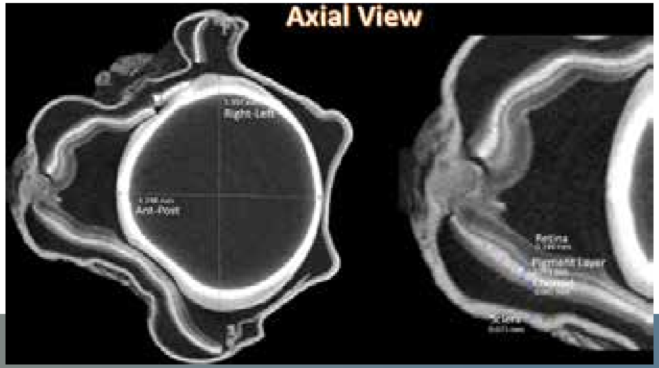


Representative micrographs of brain sections after immunostaining with anti-GFAP and AQP4 antibodies on flight (FLT) and ground control (GC) samples.

Assessment of Global Ocular Structure Following Spaceflight Using a Micro-Computed Tomography (Micro-CT) Imaging Method.

Roque-Torres, Nishiyama, Stanbouly, and Mao. **JoVE,** September 2020: e61227:61227, (https://www.jove.com/t/61227/assessment-global-ocular-structure-following-spaceflight-using-micro)

- Prolonged exposure to a spaceflight environment produces morphologic and functional ophthalmic changes in astronauts during and after an ISS mission.
- The present study was performed to **determine the impact of the spaceflight environment on ocular structures** by evaluating the thickness of the mouse retina, the retinal pigment epithelium (RPE), the choroid and the sclera layer of the C57BL/6J mice housed aboard the ISS for ~35 days using Micro-CT imaging.
- **This study demonstrated the thinning of retina and choroidal layer** and these results indicated that space environment may induce an acute and short-term response in the eye.



Axial view of a ground control mouse. Layers of the eye on the right side of the image are annotated, from top-to-bottom, retina (0.144 mm), retina pigment layer (RPE, 0.051 mm), choroid (0.041 mm), sclera (0.073 mm).

The Partial Weight-Bearing Rat Model Using a Pelvic Harness Does not Impact Stress or Hindlimb Blood Flow

Mortreux, Riveros, Semple, Bouxsein, and Rutkove. **Acta Astronautica,** March 2020: 168. https://doi.org/10.1016/j.actaastro.2019.12.024.

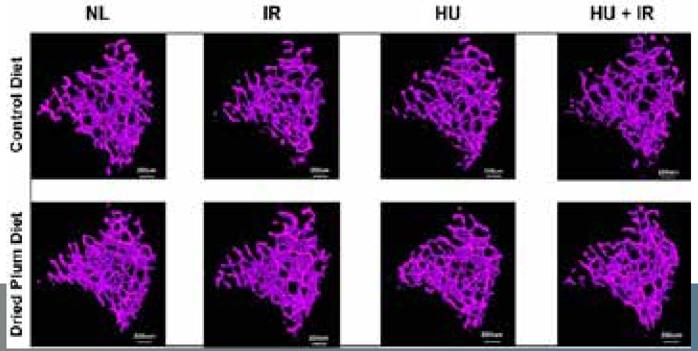
- **To study the effects of partial gravity on the Moon and Mars**, **the novel rat partial weight-bearing (PWB) model** uses a suspension jacket to partially unload forelimbs and hindlimbs so the animal retains a horizontal posture

- In this study, the use of the PWB did not lead to significant modifications of animals' blood pressure (tail blood pressure and heart rate), hindlimb oxygenation saturation, or stress markers (plasma corticosterone and serum glucose) after 28 days.
- Results suggest that **the PWB model does not induce a chronic stress regardless of the PWB level and duration**.

Dried Plums Are Effective at Preventing Bone Loss in Spaceflight Analog Study

Steczina, Tahimic, Pendleton, M'Saad, Lowe, Alwood, Halloran, Globus and Schreurs**. Sci Rep,** April 2020 10(1):6484. doi: 10.1038/s41598-020-63404-x

- Microgravity and ionizing radiation disrupt the dynamic processes of bone-destroying and bone-building cells.
- Dried plum (DP) was tested in this study as a as candidate countermeasure to mitigate spaceflight-induced bone loss from oxidative stress.
- DP diet was **shown to be effective in preventing bone loss in mice following exposure to simulated weightlessness (hindlimb unloading) and radiation exposure** and **was also effective at preventing most decrements in bone micro-architectural and mechanical properties.**
- These results show that the DP diet can protect osteoprogenitors, a population of stem cells that can differentiate into the more specialized bone-forming cells, from impairments resulting from simulated microgravity exposure.



Representative microCT images and 3D reconstructions of cancellous bone of the tibia after control diet (CD) or dried plum (DP) and either simulated weightlessness (HU), radiation exposure (IR), or a combination of the two (HU + IR).

Research In Progress

Program Element: Animal Biology

A Systems Biology Approach to Assessing the Impact of a Centrifugation Model of Spaceflight on Cross-System Communication

PI: Michael Pecaut, Ph.D., Loma Linda University

Objective: Hypergravity-induced changes in gut microbiome, SNS function, and inflammatory activity lead to a breakdown in the communication between distinct physiological systems (as measured by changes in cardiopulmonary control); and that these responses depend on the gravitational environment. **1st centrifuge run completed Nov.15, 2019**

Key Results to Date

Some measures suggest males (e.g., spleen mass, body weight loss, data not shown) are more responsive to hypergravity-induced stress than females. Our findings suggest that there are sex differences in the fecal microbiome and responses to gravitational stressors.

Research In Progress

Program Element: Animal Biology - Invertebrate

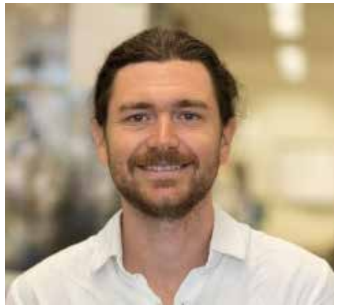
CS-04: Using Water Bears (tardigrades) to Identify Biological Countermeasures to Stress During Multigenerational Spaceflight

PI: Thomas Boothby, Ph.D, University of Wyoming

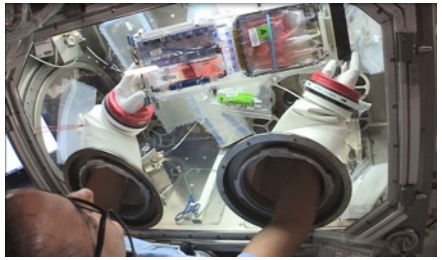
The objective of the water bear investigation is to characterize the molecular biology of short-term and multigenerational survival in the space environment by identifying genes that are required for adaptation and survival in high stress environments. Key results to date:

- Sequenced genome of the tardigrades *Hypsibius exemplaris.*
- Detected reproducible changes in tardigrade gene expression induced by stress.
- Developed and validated experimental and computational approaches for measuring the effect of different environmental conditions on tardigrade gene expression.
- Developed a reverse genetic approach, RNA interference, for tardigrades, which allows to directly investigate the role of a gene in conferring tolerance to an environment.

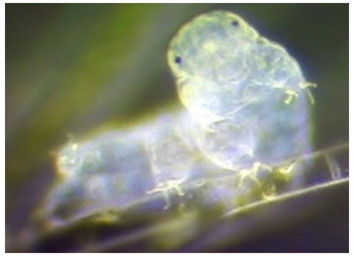
Bioculture System



Thomas Boothby, Ph.D.



CS-V imagery: Bioculture System Cassette in LSG



Tardigrade (water bear)

Currently Scheduled to launch in 2021

New Capabilities

Program Element: Animal Biology

Ground Facilities:

Roskamp - Sarasota, Florida (alternate animal care facility for KSC)

Eastern Virginia Medical School (EVMS) - Norfolk, Virginia

- Enabling launch of rodents on Northrop Grumman vehicles out of Wallops Flight Facility)

Flight Facilities:

Working with ISS Program to support potential development pathways for rodent research, which may include:

- Next generation rodent flight habitat/transporter
- Transition to commercially developed services and facilities

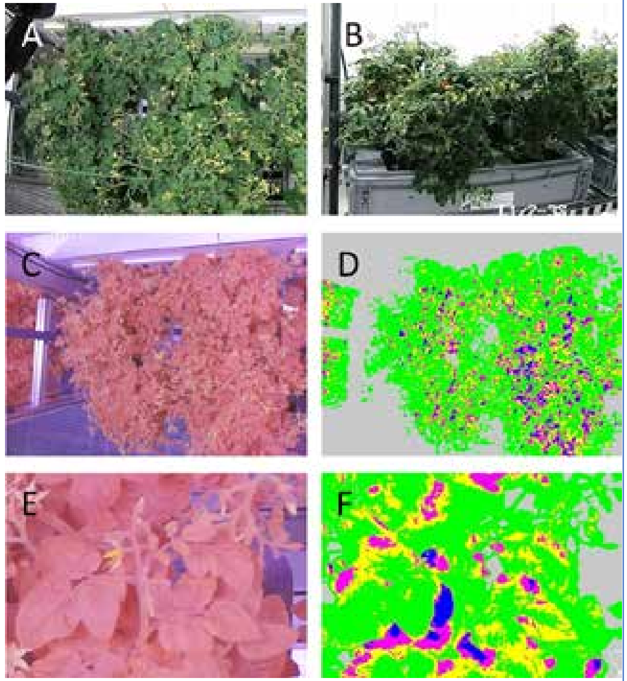
Research Highlights: PLANTS

16 New Publications

NDVI imaging within space exploration plant growth modules – A case study from EDEN ISS Antarctica

Tucker, Callaham, Zeidler, Paul, and Ferl. **Life Sciences in Space Research**, August 2020: doi: 10.1016/j.lssr.2020.03.006

- **Normalized Difference Vegetative Index**, or NDVI, presents relative reflectance of Near IR from plant leaves to measure relative plant health.
- In support of human space exploration, **remote assessment of plant health** within exploration habitats becomes critical.
- Project examines the deployment of NDVI-like capabilities within a planetary analog greenhouse on the Antarctic ice shelf with remote monitoring at UF.
- Results provide insights into the potential use of specific imaging wavelengths to monitor plant health and enhance crop production in space exploration.



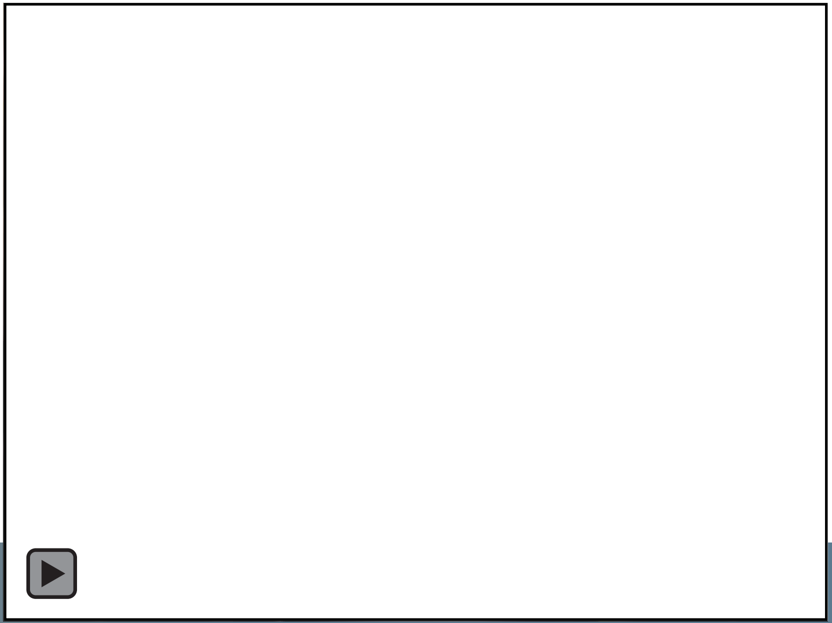
EDEN orange cherry tomato plants in from the survey cameras, top view (A), side (B). Images from modified dual bandpass camera-unprocessed image (C) and in false color after NDVI processing (D). A cropped and zoomed selection is shown in (E and F).

The EDEN ISS Greenhouse in Antarctica.

Hardware Validation of the Advanced Plant Habitat on ISS: Canopy Photosynthesis in Reduced Gravity

Monje, Richards, Carver, Dimapilis, Levine, Dufour and Onate. **Frontiers in Plant Science**, June 2020: https://doi.org/10.3389/fpls.2020.00673

- **Advanced Plant Habitat (APH) is a fully enclosed, closed-loop plant life support system with an environmentally controlled growth chamber** designed for conducting both fundamental and applied plant research.
- In 2018 a hardware validation test was conducted after installation on the ISS and this test utilized both Arabidopsis and wheat plants.
- Test examined the ability to control light intensity, spectral quality, humidity, CO_2 concentration, photoperiod, temperature, and root zone moisture using commanding from Kennedy Space Center.
- Wheat was also used to validate the ability to measure gas exchange of plants from non-invasive measurements (i.e., canopy photosynthesis and respiration).
- **This hardware validation test confirmed that APH can measure fundamental plant responses to spaceflight conditions**.

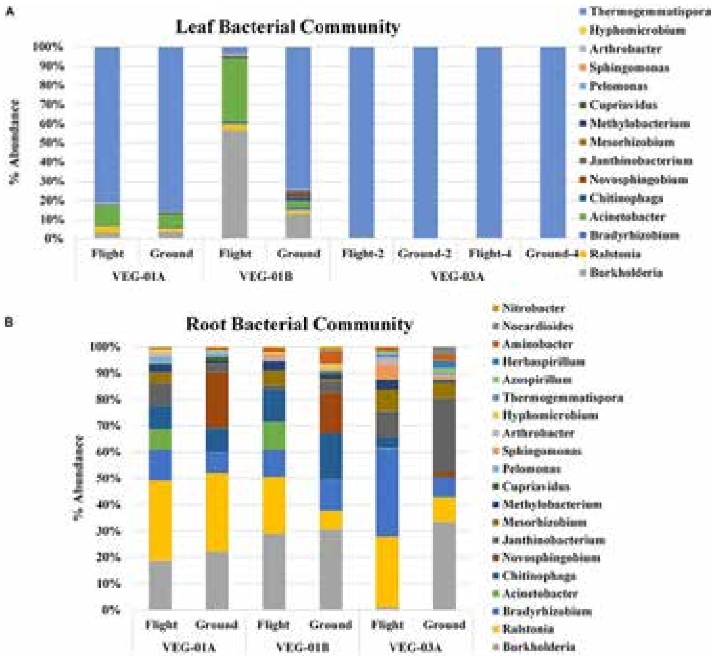


A time-lapse video showing the growth of both plant types on ISS.

Microbiological and Nutritional Analysis of Lettuce Crops Grown on the International Space Station

Khodadad, Hummerick, Spencer, Dixit, Richards, Romeyn, Smith, Wheeler, and Massa. **Frontiers in Plant Science**, March 2020: https://doi.org/10.3389/fpls.2020.00199

- Characterization of food safety, microbial communities, and nutritional content of three lettuce crops grown over three years.
- **Indicated that leafy vegetable crops can produce safe, edible, fresh food to supplement to the astronauts' diet,** and provide baseline data for continual operation of the Veggie plant growth units on ISS.



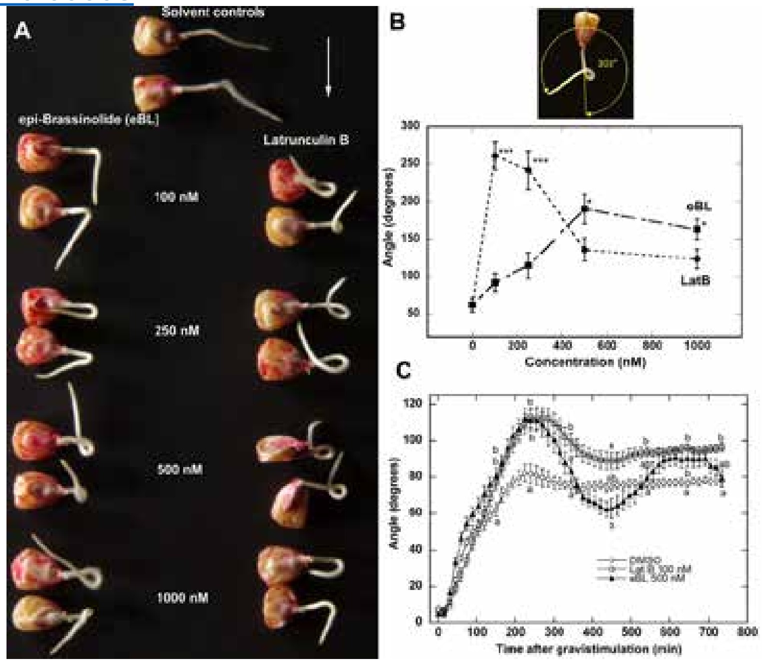
Readership Metrics (10/19/20):

- **Attention Score of 373**
- **Top 5%** of all research outputs tracked by Altmetric
- **#1 of 11,337** outputs from Frontiers in Plant Science
- **106 additional articles** from 95 news outlets generated from this article
- **39,574 total views**
- Additional metrics and news stories at https://frontiers.altmetric.com/details/77108228

Brassinosteroids Inhibit Autotropic Root Straightening by Modifying Filamentous-Actin Organization and Dynamics

de Bang, Paez-Garcia, Cannon, Chin, Kolape, Liao, Sparks, Jiang and Blancaflor. **Frontiers in Plant Science**, February 2020: https://doi.org/10.3389/fpls.2020.00005

- Gravitropism, the directional growth of plant organs in response to gravity, is influenced by plant hormones, including brassinosteroids.
- **Tested hypothesis that eBL, a brassinosteriod, enhanced root responses to gravity by impacting the cytoskeletal component F-actin**, similarly to the actin inhibitor LatB.
- Using clinostats to simulate microgravity, eBL was shown to inhibit autotropism (root straightening after a gravity stimulus was removed) by altering F-actin dynamics and organization.

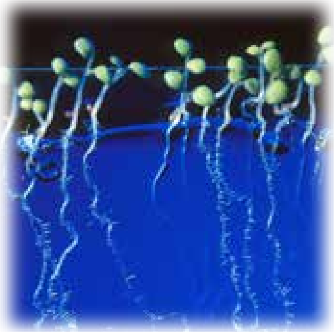


(A) Representative images of *Z. mays* roots grown on a 2-D clinostat for 20 h to test autotropic straightening after withdrawal of the gravity signal. **(B)** Dose response of root curvature on a clinostat after eBL and LatB treatment. **(C)** Time course of root gravitropism after vertically growing roots were placed horizontally without clinorotation

RNAseq Analysis of the Response of Arabidopsis thaliana to Fractional Gravity Under Blue-Light Stimulation During Spaceflight

Herranz, Vandenbrink, Villacampa, Manzano, Poehlman, Feltus, Kiss, and Medina. **Frontiers in Plant Science**. November 2019 26;10:1529. doi: 10.3389/fpls.2019.01529

- The objective of this flight study was to **induce subtle blue-light phototropism in roots in spaceflight to discern the transcription responses to different tropisms on-orbit** and to detect green expression differences and gravity thresholds of the cellular effects under microgravity and light stimulation.
- Removal of the gravity influence on blue-light-illuminated seedlings showed a reduction in gene expression in multiple pathways associated with photosynthesis.
- Transcriptional analyses of plants under blue light stimulation suggests that root blue-light phototropism may be sufficient to reduce the gravitational stress response caused by the lack of gravitropism in microgravity.



Spaceflight induces novel regulatory responses in Arabidopsis seedling as revealed by combined proteomic and transcriptomic analyses

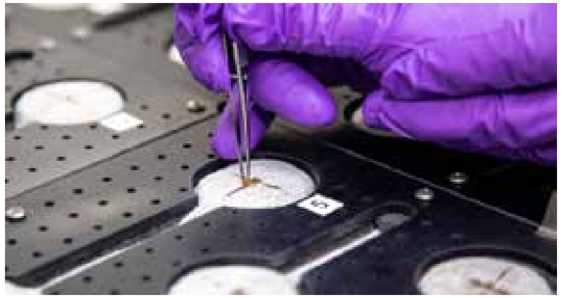
Kruse, Meyers, Basu, Hutchinson, Luesse, Wyatt. **BMC Plant Biol**. May 2020: 20(1):237. doi: 10.1186/s12870-020-02392-6.

- Proteomic and transcriptomic (RNA-seq) analyses simultaneously quantified protein and transcript differential expression of three-day old, etiolated *Arabidopsis thaliana* seedlings grown aboard the International Space Station along with their ground control counterparts.
- **The datasets gathered from Arabidopsis seedlings exposed to microgravity revealed marked impacts on post-transcriptional regulation, cell wall synthesis, redox/microtubule dynamics, and plastid gene transcription.**
- Omics data from this experiment were funded by GeneLab and can be found in the GeneLab Data Repository.

Advanced Plant Habitat (APH) ISS Experiments

- PH-01: Arabidopsis plant tissue returned to Earth in Jan. 2020
- PH-02: Radishes – Ground tested and **now on ISS to begin growth Oct.-Nov. 2020**

PH-02 Radish Ground Studies



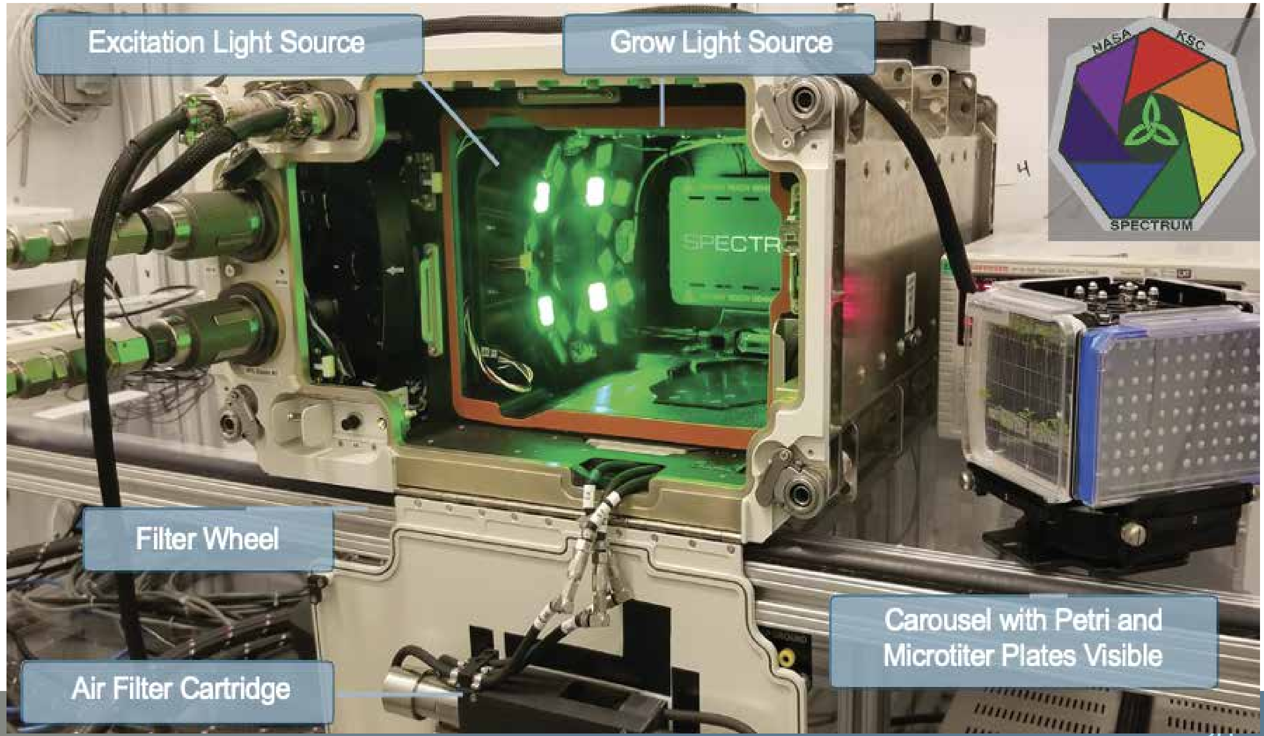
PH-02 PI Dr. Karl Hasenstein plants seeds for NG-14 launch

- PH-03: Arabidopsis multi-generational epigenetic study undergoing ground testing

PH-03 Arabidopsis plants with seed containment bags

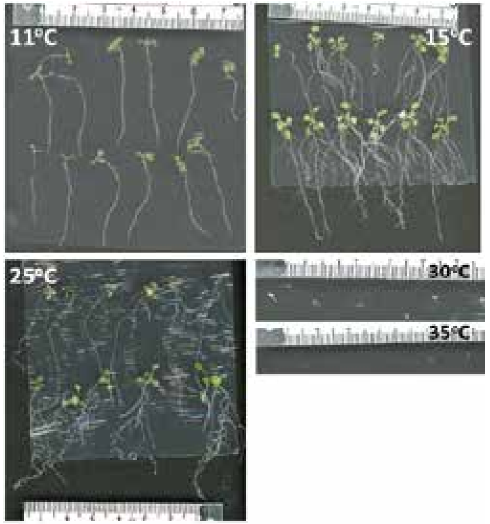
Spectrum Multi-Spectral Fluorescence Imager

- **Imaging system designed for capturing *in vivo* genetic expression on ISS installed on the ISS in June 2020**
- Suitable for model organisms including unicellular organisms, plants, and invertebrates
- Controlled environment chamber with reconfigurable imaging capabilities for high resolution imaging in various wavelengths
- Following successful checkout, Science validation experiment (Spectrum-001) launched to ISS in Oct. 2020



Research In Progress

Program Element: Plant Biology



Growth and Development of Ecotypes of Arabidopsis thaliana: Preliminary Experiments to Prepare for a Moon Lander Mission

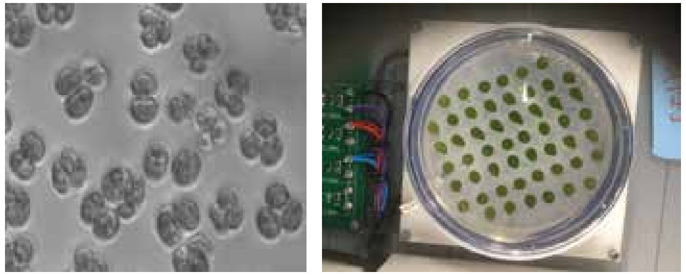
PI: John Z. Kiss, Ph.D, University of North Carolina at Greensboro

Space Biology PI Dr. John Kiss of the University of North Carolina-Greensboro has proposed sending **a green-House housed in a 1U CubeSat on a robotic mission as part of the Artemis lunar program**. Due to the extremely limited power resources, a very narrow temperature range will be available for growing plants. In anticipation of this, Dr. Kiss has conducted a series of experiments where he tested a mixture of ecotypes of the model plant *Arabidopsis thaliana* from colder and warmer climates to see which would grow best. Their investigation found that **there is one single genotype, *Columbia* (Col-0), that had the best seed germination, growth, and development at the widest temperature range (11–25 ºC)**.

Artemis I BioExpt-1

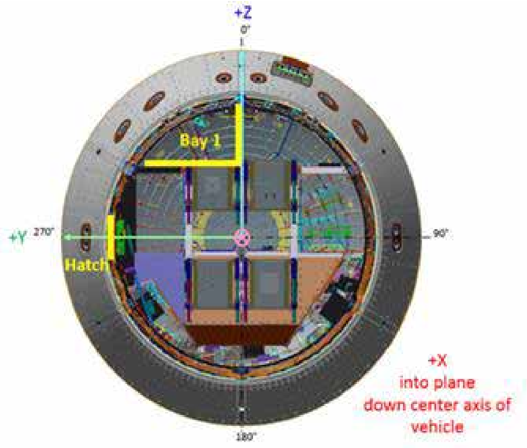
- Four Projects Selected:
  - ***Arabidopsis thaliana* plants**: ***Life Beyond Earth: Effect of Space Flight on Seeds with Improved Nutritional Value***; PI Federica Brandizzi, Michigan State University
  - **Algae *Chlamydomonas reinhardtii***: ***Fuel to Mars***; PI Timothy Hammond, Institute for Medical Research
  - **Fungi *Aspergillis niger***: ***Investigating the Roles of Melanin and DNA Repair on Adaptation and Survivability of Fungi in Deep Space***; PI Zheng Wang, Naval Research Laboratory
  - **Yeast *Saccharomyces cerevisiae***: ***Multi-Generational Genome-Wide Yeast Fitness Profiling Beyond and Below Earth's Van Allen Belts***; PI Luis Zea, University of Colorado-Boulder

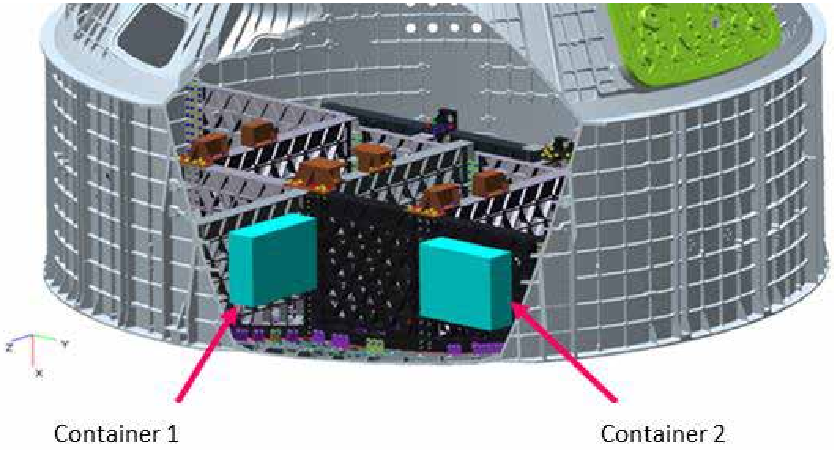
Arabidopsis seeds will be tested for germination post-flight



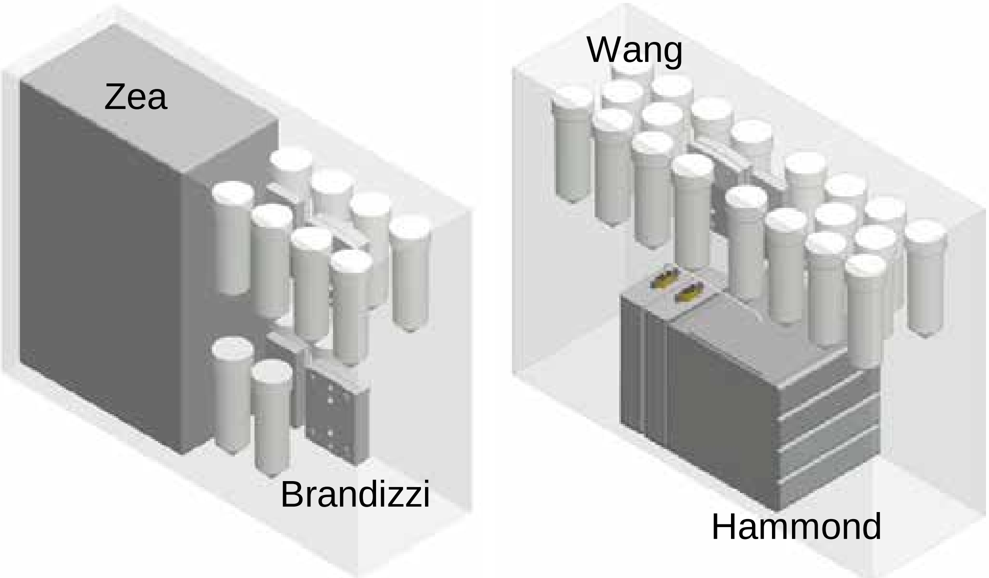
Chlamydomonas wt and deletion clones cultured for flight

Yeast culture bags for flight





The SBP Payload Container Assemblies are installed onto backbone panels 1 and 2 in Bay 1 of the Orion capsule.



SBP Payload Container Assembly configuration for Drs. Zea & Brandizzi (left) and Drs. Wang & Hammond (right).

Growing Beyond Earth - Fairchild Tropical Botanic Gardens

Citizen science program with **thousands of US middle and high school students helping NASA collect data on Space Crop Production**

- Students test crops in Veggie-like growth chambers in their classrooms
- Candidate crops feed into NASA's high-fidelity crop testing at Kennedy Space Center
- Two student-selected plant varieties have been tested in Veggie on the ISS
- Current testing is remote/at home and students are testing Chile pepper pollination for an upcoming flight experiment.

https://fairchildgarden.org/science-and-education/science/growing-beyond-earth/

- Maker contest for students and professionals to help design crop growth systems for space

https://fairchildgarden.org/science-and-education/innovation/maker-challenge/

Research Highlights: MICROBES

16 New Publications

Crewmember Microbiome may Influence Microbial Composition of ISS Habitable Surfaces

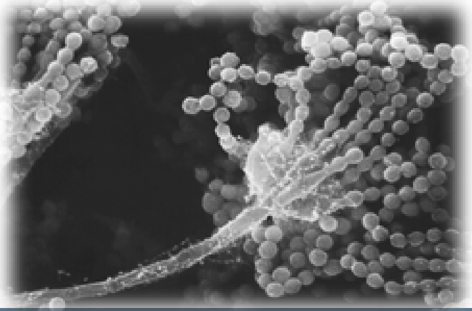
Avila-Herrera, Thissen, Urbaniak, Be, Smith, Karouia, Mehta, Venkateswaran, and Jaing **PLoS One**, April 2020: 15(4):e0231838. doi: 10.1371

- Research objective was to **investigate the interplay between the microbial communities onboard the ISS and its crew to understand microbiome changes over time, and the potential impact of pathogens to crew health.**
- Samples from one crew member over eight time points indicate that skin, nostril, and ear samples are more similar to the microbe populations found on ISS surfaces than mouth and saliva samples.
- The microbial composition of the crewmember's skin samples are more closely related to the ISS surface samples collected by the crewmember on the same flight than ISS surface samples collected by crewmembers on different flights.
- SourceTracker **results predicted that the crewmember microbiome contributed to 42 - 55% of the Flight surface microbiome** across all eight locations.

Contributions of Spore Secondary Metabolites to UV-C Protection and Virulence Vary in Different Aspergillus fumigatus Strains

Blachowicz, Raffa, Bok, Choera, Knox, Lim, Huttenlocher, Wang, Venkateswaran, and Keller. **mBio,** February 2020**:**11(1):e03415-19. doi: 10.1128/mBio.03415-19

- The **human pathogen Aspergillus fumigatus** is a known contaminant on the ISS. Melanin, a secondary metabolite in spores, has been shown to be critical for virulence in A. fumigatus, and to be protective against UV radiation in other fungi.
- Three diverse strains of A. fumigatus, including one ISS strain (IF1SW-F4), were examined in this lab-based investigation.
- **DHN-melanin provides protection from UV-C and virulence but is strain dependent. Fumiquinazoline, another secondary metabolite produced by A. fumigatus, also showed protective properties from UV-C radiation.**

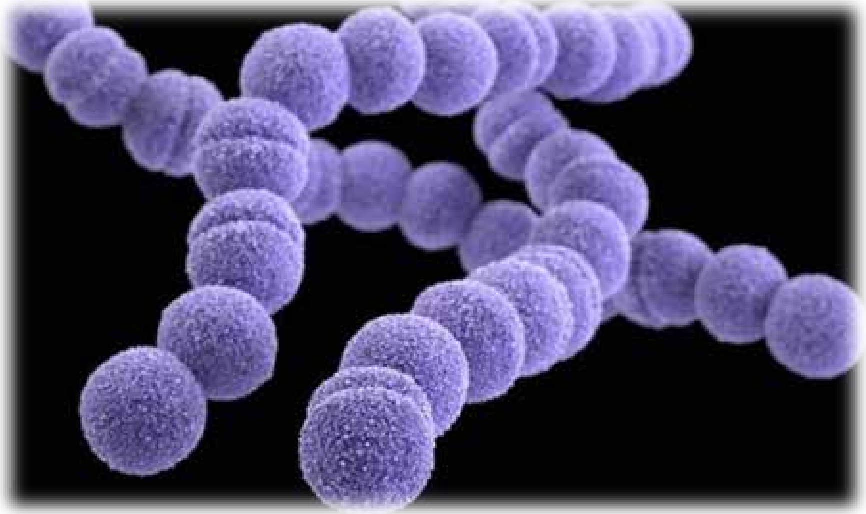


The Influence of Spaceflight on the Astronaut Salivary Microbiome and the Search for a Microbiome Biomarker for Viral Reactivation

Urbaniak, Lorenzi, Thissen, Jaing, Crucian, Sams, Pierson, Venkateswaran, and Mehta. **Microbiome,** April 2020: 8(1):56. doi: 10.1186/s40168-020-00830-z.

(Study sponsored by NASA's Human Research Program with postdoctoral support from Space Biology for environmental sample analyses)

- An investigation into whether and how the human salivary microbiome is affected pre-flight, in- flight (ISS), and post-flight (upon return to Earth).
- Microbiome (alpha) diversity and richness was significantly increased during exposure to spaceflight, as compared to pre-flight and post-flight data.
- Beta diversity of the salivary microbiome showed no distinct clustering, suggesting no microbiome differences between subjects and flight status.
- *Streptococcus* was the most abundant organism in the saliva.



Science Accomplishments

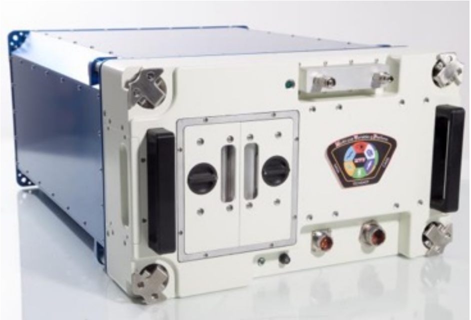
Program Element: Microbiology (Experiments conducted on the ISS)

Inc.58 → Inc.59 → Inc.60 → Inc.61

SpX-16
Micro-14
Nielsen
Pt.1 (GAP)

SpX-17
Micro-14
Nielsen
Pt.2 (BioCells/FEP)

SpX-18
MVP-Cell-02
Everroad



Multi-use Variable-gravity Platform

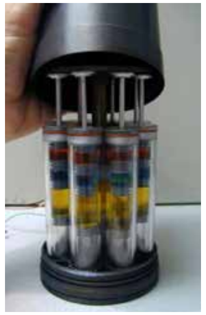
Characterizing the Effects of Spaceflight on the *Candida albicans* Adaptation Response
PI: Sheila Nielsen, Ph.D. Montana State University

Aim 1: Evaluate the microgravity-induced **alterations in biosynthetic regulation, cellular content, and subcellular localization of ergosterol and b-glucans**.

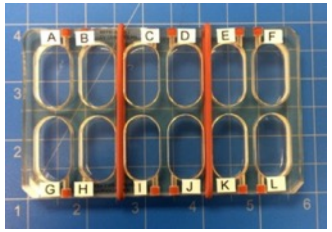
Aim 2: Delineate the contributions of low fluid shear associated oxygen depletion and carbon dioxide enrichment in the microenvironment to cellular adaptation responses.

Aim 3: Characterize the effect of spaceflight on *C. albicans* virulence using a human monocyte host.

FPAs / GAPs



FEP Bags



12-well BioCells

Experimental Evolution of *Bacillus subtilis* Populations in Space: Mutation, Selection, and Population Dynamics
R. Craig Everroad, Ph.D.; NASA Ames Research Center

Aim 1: Document **adaptive changes in growth rate of *B. subtilis* in the space environment** compared to 1g and ground controls

Aim 2: Identify targets of selection in space-adapted lines of mutator and competent strains of *B. subtilis* by examining exogenous DNA-uptake and de novo mutations through deep sequencing

37

Research In Progress

Program Element: Microbiology

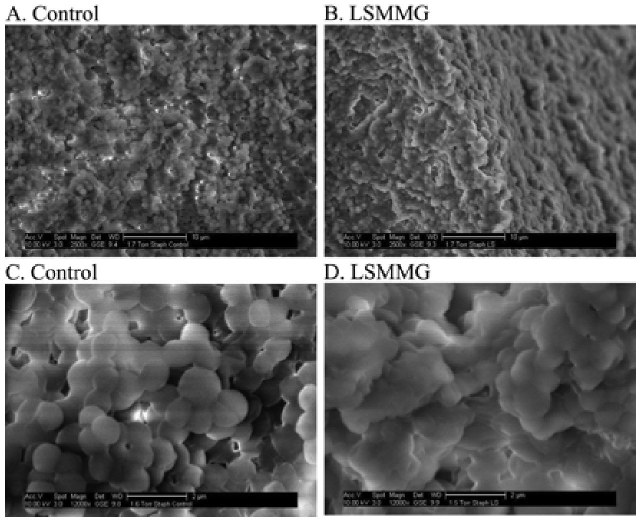
Inc.64 → **Inc.65** → **Inc.66**

SpX-21
Micro-14
Nielsen (re-flight)
&
Bacterial Adhesion and Corrosion

SpX-22
MT-3

SpX-23
MVP-Cell-02
Everroad (re-flight)
MT-3

SpX-24
Microbial Tracking-3 [MT-3] (completion)



Bacterial Adhesion and Corrosion will study biofilm formation and their impacts on stainless steel. (PI: Robert McLean, Ph.D., Texas State University–San Marcos)

Microbial Tracking-3 will sample eight locations on the ISS over 3 separate flights (PI: Jack Gilbert, Ph.D. University of California – San Diego)

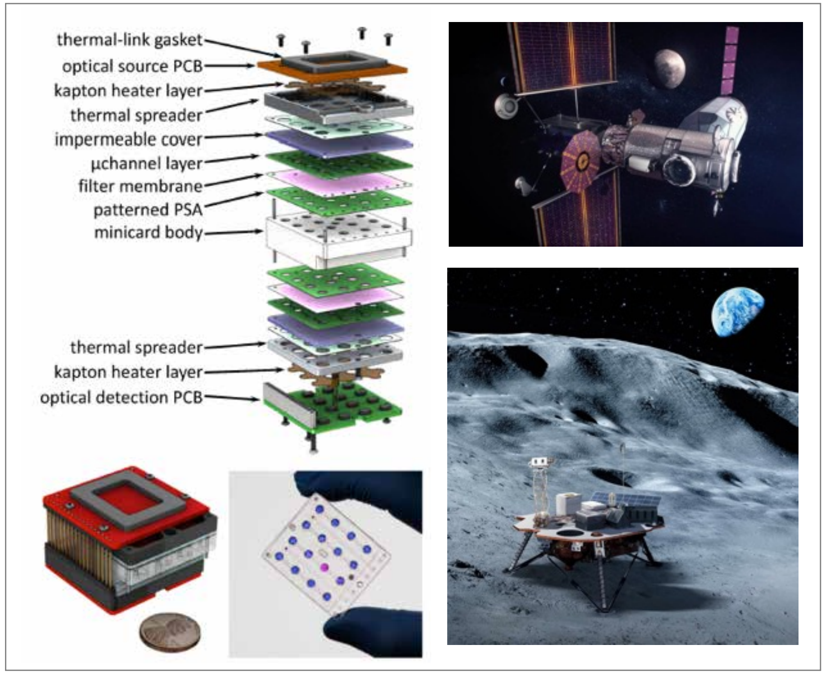
Lunar Explorer Instrument for space biology Applications (LEIA)

Project Manager: Brandon Maryatt
Project Scientist: Jessica Audrey Lee
Mission Scientist: Matt Lera

Objective:
Biological research beyond LEO using autonomous, radiation-tolerant research hardware, compatible with a wide range of flight opportunities.

Relevance / Impact:
- Transportation flexibility to take advantage of all opportunities for conducting science in flight in deep space and planetary environments.
- Increased understanding of the biological effects of the unique environment(s) beyond LEO.
- Contribution to advancing technologies on Earth and technology transfer to the commercial space sector.

Project Development Approach and current status:
- Hardware basis has been selected: the BioSentinel BioSensor payload.
- Research solicitation and and awards will take place through NRA process
- Will support launch opportunities in FY23 and FY25

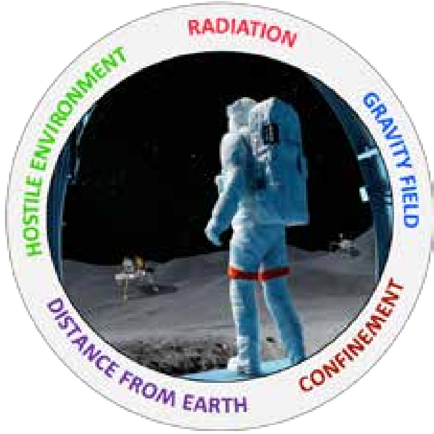


*Left: diagrams of exploded (**top**) and assembled (**bottom**) microfluidic minicard from BioSensor payload, with thermal control and optical measurement components. From Ricco et al. (2020) DOI:10.1109/MAES.2019.2953760*

Forecasting Science Directions in the Community

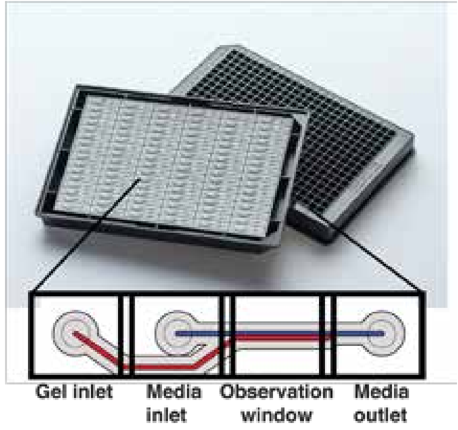
Future Science Directions

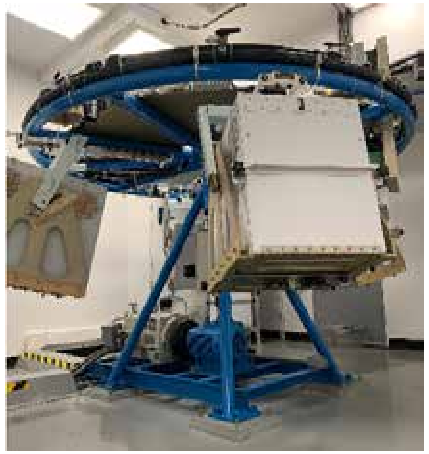
Program Element: Animal Biology – Vertebrates & Invertebrates



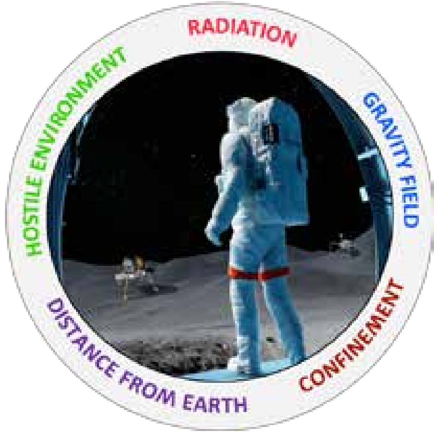
Continued animal research is critical to support future human exploration and habitation missions.

- Perform longitudinal studies in animal model systems to parameterize predictive computational modeling.
- Provide leadership in gathering comprehensive datasets and archiving metadata; promote data sharing to ensure the broadest access and support predictive science (e.g. systems biology).
- Utilize animal models to evaluate the long-term effects of spaceflight on development, physiology, and neurobehavior, of the combined effect of deep space radiation and altered gravity environments, along with evaluation of other spaceflight stressors.
- Use of animals as translational and accelerated disease models.
- Interactions of animals with microbial environment and assessing potential pathogenesis outcomes.
- Development and testing of biomarkers in animal models for early detection and screening of the effects of space radiation and microgravity on human health.
- Development and testing of novel radiation countermeasures using animal models.
- Support the development and flight implementation of novel approaches such as High-throughput 3D human blood-brain barrier on-chip: Organ-on-chip approach.





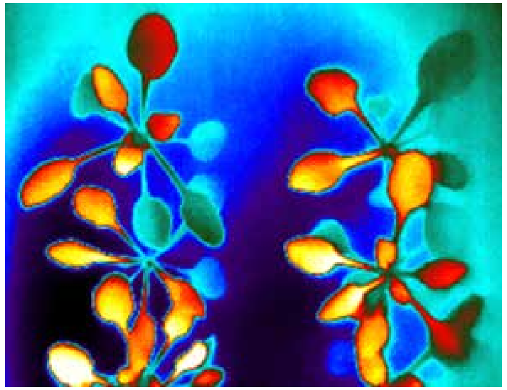
Future Science Directions



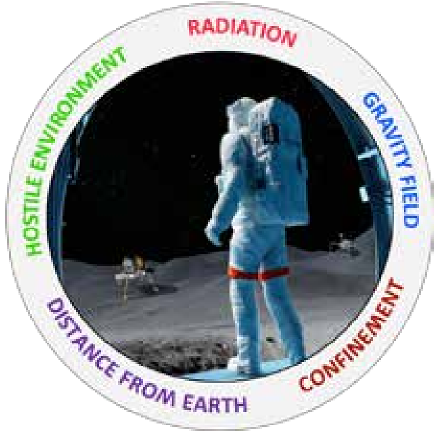
Program Element: - Plant Biology

Plant Biology is a critical research area for enabling space crop production to maintain crew health during long-duration deep space missions.

- Study plant cellular and molecular responses and adaptations to spaceflight.
- Plant microbiome dynamics and evolution in the built space environment.
- Understand plant disease responses under deep space conditions.
- Develop microbial biocontrols for human and plant pathogens in space systems.
- Investigate seed microbial biopriming to promote crop growth in space.
- Develop effective, sustainable methods for delivering water and nutrients to plants in micro and partial gravity.
- Develop non-destructive plant health monitoring technologies for spaceflight.
- Use targeted breeding / genetic engineering approaches to optimize space crops.
- Improve space crop photosynthesis, including carbon fixation, light interception, and assimilate partitioning.
- Identify space radiation concerns for plants and develop countermeasures.
- Understand gravity and atmospheric pressure thresholds related to plant physiological responses and space crop production.
- Characterize an array of crops that will provide safe human dietary supplementation in spaceflight.
- Characterize changes during multigenerational plant growth off-Earth.

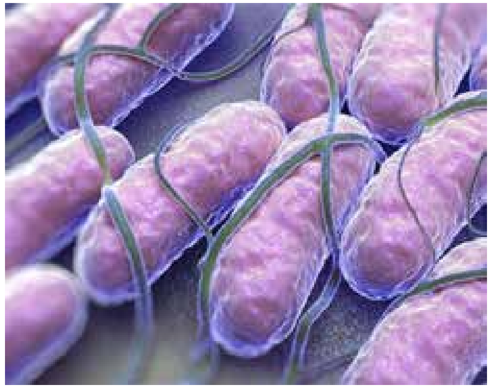


Future Science Directions



Program Element: - Microbiology

- Apply high throughput screening and advanced bioinformatics analysis to develop a comprehensive understanding of microbial diversity and dynamics in space.
- Comprehensive analysis of the effect of spaceflight on astronaut microbiome.
- Study dysbiosis (change in microbiome composition) during the spaceflight and upon return on earth to understand links with health consequences.
- Develop and implement the microbes-on-chip approach to study high-throughput and multi-factor effects on microbial isolates.
- Development and testing of 'microbial biocontrol' approaches to control potential pathogens on ISS and human habitation.
- Develop space tolerant plant-microbiome systems for significant plant growth and disease prevention.
- Monitoring and control of indoor microbiome for the sustained presence of humans in space and future habitations.
- Development of novel and useful antimicrobial agents to prevent biofilms' development on ISS surfaces and water treatment plants.
- Selecting and applying microbes appropriate for biomining (regolith) and waste processing.



Challenges of interest – spaceflight stressors

OVERVIEW OF THE EFFECTS OF **SPACEFLIGHT AND RADIATION**

Combined effects of spaceflight stressors

- Spaceflight stressors can include:
  - Elevated levels of ionizing radiation
  - Altered gravity conditions
    microgravity, hypergravity, partial gravity on planetary surface
  - Elevated CO_2 levels
  - Altered day/night light cycles
  - Social isolation, etc.

- Ionizing radiation and altered gravity are two important, novel spaceflight stressors that show various responses when applied together, depending on the organism, tissue type and assay. When combined, a given response to stressor may be:
  - Amplified (additive, synergistic)
  - No effect
  - Suppressed (data not shown)

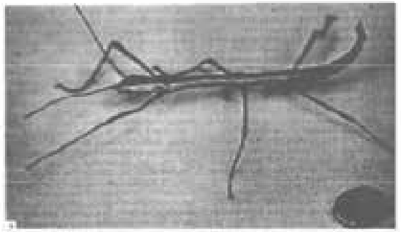
- **Bottom line:**
  - **We still have a very limited understanding of how living organisms respond to the various spaceflight stressors.**
  - **So it is critical to expand our knowledge base, enabling us to better prepare for possible risks to astronaut health posed by long-duration spaceflight missions to the Moon and Mars.**

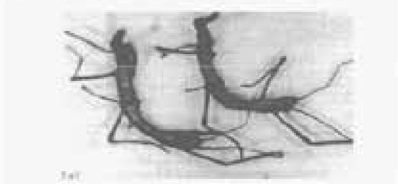
The combined effects of radiation and microgravity can be greater than either condition alone

1. Significant increase in early developmental abnormalities in Carausius morosus (stick insect) during space flight and increased with heavy ion hits (Biostack concept - sandwich with nuclear track detectors)

G.Reitz, H.Bucker, R.Facius, G.Horneck, E.H. Graul, H.Berger, Ruther et al. (1981). *Adv Space Res* 10:161. {Shown on Spacelab Mission D1 (7-day mission) and reconfirmed on Biosatellite Mission Cosmos 1887(13-day mission)}

Normal larvae





Abdominal segment anomalies from flight

Antenna anomalies from flight

- Larval anomalies detected from space flown samples plus heavy ion hits at early embryonic stages of development
- Very few animals with developmental anomalies from microgravity alone *(in space but not hit by radiation as verified by CR39 nuclear track detectors)*
- No animals with anomalies detected on 1g centrifuge in space flight even though hit with heavy ions.
- Zero anomalies in ground controls

Combined effect of radiation and spaceflight can differ in the same animal population depending on the tissue studied

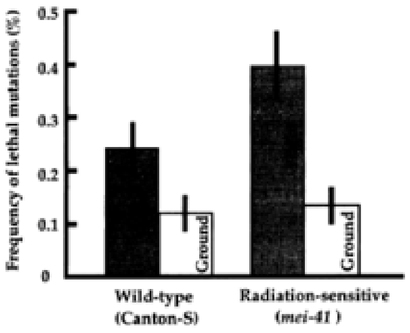
3. Significant increase in frequency of lethal mutations induced in Drosophila (fruit fly) male germ cells during spaceflight (sex-linked recessive F2 screen)

M.Ikenaga, I. Yoshikawa, M.Kojo, T. Ayaki, H.Ryo, K. Ishizaki, T. Kato, H.Yamamoto and R. Hara (1997). *Biological Sciences in Space* 11:346.
(Space shuttle Endeavor flight)

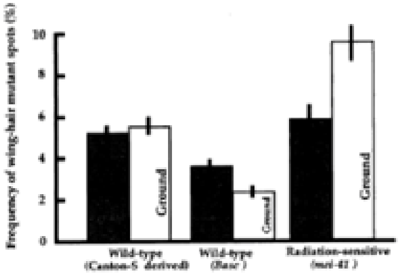
Adult males sent into space for 8 days, mated after return. Analyzed F2 offspring for lethality of irradiated X chromosome inherited from grandfather from space.

Strain	Exp. group	No. of X-chromosomes tested	No. of lethal X-chromosomes detected	Frequency of lethal mutations* (% ±SE)
wild type Canton-S	flight	9,176	22	0.24 ±0.051#
	ground control	9,177	11	0.12 ±0.036
radiation-sensitive *mei-41*	flight	9,355	37	0.40 ±0.065§
	ground control	8,975	12	0.13 ±0.037

*Number of lethal X-chromosomes divided by the number of X-chromosomes tested.
#Significantly different at the 5% level from the ground control group.
§Significantly different at the 1% level from the ground control group.



……but not in somatic tissue that can be repaired during development or in flies that do not survive due to selection of the fittest in the population



Larvae sent into space, pupae returned, adults eclosed on the ground post-flight. Analyzed wing hair somatic mutations of adults arising from mutations that would have occurred in wing imaginal disc of larvae while in space. Mutants may either die as larval or pupal lethals or be corrected during growth and developmental stages, in which case these mutations would not be scored by this assay

Effects of radiation and spaceflight on *Arabidopsis thaliana* plant seeds can differ depending on flight duration and other factors

1. Long Duration Mission (5.8 yr) Arabidopsis thaliana seeds with ambient radiation

M.W. Zimmerman, K.E. Gartenbach, and A.R. Kranz (1994). *Adv Space Res* 14:51 (LDEF mission)

- Very long duration mission, at higher than usual orbit (324-479 km compared to 350-390 km for ISS)
- Found significant changes that correlate with radiation hits (Biostack concept)
- Significant decrease in germination, increased delays in germination
- Increased mutation frequencies

Long duration study with ambient radiation, shows significant spaceflight effects on seed germination and mutation frequencies when seeds hit with radiation in flight.

2. Effects of preflight gamma radiation & spaceflight on Arabidopsis thaliana seeds

E.N. Vaulina, I.D. Anikeeva, L.N. Kostina, I.G. Kogan, L.R. Palmbakh and A.L. Mashinksky (1981). *Adv Space Res* 1:163 (Salyut 6 orbital station)

Group	Seed Germination	Seedling Death in the Cotyledon Phase	Number of Fertile Plants	Number of Non-fertilized Seedbuds	Number of Recessive Mutants
49 Days Flight Exposure					
Ground Control	91.03 ± 1.37	11.62 ± 1.61	69.28 ± 2.22	6.55 ± 0.28	10.19 ± 0.36
Flight Control	86.65 ± 1.52	19.77 ± 1.91[a]	60.56 ± 2.70	8.31 ± 0.32[a]	9.30 ± 0.35
γ-irradiation Control	94.08 ± 1.04	13.31 ± 1.54	72.56 ± 2.01	22.47 ± 0.50	12.70 ± 0.45
γ-irradiation + Flight	91.57 ± 1.14	12.52 ± 1.42	75.25 ± 1.78	22.46 ± 0.44	9.75 ± 0.35[a]
226 Days Flight Exposure					
Ground Control	94.09 ± 1.06	18.83 ± 1.82	60.69 ± 2.20	8.85 ± 0.30	3.78 ± 0.22
Flight Control	87.07 ± 1.51[a]	24.83 ± 2.08	53.33 ± 2.24	12.12 ± 0.35[a]	14.87 ± 0.40[a]
γ-irradiation Control	92.78 ± 1.91	26.77 ± 2.12	43.10 ± 2.28	34.35 ± 0.58	21.78 ± 0.63
γ-irradiation + Flight	81.89 ± 1.92[a]	44.55 ± 2.74[a]	20.70 ± 2.02[a]	42.09 ± 0.80[a]	31.39 ± 0.98[a]

Study with preflight gamma radiation shows time dependence of spaceflight effects on seeds (caveat: 30 krad/300Gy dose of gamma used for preflight irradiation).

3. Other missions have yielded various results with Arabidopsis seeds, some showing no germination effects but increased mutation frequency, some showing no overall effects. However, there were differences in flight duration, procedures, and hardware.

Many prokaryotic and eukaryotic cells in culture indicate normal DNA repair in space

Repair of radiation induced DNA damage progresses normally under microgravity conditions

G.Horneck (1999). *Mutation Res* 430:221.

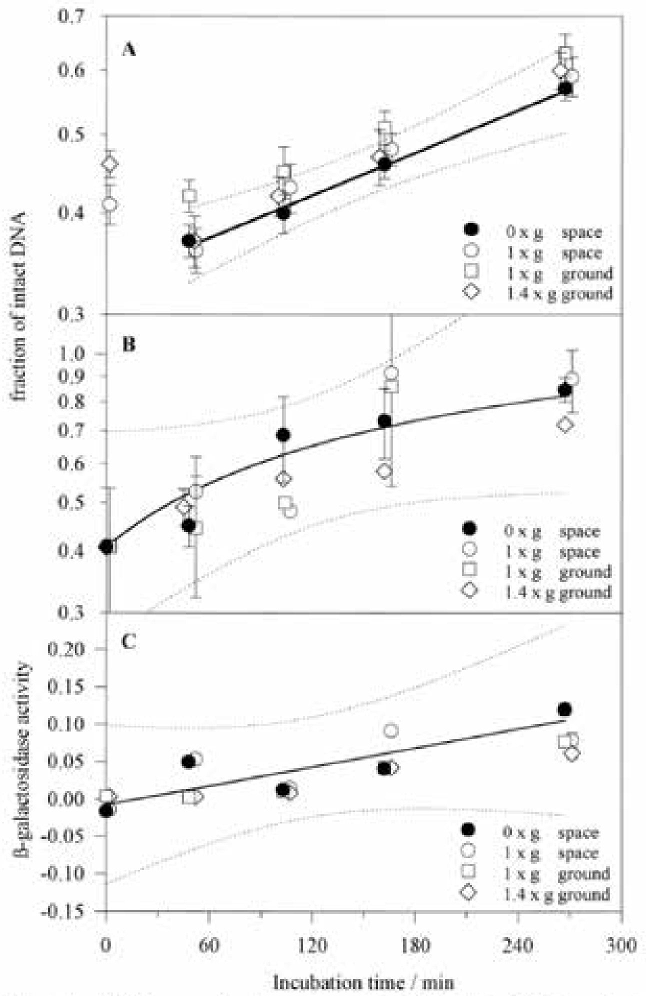


Fig. 3. Repair of radiation-induced DNA damage under microgravity conditions. (A) Rejoining of DNA strand breaks in cells of *E. coli* B/r. (B) Rejoining of DNA strand breaks in human fibroblasts. (C) Induction of SOS response in cells of *E. coli* PQ37 (modified from Ref. [25]).

Rejoining DNA strand breaks in E.coli

Rejoining DNA strand breaks in human fibroblasts

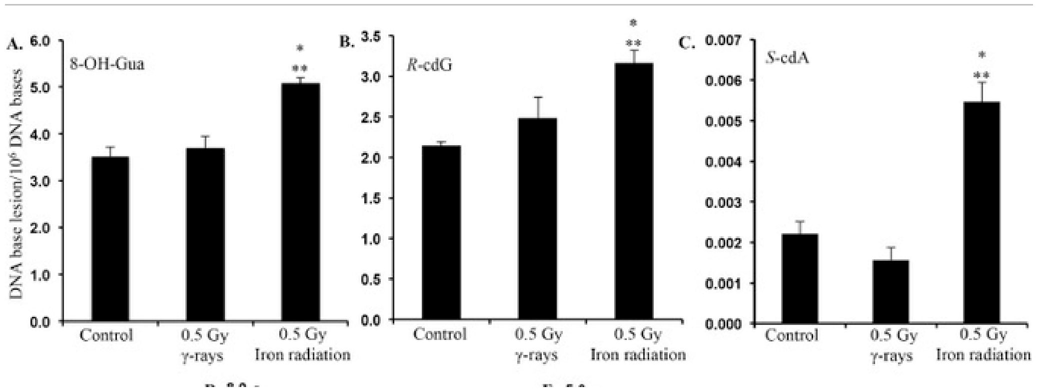
Induction of SOS response in E.coli

Similar data showing normal repair of radiation damaged DNA in space in Saccharomyces cerevisiae (yeast) cells (data not shown), in addition to E.coli and human fibroblasts (data shown here).

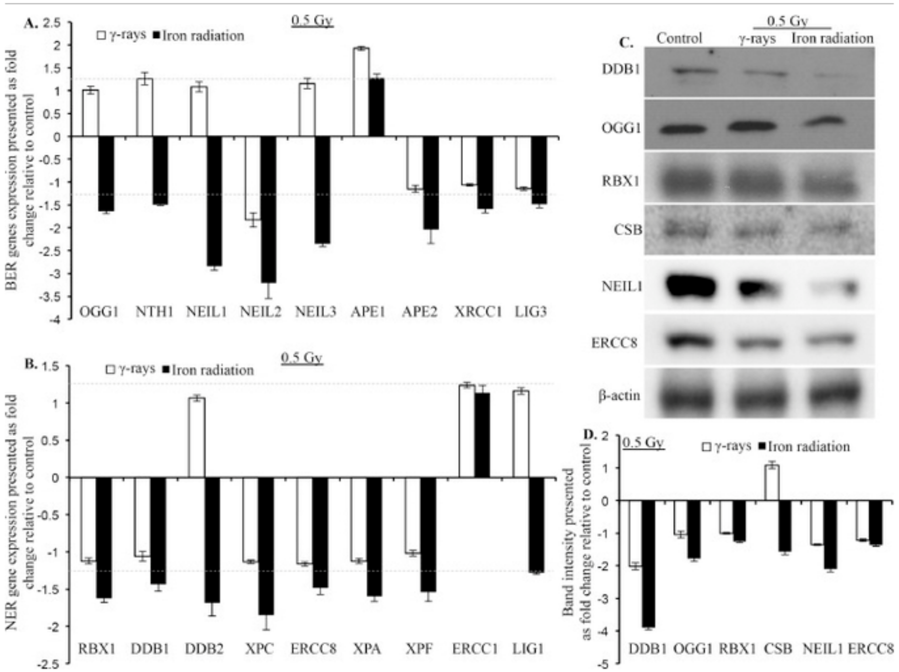
Therefore, combined effects of microgravity and radiation during spaceflight in larger, multicellular organisms, cannot be explained solely by reduced efficiency of cellular DNA repair mechanisms (as initially postulated).

Heavy ion space radiation (^{56}Fe) triggers ongoing DNA base damage by downregulating DNA repair pathways in mice *in vivo* (a ground-based, radiation-only study)

S. Suman, P. Jaruga, M. Dizdaroglu, A.J. Fornace, K. Datta. (2020) *Life Sciences in Space Res*. 27:27-32.



Levels of DNA base lesions 2 months after 0.5 Gy of γ-irradiation and 0.5 Gy of ^{56}Fe irradiation with accompanying sham irradiation. A) 8-OH-Gua. B) R-cdG. C) S-cdA.



Radiation exposure altered expression of genes associated with repair of DNA base lesions two months after 0.5 Gy of γ-irradiation and 0.5 Gy of heavy ion irradiation. A) Fold change in the expression of BER pathway genes. B) Fold change in the expression of NER pathway genes. Dotted lines indicate fold change cut off of 1.25-fold. C) Greater decrease of BER and NER repair proteins after iron- relative to γ-irradiation. D) Quantification of immunoblots shows decreased BER and NER proteins after iron irradiation.

- **Measured DNA base modifications in mouse intestine after gamma and heavy ion irradiation of whole animals (assessed BER and NER alterations afterwards)**
- **Increased DNA base modifications and downregulation of BER and NER gene transcripts and protein expression even 2 months after heavy ion irradiation**
- **Radiation downregulated DNA repair pathways in mouse GI system**
- **Implications for chronic gastrointestinal disease risks after heavy ion radiation exposure.**
- **This is a ground-based study, not tested in conjunction with simulated weightlessness (HU) or spaceflight.**

Nitric oxide and oxidative damage-related pathways implicated in some murine responses to simulated microgravity and radiation

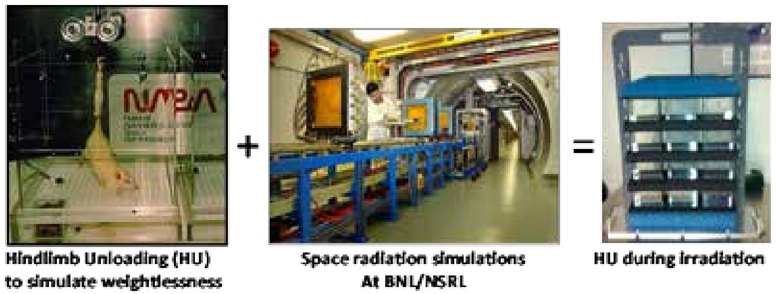
- **Only one rodent experiment has been performed using an external radiation source in space: the Russian Cosmos-690 Mission included groups exposed to relatively high radiation doses (2.2 & 8Gy of ^{137}Cs); findings were inconclusive.**

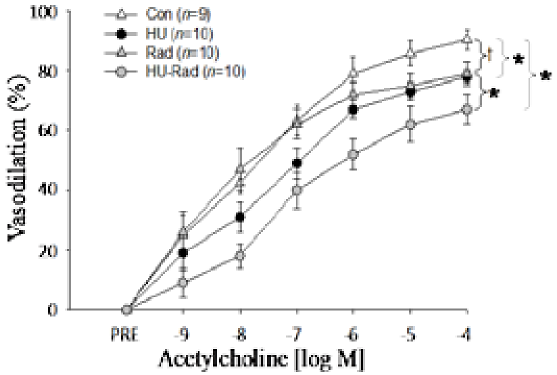
  Portugalov, V.V., E.A. Savina, A.S. Kaplansky, V.I. Yakovleva, G.N. Durnova, A.S. Pankova, V.N. Shvets, E.I. Alekseyev, and P.I. Katunyan, Aviat Space Environ Med, 1977. **48**(1): p. 33-6.

- **Therefore, a ground-based rodent model of hindlimb unloading (HU) is used to determine effects of simulated microgravity, alone or combined with radiation, in vascular, immune, CNS and musculoskeletal systems.**

1. Vascular dysfunction: Heavy ion radiation and simulated weightlessness impairs vascular reactivity in hindlimb blood vessel to greatest extent when combined via a nitric oxide-dependent mechanism.

Ghosh P, Behnke BJ, Stabley JN, Kilar CR, Park Y, Narayanan A, Alwood JS, Shirazi-Fard Y, Schreurs AS, Globus RK, Delp MD. Radiat Res. 2016;185(3):257-66

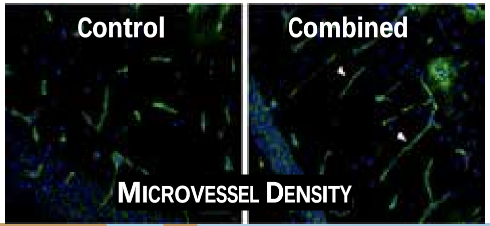




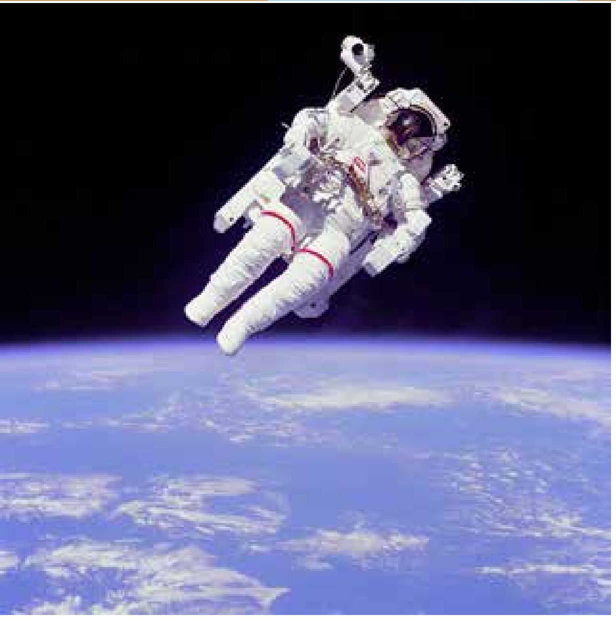
- ^{56}Fe (1 Gy) and simulated microgravity over 2wk each impair peak endothelium-mediated vasodilation in microvessel (PNA), and effect is greatest when combined.
- NO inhibitor abolishes all functional effects of radiation and simulated microgravity.
- Radiation and simulated microgravity differentially regulate oxidative enzyme expression (eNOS, SOD1, XO oxidase).
- Results suggest that the combined challenges of radiation and microgravity during spaceflight impair vasodilator function of resistance arteries mediated by a deficit in NOS signaling.

2. CNS: Simulated microgravity and low-dose/low-dose-rate radiation induce oxidative damage in the mouse brain.

Mao XW, Nishiyama NC, Pecaut MJ, Campbell-Beachler M, Gifford P, Haynes KE, Becronis C, Gridley DS Radiat Res 2016 Jun;185(6):647-57



- ^{57}Co (0.04 Gy at 0.01 cGy/h) combined with simulated weightlessness over 3wk appeared to reduce microvessel length density in cerebral cortex 9 mo. later.
- Greatest effects of ^{57}Co on oxidative damage marker in cortex and hippocampus if radiation and simulated microgravity are combined compared to controls.
- Additional recent evidence for adverse effects of spaceflight, as well as combined simulated weightlessness and radiation, on markers of Blood Brain Barrier, behavior, and oxidative stress in /eye.



Increased chromosomal aberrations previously observed in lymphocytes of astronauts (*in vivo*) after spaceflight

- **Increase in chromosomal aberrations previously seen in astronauts even at low Earth orbits during ISS, Mir and STS (shuttle) missions.**
- **80% or more of organ dose equivalents on the ISS are from galactic cosmic rays (e.g. charged carbon, iron, silicon ions etc.) which are difficult to shield, and will be more abundant as astronauts go beyond the Van Allen Belt**

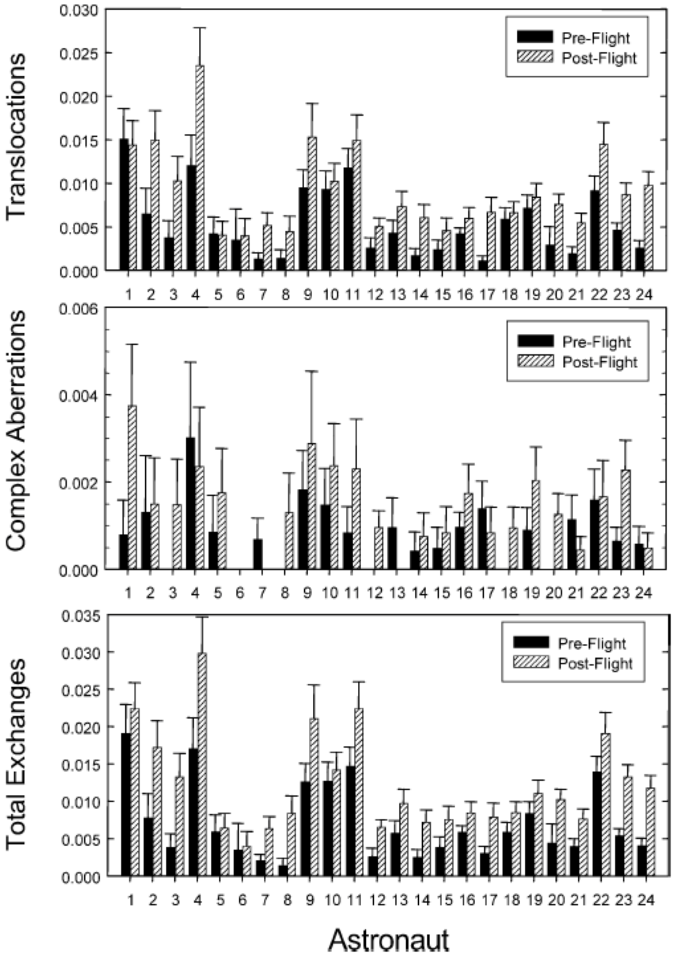


FIG. 4. Frequency of translocations, complex aberrations or total chromosome exchanges measured in each astronaut's blood lymphocytes before and after his or her respective space mission on ISS, Mir or STS. Increases in total exchanges were observed for all astronauts. Translocations (22 of 24) and complex aberrations (17 of 24) were increased in the majority of astronauts.

TABLE 2
Test of Possible Increase in Frequencies of Complex Chromosomal Aberrations or Translocations for Combined Astronaut Samples from ISS Missions or all Mir, STS and ISS Missions using Biodosimetry

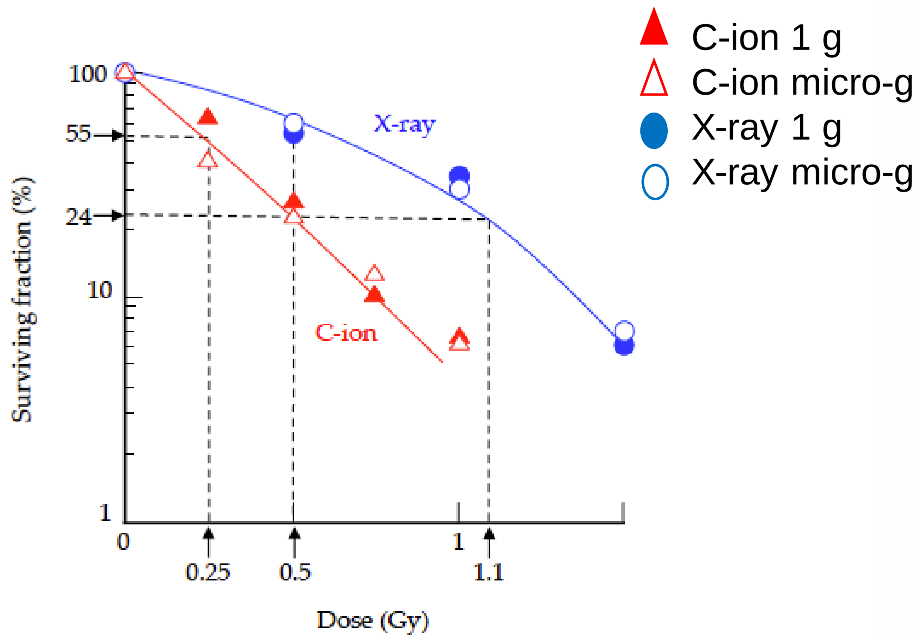
Mission(s)	Cells scored pre-flight	Cells scored post-flight	Aberrations (WGE) pre-flight	Aberrations (WGE) post-flight	Relative number and 95% CL	*P* value
Complex aberrations						
ISS	143,505	164,856	113	143	1.58 [1.26, 1.99]	$<10^{-4}$
ISS, Mir and STS	151,591	188,988	189	248	1.51 [1.23, 1.85]	$<10^{-4}$
Translocations						
ISS	143,505	164,856	636	1181	1.76 [1.60, 1.93]	$<10^{-4}$
ISS, Mir and STS	151,591	188,988	823	1654	1.75 [1.61, 1.90]	$<10^{-4}$

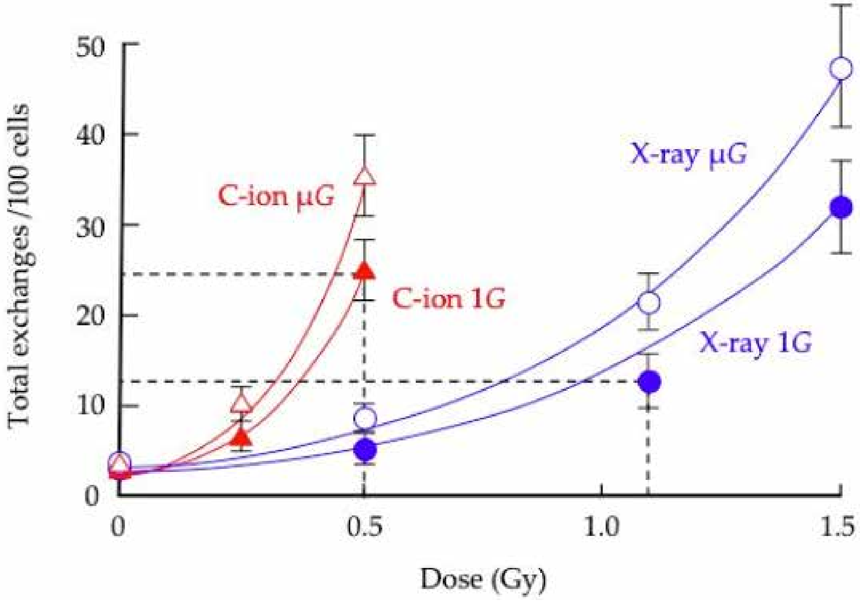
Note. The number of complex aberrations or translocations is represented as whole genome equivalents (WGE) to account for variations in painted chromosomes at different missions.

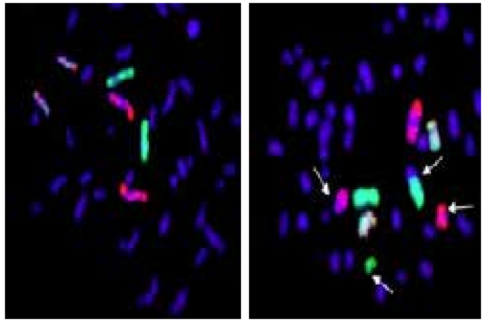
F.A. Cucinotta, M.Y.Kim, V. Willingham, and K.George. (2008) *Rad Res*. 170:127. (Studies in ISS, Mir and STS astronauts)

Cell culture studies suggest that simulated microgravity treatment can increase the frequency of chromosomal aberrations in irradiated human lymphocytes, resembling results seen previously in astronauts

S. Yamanouchi, J. Rhone , J. Mao, K. Fujiwara, P.B. Saganti , A. Takahashi ,* and M. Hada 2.*. 2020. Life **10**: p. 187.







Fluorescence In-situ Hybridization (FISH) visualization of chromosomes in human lymphocytes.

- Radiation exposure decreases cell viability
- Carbon ions are more lethal than x-rays
- Simulated microgravity has no additional effect

- Radiation exposure increases frequency of chromosomal aberrations (CA)
- Carbon ions show more CA than x-rays
- **Simulated microgravity further increases the number of chromosomal aberrations in irradiated samples**

Summary of results from the combination of radiation and microgravity in spaceflight

- Significant effects of radiation observed in many multicellular organisms during spaceflight.
    - Assay choice is important since radiation hits are low probability events.

- Multicellular and embryonic systems, including plants, insects, and other invertebrate and vertebrate animals, can be acutely sensitive to combined effects of reduced gravity and radiation
    - Organism and tissue choices are important to generate sufficient sample size, “n”.
    - Use of in-flight centrifuged 1g controls can help distinguish the effects of radiation from altered gravity

- Spaceflight does not impair DNA repair mechanisms at the cellular level in yeast and bacteria, which appear to function normally in space. However, heavy ion irradiation on Earth causes persistent DNA damage in mice.
    - Therefore, how the combination of microgravity and radiation affects DNA repair may differ in single cell vs multicellular organisms.

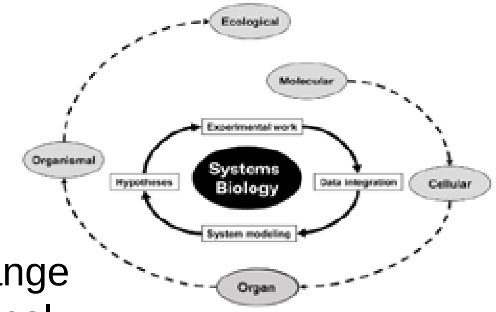
- Human lymphocytes from astronauts show significant increases in chromosome damage even on ISS missions (low Earth orbits), with its relatively low radiation exposures. However, GCR effects are significant and responsible for 80% of the damage at these orbits.

- Possible mechanisms responsible for combinatorial effects of microgravity and radiation on multicellular organisms, such as signal transduction, metabolic/physiological states, oxidative stress, cell migration, intercellular communication, pattern formation, bystander effects, and genomic instability, need further study.

- Higher orbits or interplanetary travel introduce greater radiation exposures, including GCR, and likely also greater effects on all organisms, including humans.
    - Ground based accelerators do not accurately reproduce space radiation in terms of long-term, low dose rate, variability and combination of radiation species.

Therefore, we need to use various organisms amenable to spaceflight experimentation to obtain statistically reliable data on the combined effects of space radiation, altered gravity, and other spaceflight stressors.

Challenges of Interest for the future



New topic areas

- Systems Biology: summing the component parts to create a model for predicting how the system will change over time and under varying environmental conditions. This approach could be applied to multiple biological systems to develop predictive models of the effect of long-duration spaceflight.
- Effects of combined spaceflight stressors: e.g. deep-space radiation and altered gravity

Research Campaign: *Are there research campaigns ($50-100M/yr) to consider?*

Use well-characterized biological model organisms to understand the complex biological consequences of exposure to deep space's unique environment:

- **Approach:** Conduct a sequence of Earth-based, low Earth orbit (LEO), and lunar/cis-lunar investigations, with transit to and on Mars research as the goal. Populations of biological model organisms of increasing complexity will be used to gather data on changes in the underlying responses to space-unique environmental stressors, which do not occur in everyday Earth-based systems. The data from cells, organs, and the whole organism will be compared within populations and across organisms to create a database that ultimately enables predicting physiological responses of humans to the deep space environment.
- Resources for ground-based studies could include ionizing radiation facilities such as NASA's Space Radiation laboratory at Brookhaven National Laboratories, hypergravity facilities, and simulated microgravity platforms. Flight studies could compare results between ground (1g, baseline radiation), the ISS (microgravity, low radiation environment), autonomous free flyers (microgravity, high radiation environment), and lunar surface (1/6g, high radiation environment), in long-duration multigenerational studies with biological model organisms. Appropriate radiation sensors and flight hardware would be developed for free flyers and lunar missions.

Challenges of Interest for the future (continued)

Keystone Capabilities: *Are there large ($50-150M) hardware facilities to consider?*

Ground Facilities: Gravity Radiation PlanEt Simulator (GRAPES)

- Ground facility that replicates conditions on the Moon and Mars to conduct realistic ground studies mimicking the type of environment that organisms (and supporting hardware) will have to endure under those harsh conditions. Large environmental chambers with chronic low dose-rate radiation capabilities, planetary conditions such as light cycle, simulated partial gravity, exposure to lunar/martian dust simulants, and atmospheric conditions (CO_2, O_2, humidity, temperature) as experimental cofactors
- ***Approach***: The implementation sites would be (a) NASA Ames benefitting from their unique centrifuges to simulate gravity as a continuum (simulated reduced gravity to hypergravity) combined with chronic gamma irradiation sources of low-LET low dose-rate ionizing radiation, and (b) a "sister site" could be near NASA Space Radiation Lab at Brookhaven National Lab where similar simulators would be used in conjunction with low dose rate of simulated high-LET cosmic radiation (no hypergravity simulation). (*Implementation of the second site at BNL will require some engineering design. While BNL has made great progress on mixed field exposures, there is not yet any availability of low dose rate exposures, and thus a feasibility question remains to be established for the BNL site. The first site alone would provide a valuable venue for pre-flight investigations in preparation for lunar, Gateway, and deep space missions)*

ISS Facilities:

- **Expand and test automated sampling and analyses tools** within facilities that support habitats with centrifuges, incubators and automated devices for bioprinting, organ-on-a-chip type assays and analyses. Provide conditioned environments with a view to using these in future on the lunar surface, Gateway, free flyers etc.

Lunar Science Facilities:

- **Developing a miniaturized version of the of ISS's science capabilities for use on the Lunar surface:** Multi-organismal facilities with habitats, automated science capabilities, conditioned environments, video cameras, lighting, radiation sensors etc. to test biological systems/organism responses, in the absence or presence of radiation shielding, lunar dust exposure, or 1g centrifugation, etc.

I thank Anthony Hickey, Nicole Rayl, Gioia Massa, Parag Vaishampayan, Ruth Globus, Denise Freeland, Ye Zhang, Howard Levine, Jonathan Galazka, Yasaman Shirazi, April Ronca, Joshua Alwood, Bryan Onate, Aubrie O'Rourke, Raymond Wheeler, Matt Romeyn, Ralph Fritsche, Sylvain Costes, Marianne Sowa, Jared Broddrick, Jessica Lee, Elizabeth Keller, for helping me prepare this presentation. I am grateful to all of them, as well as those who are unnamed here.

THANK YOU!

BACKUP SLIDES

Decadal Survey Initiative

NASA Space Biology Centers have initiated efforts to support the development of Decadal Survey White Papers

Engage our communities to solicit White Paper topics
- Space Biology grant awardees
- GeneLab Analysis Working Groups
- Center for the Utilization of Biological Engineering in Space (CUBES)

Promote and ***Participate*** in the ASGSR activities
- Leading and/or participating in workshops
- Producing materials in preparation for the workshops

Contribute White Papers where we have unique experiences and insight
- multiple orthogonal topics relevant to microbial, plant, and animal research in space
- Programmatic perspectives on the implementation of Research Campaigns
- Flight implementation challenges and opportunities
- CUBESATs and instrument development
- Lunar payloads and science opportunities
- High-performance computing and Open Science
- Education and developing the next generation of Space Biologists