EXPLORE

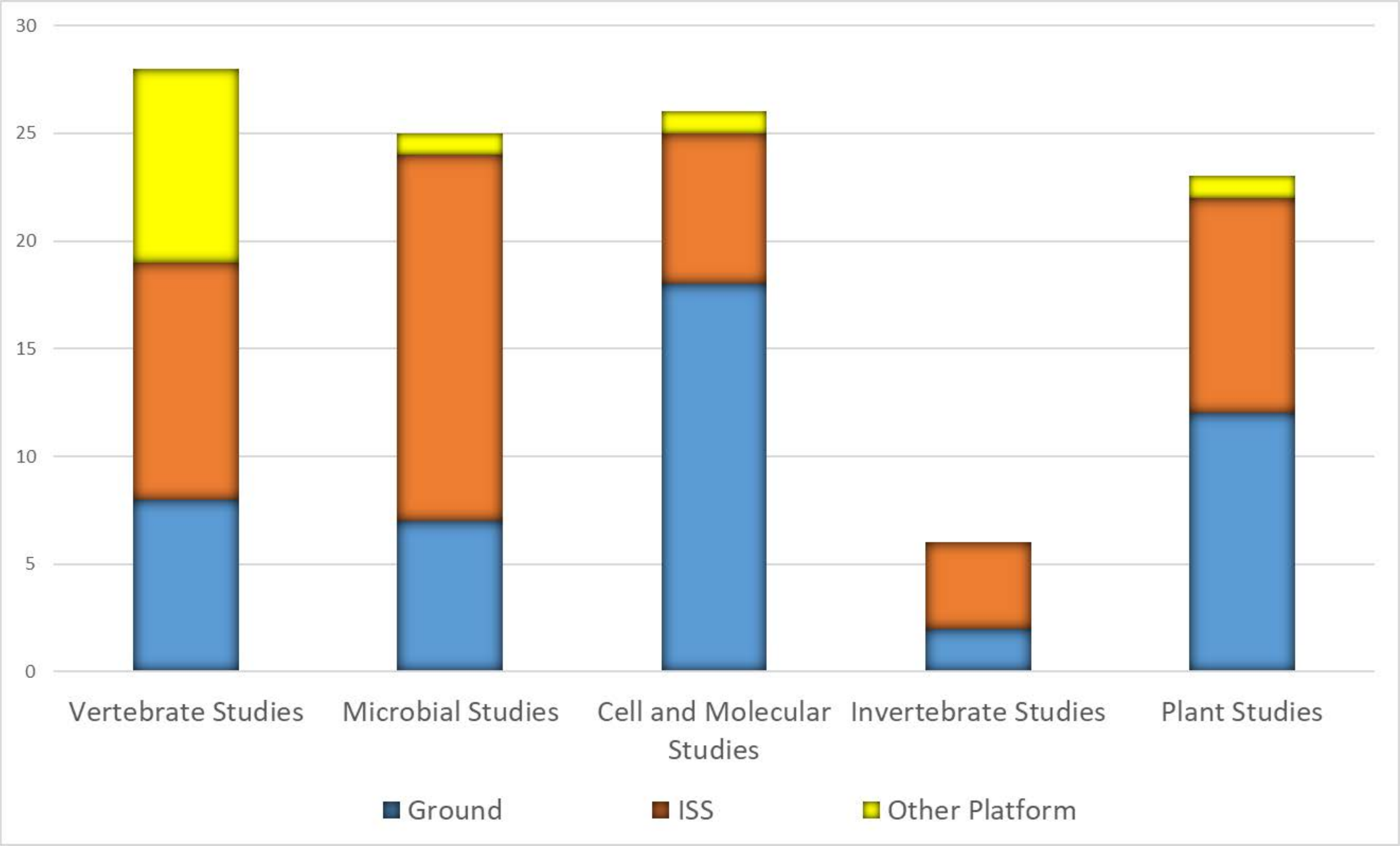
Committee on Biological and Physical Sciences in Space

David L. Tomko, PhD
SLPSRA Space Biology Program Overview and Status
Irvine, CA

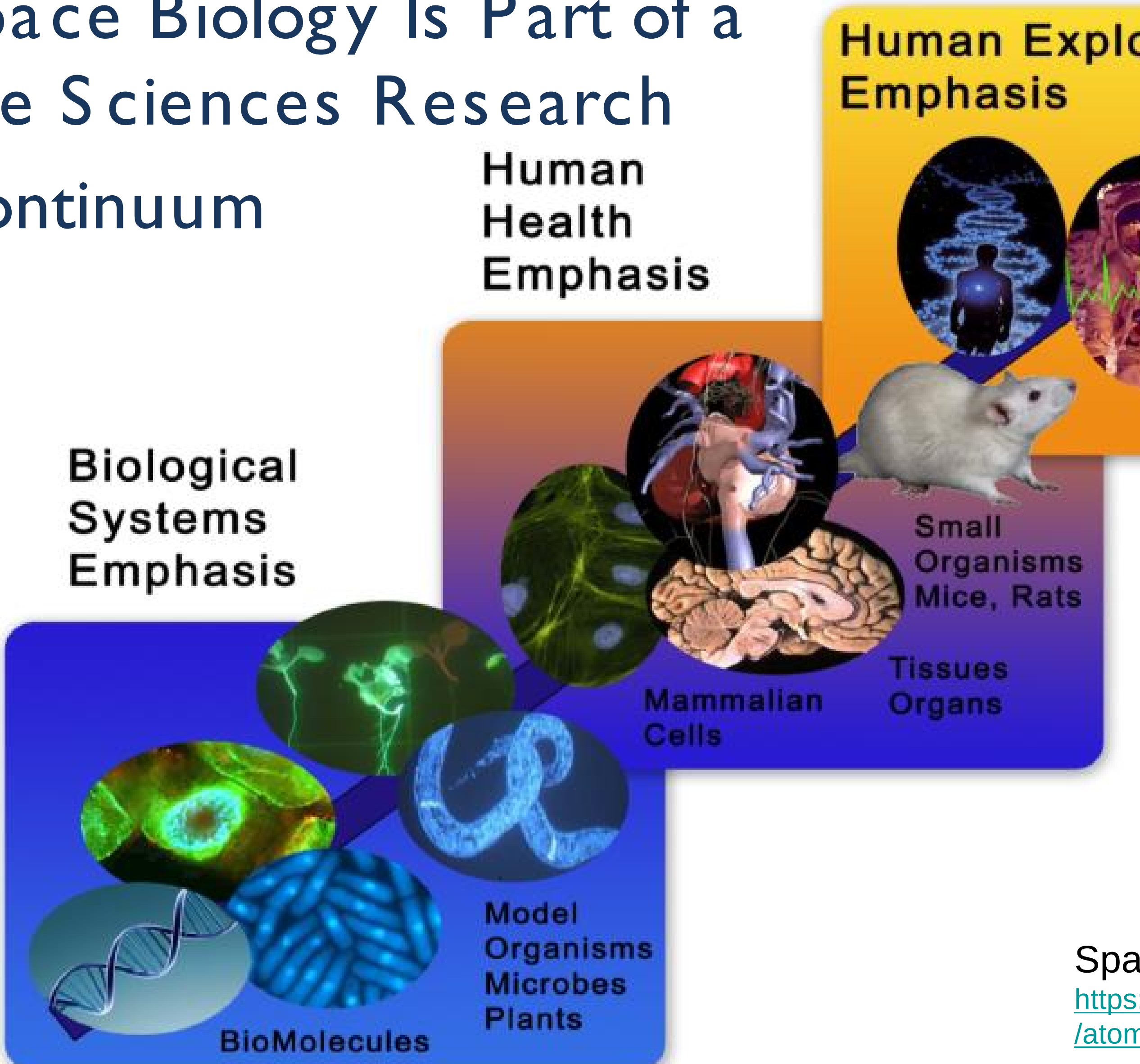
29 October 2019

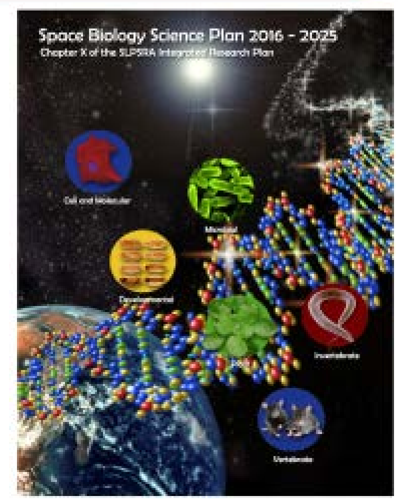
THE BOTTOM LINE

Current Space Biology Funded Tasks



Space Biology Is Part of a Life Sciences Research Continuum





Space Biology Science Plan
https://www.nasa.gov/sites/default/files/atoms/files/16-05-11_sb_plan.pdf

Translational Research by Design: Space Life Sciences at

Space Biology | Basic | Translational Research | Applied | **Human Research**

Fundamental Science

Study how life responds, adapts, develops, interacts and evolves in the space environment and across the gravitational continuum:

- Cell and Molecular Biology
- Microbiology
- Animal Biology
- Plant Biology
- Developmental & Reproductive Biology

Science Exploring the Unknown

Space Biology provides knowledge and collaborates with HRP to reduce risks and develop countermeasures:

- Animal Research
- Cells & Tissues
- Immunology
- Wound healing & fracture repair
- Bone & muscle
- Radiation/micro-g interactions
- Microbiome of the built environment

← *Critical Link!* →

Identify, characterize and mitigate the risks to human health and performance in space:

- Exercise Countermeasures
- Physiological Countermeasures
- Space Radiation Biology
- Behavioral Health and Performance
- Space Human Factors and Habitability
- Exploration Medical Capability
- Environmental Monitoring

Science Addressing The Known Risks

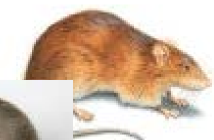
Medical Operations

Space Biology RESEARCH FOR HUMAN EXPLORATION

National Aeronautics and Space Administration

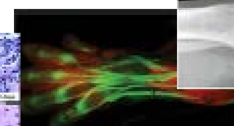
Gravity as A Continuum: Exploration and Knowledge

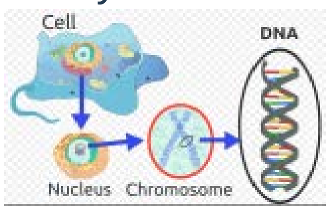
The Gravity Dose Response Curve: Threshold or Continuum?

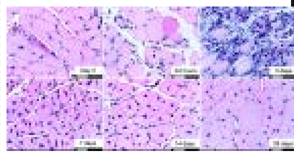


- Gravity induces biological responses at gene expression, cellular, systems and whole organism levels

- Dose response curves of any of these responses are not fully characterized







- Are responses continuous or driven by thresholds?
- Do responses require continuous or intermittent exposures?
- Does the sensitivity/dose response change during development?
- What are Mars and moon gravity level effects?

Gravity as a Continuum ToolBox - ground & flight research on a variety of organisms to define dose response curve & adaptation mechanisms from 0 to >2+g

Ground-based Centrifuges

In-flight Centrifuges (KUBIK, JAXA mouse centrifuge, Free-flyers, MVP)

Suborbital and Parabolic Flight (Fractional and low gravity)

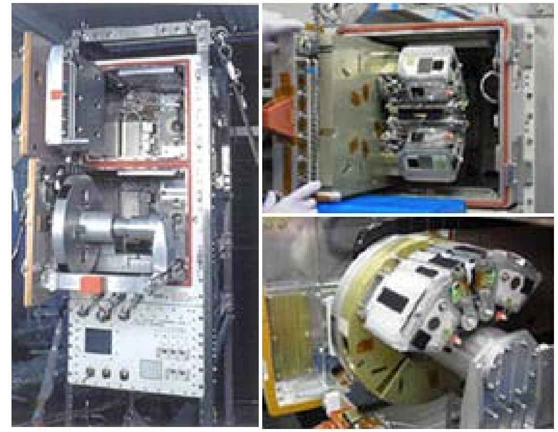
Ground-Based Fractional-G Simulations (Clinostats, RPMs, HARVs, etc)

Partial Unloading in Animals and Humans

Studying the Continuum of Gravity on Earth and on ISS Using Rodents

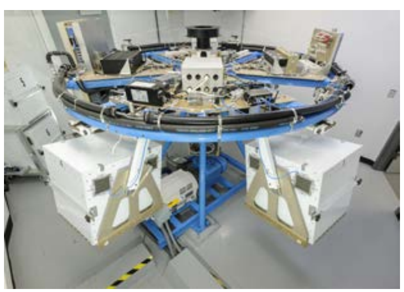
ISS Habitats for Rodents

NASA Rodent Habitat: Microgravity



JAXA Mouse Habitat Unit: Microgravity to 2xg

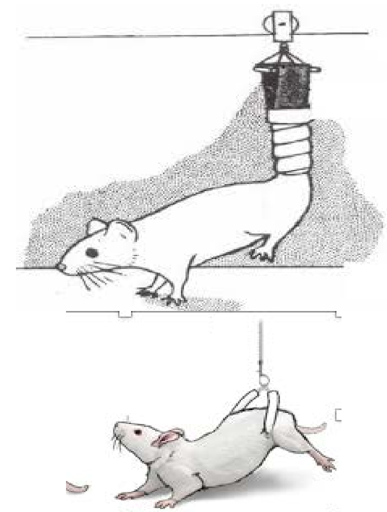
Ground Analogs



NASA ARC 8 ft Centrifuge
1xg to 4xg

Standard Housing
1xg

Partial Weight-Bearing
Horizontal Unloading
> 0xg to <1xg

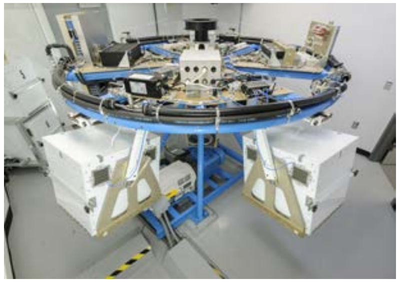


Hindlimb Unloading
Simulated Microgravity

Michael Pecaut
Loma Linda University

A systems-biology approach to assessing the impact of a centrifugation model of spaceflight on cross-system communication

(Team Investigation; Grant# 80NSSC19K1038)

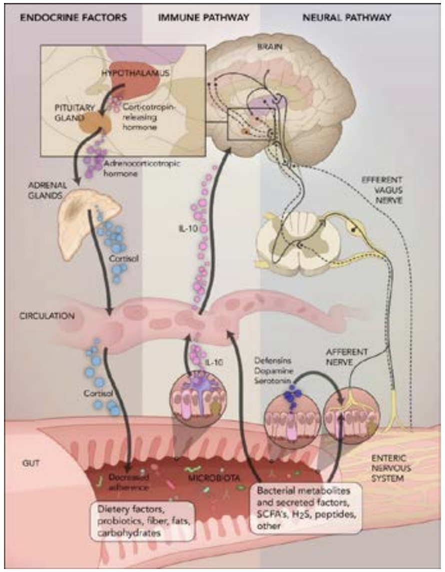


8 ft Diameter Centrifuge
NASA Ames Research Center

Ground-based study to understand how psychological and physiological stress due to hypergravity impacts different physiological systems and activities and cross-systems communication. In addition a probiotic treatment with bacterial species known to be stress protective will be tested as a countermeasure

Specific Aims:

1. Confirm that centrifugation results in detectable changes in gut microbiome, as well as in sympathetic and inflammatory activity, indicative of increased health risk
2. Confirm that prolonged exposure to hypergravity leads to a breakdown in communication between distinct physiological systems, causing decrements in function
3. Determine that centrifugation-induced changes in health status signaling are linked to changes in microbiome



Example of cross-systems communication

Specimen: Mouse; stress protective gut bacteria
Treatment: 1.5xg or 2.0xg for 4 weeks

Image credit: Wellcome Library, London

RODENT RESEARCH
BION

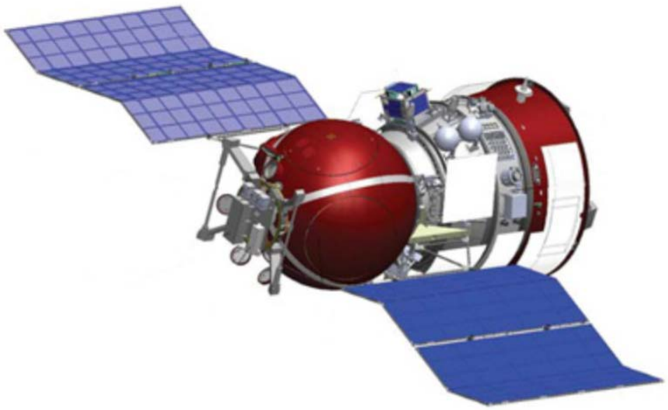
Bion-M2 Free Flyer Mission: Russian/US Collaboration (Appendix C)

Goal: Investigate systemic, cellular and molecular mechanisms underlying adaptation of mice to combined effects of microgravity, cosmic radiation and other spaceflight factors as well as their readaptation on return to Earth.

- Bion-M2 experiments will be performed on space-flown C57BL/6 mice as well as ground controls housed in flight habitats and exposed to a simulated flight environment (and vivarium controls).
- Biosamples will be collected to detect acute spaceflight effects 1) approximately 2-3 hours after landing; 2) 14-17 hours after landing; 3) 3 days and 4) 7 days after landing.

Mission Characteristics

- 2.5 m Sphere used for spaceflight experiments
- 700 kg of payload mass (4 cubic meters) within recoverable module
- 800-1000 km circular orbit for 30 days
- Internal Pressure: 660-960 mm Hg (typically 720-760 mm Hg)
- pO_2: 140-180 mm Hg
- pCO_2: <7 mm Hg (typically <1 mm Hg)
- Relative Humidity: 30-80%
- Capsule Temperature: 18-28C (targets 25 +/- 0.3C
- Average Power: 350+ Watts
- Inclination Angle: 62.8° or 82.3 °
- Period: Approximately 90 minutes
- Flight Duration: 21-60 days



Space Biology Solicitation Activity

NRA NNH16ZTT001N-BION Appendix C: "Solicitation of Proposals for Possible Inclusion in the Russian Bion-M2 Mission" - Released: July 14, 2017

- NASA solicited proposals for Russian Bion-M2 mission experiments in 2020-21. NRA solicited pre-proposals compatible with those to be flown by Russian PI's.
- NASA used a two-phase submission and review process for investigators wishing to submit proposals to this Appendix:
- **34** pre-proposals were submitted by Sept 21, 2017 deadline. Pre-proposals were evaluated by NASA for suitability for inclusion in mission planning.
- 24 Investigators with pre-proposals that satisfied evaluation criteria were invited to submit full proposals on Sept, 09, 2018. Proposals were due Nov, 5, 2018
    - <u>3 Microbiology Investigations</u>
    - <u>1 Animal Biology: Invertebrate Investigation</u>
    - <u>20 Animal Biology: Rodent Investigations</u>
- Phase 2 full proposals underwent scientific merit and technical feasibility review by the NASA Space Biology Program and IPs
- 9 awards were made for rodent investigations

Selections for NRA NNH16ZTT001N-BION/NNH18ZTT002N: "Solicitation of Proposals for Possible Inclusion in the Russian Bion-M2 Mission"

Elizabeth Blaber, Ph.D. NASA ARC (Now RPI)

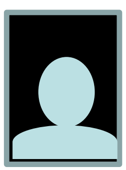
Single cell analysis of bone marrow progenitor & differentiated progeny populations in response to long-duration spaceflight

Mary Bouxsein, Ph.D. Beth Israel Deaconess

The Effects of Spaceflight and Reloading on Skeletal Muscle and Bone

Michael Delp, Ph.D. Florida State

High Altitude Spaceflight on the Bion-M2: Effects on Arterial and Venous Vessels



Lesya Holets, Ph.D. U Kansas Medical Ctr.

The impact of the prolonged LEO on male reproductive health and fertility in mice on the Bion-M2 mission.

Peter Lee, Ph.D. Ohio State

Biomarkers Associated with Spaceflight-induced Cardiac Dysfunction

Selections for NRA NNH16ZTT001N-BION/NNH18ZTT002N: "Solicitation of Proposals for Possible Inclusion in the Russian Bion-M2 Mission" (Cont.)

Xiao Wen Mao, Ph.D. Loma Linda University

Spaceflight-induced Effects on Neurovascular Remodeling & Blood-retina Barrier Function: Role of Oxidative Stress

Kanokporn Rithidech , Ph.D., SUNY Stony Brook,

Proteomic analysis of mouse plasma after space flight with the Bion-M2 Mission

Candice Tahimic, Ph.D., NASA ARC

Re-adaptation after spaceflight: mechanisms and impact on long-term tissue health

Russell Turner, Ph.D., Oregon State "*Time Course for re-adaption of thermoregulation and bone following spaceflight*

RODENT RESEARCH MHU

NASA investigator team from Human Research and Space Biology Programs

ISS flight experiment collaboration between HRP, Space Biology, and JAXA (OP3) using the JAXA Mouse Habitat Unit (MHU) to investigate mouse physiological responses to partial gravity

- Multi-PI integrated objective: Define the relationships between physiological response, adaptation, and gravity for multiple physiological systems and functions: musculoskeletal system, neuromotor performance, central nervous system, circadian rhythm system, metabolism, gastrointestinal (GI) microbiome, GI inflammation, and immune system
- C57BL/6 male mice; ~12-weeks old at launch
- On-orbit centrifugation priorities: microgravity, 0.25xg, 0.5xg, 0.75xg, and 1xg
- Habitat and vivarium ground controls

HARVARD MEDICAL SCHOOL
Beth Israel Deaconess Medical Center

Mary Bouxsein, Ph.D.

Dose-Response Study of Musculoskeletal Outcomes Following Centrifugation in Adult Mice on ISS

Determine the effects of partial gravity on neuromotor performance and bone, muscle and joint structure function, including determining the cellular and molecular mechanisms underlying musculoskeletal and neuromotor responses

UCDAVIS

Charles Fuller, Ph.D.

Adaptation of Mouse Systems Physiology to Artificial Gravity via Centripetal Acceleration: Timing, Metabolism & Aging

Determine the effects of partial gravity on circadian rhythm and associated physiological changes, clock genes, and biomarkers of metabolism and aging

Center for Sleep & Circadian Biology
NORTHWESTERN UNIVERSITY

Martha Vitaterna, Ph.D.

Impact of the Martian Solar Day and Gravity on Microbiota in Mice: Mechanisms and Multi-System Physiology

Determine the relationship between partial gravity, light/dark cycle, the murine gut microbiota, and metabolic functions, including interactions between the gut microbiota and immune and metabolic functions

GROUND-BASED RODENT RESEARCH PARTIAL UNLOADING (FOUR LIMB AND HINDLIMB)

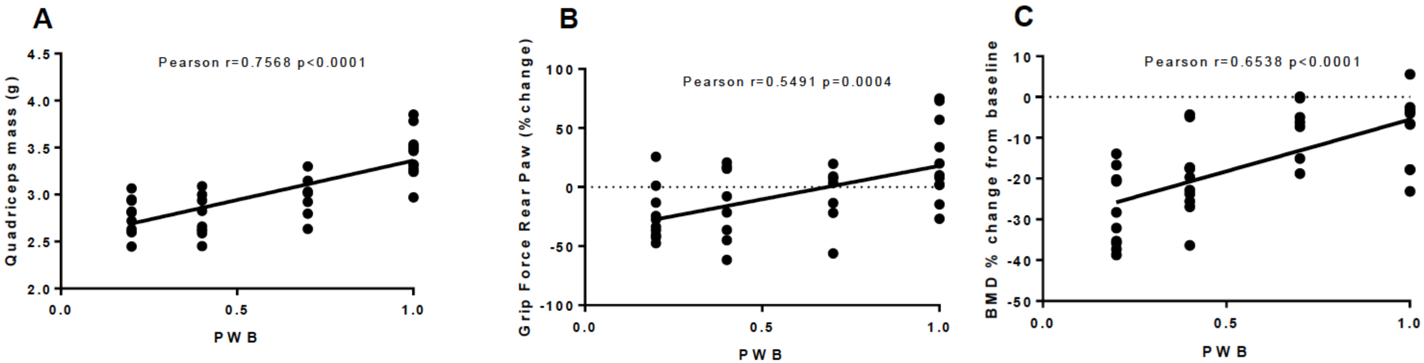
Space Biology / HRP Sponsored Publication

Mortreux, M., Nagy, J.A., Ko, F.C., Bouxsein, M.L.3, Rutkove, S.B. "A novel partial gravity ground-based analogue for rats via quadrupedal unloading" J Appl Physiol. https://doi.org/10.1152/japplphysiol.01083.2017

Dr. Seward Rutkove, MD, Beth Israel Deaconess Medical Center Boston (Harvard Medical School)

- Effects of partial gravity (*i.e.* lunar -0.16g; or Martian -0.38g) on musculoskeletal health are unknown.
- Developed a novel partial gravity analogue (partial unloading) model to use in rats over extended time periods.

Findings: 2 weeks of partial weight bearing at 20, 40, or 70% of normal loading affects musculoskeletal health - decreased trabecular bone density, hind limb muscle mass, and impaired muscle function characterized by grip force.

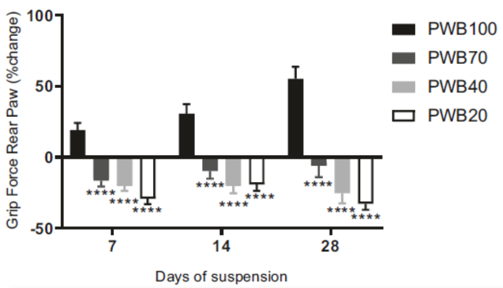


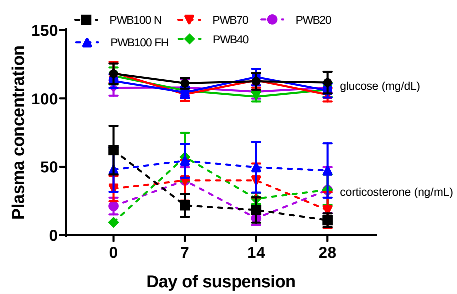
- Musculoskeletal deconditioning severity correlated with degree of partial unloading, indicating that biological responses to varying levels of gravity may occur as a continuum.
- Model will enable analog studies of physiological changes across the body from mechanical unloading of partial gravity and allow countermeasure design/test.

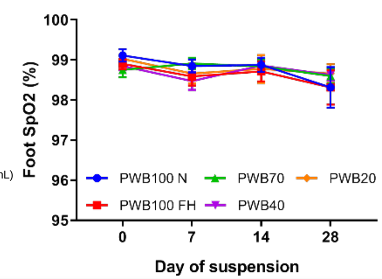
Space Biology / HRP Sponsored Publication

- Developed a novel partial gravity analogue (partial unloading) model to use in rats over extended time periods or to use in association with hindlimb unloading.

Findings: Partial unloading leads to dose-dependent musculoskeletal disuse after 7, 14, and 28 days (Mortreux M. et al., Npj microgravity 2019 Aug 22;5:20)
These findings were not due to or exaggerated by stress or hindlimb perfusion (Mortreux M. et al., Acta Astronautica *Under review*).

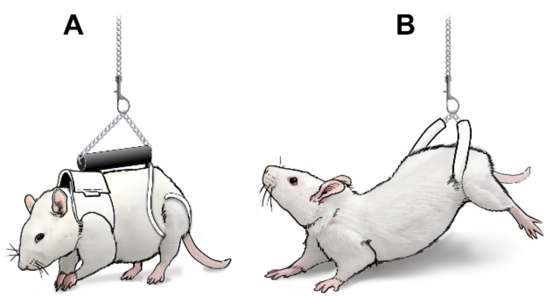


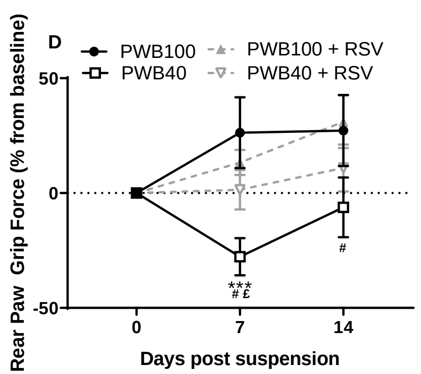


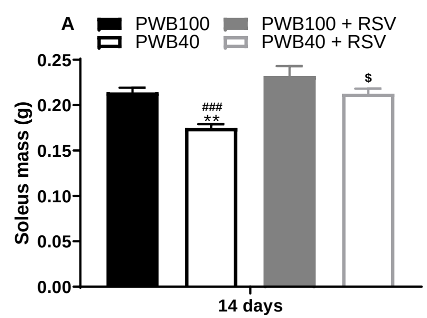


Dr. Marie Mortreux, PhD, Harvard Medical School (Beth Israel Deaconess Medical Center)

- Model already used to mimic a landing on Mars (Mortreux M. et al., J Vis Exp 2019, Apr 4;(146)) and to assess nutraceutical countermeasures (resveratrol) to prevent muscle deconditioning (Mortreux M. et al., Frontiers Physiol 2019 Jul 18;10:899).







New Rat Partial Weight-Bearing Analog Studies: Physiology, Recovery, Countermeasures

Sardar Uddin, Ph.D.
Stony Brook University

Bone remodeling under differential gravitational environments
(Grant# 80NSSC19K0431)

Ground studies to understand bone remodeling in response to partial weight-bearing (PWB), including identifying pathways associated with the partial gravity environment, and to assess if periodic treatment with hypergravity during a 30-day simulated microgravity treatment may mitigate microgravity-induced bone loss

Specific Aims:

1. Determine the effects of simulated microgravity or PWB (1/6xg and 1/3xg) on bone loss and long-term recovery

2. Assess the efficacy of periodic hypergravity (2xg) treatments during long term simulated microgravity treatment as a countermeasure to microgravity-induced short-term and long-term effects on bone integrity
   - 2xg (2x weight of the rat) will be created by adding weights to the to the partial weight-bearing vest

Specimens: 16-week old, skeletally mature Sprague Dawley rats

New Rat Partial Weight-Bearing Analog Studies: Physiology, Recovery, Countermeasures

Michael Delp, Ph.D.
Florida State University

Effects of simulated microgravity and partial unloading on organ systems of the body (Grant# 80NSSC19K1599)

Ground studies to identifying how the adaptation of individual organ systems to changes in gravity (e.g. simulated microgravity and horizontal unloading via partial weight-bearing) may influence other organ systems, as spaceflight leads to systemic, integrative physiological adaptations to weightlessness

Specific Aims:

1) Determine how fluid shifts influence multiple organ system by comparing tissue and organ function and structure following 30-d head-down and horizontal unloading/PWB treatments

2) How these same physiological systems adapt to varying degrees of gravitational loading (e.g., simulated microgravity, Lunar [1/8xg], Mars [3/8xg]and Earth [1xg]) in order to determine whether partial gravity may serve as a potential countermeasure to the effects of microgravity

Specimens: 10-month old male and female Fischer-344 rats
Tissues Systems: arteries, lymphatics, digestive, bone, skeletal muscle, visual, and brain neural systems

New Rat Partial Weight-Bearing Analog Studies: Physiology, Recovery, Countermeasures

Seward Rutkove, Ph.D.
Beth Israel Deaconess Medical Center
Harvard University

Approaching gravity as a continuum: musculoskeletal effects of fractional reloading (Grant# 80NSSC19K1598)

Ground studies that employ both partial weight-bearing and hindlimb unloading models sequentially to investigate gravity as a continuum and its impact on musculoskeletal adaptation to reloading by studying the resulting musculoskeletal alterations in transitioning from 2 weeks of 0xg to 0.2x, 0.4x, and 0.7xg, hypothesizing that there is a dose-dependence to the reloading, including recovery and associated injury; also assess the potential benefit of using these three levels of partial weight-bearing as intermediate steps on the way to transitioning back to 1g.

Specific Aims:

1. To determine the physiological adaptations of the musculoskeletal system in males to the fractional gravity of either the Moon or Mars after experiencing microgravity in transit.
2. To determine the physiological adaptations of the musculoskeletal system in females to the fractional gravity of either the Moon or Mars after experiencing microgravity in transit
3. To investigate the potential musculoskeletal benefits of artificial gravity in-flight before returning to Earth

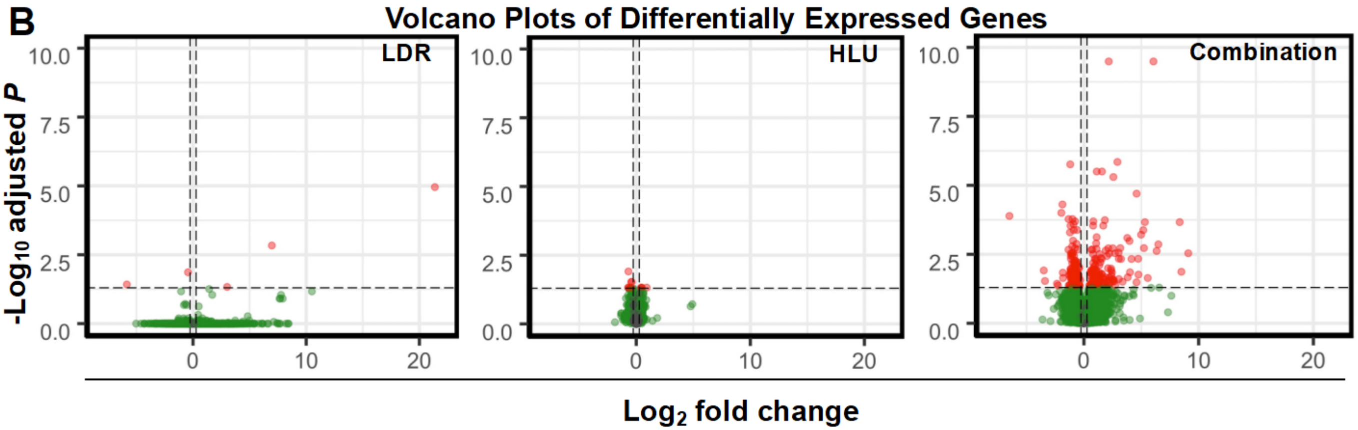
Specimens: 14-week old male and female Wistar rats

Space Biology Sponsored Publication

Overbey EG, Paul AM, da Silveira WA, Tahimic CGT, Reinsch SS, Szewczyk N, Stanbouly S, Wang C, Galazka JM, <u>Mao XW</u>. Mice Exposed to Combined Chronic Low-Dose Irradiation and Modeled Microgravity Develop Long-Term Neurological Sequelae Int J Mol Sci. 2019 Aug 22;20(17). pii: E4094. doi: 10.3390/ijms20174094.

Dr. Xiao Mao, MD, Loma Linda School of Medicine

- Combined unloading (simulating microgravity exposure) and low dose irradiation (simulating radiation exposure during spaceflight) lead to heighted transcriptional changes in the brain as compared to single exposures
- Changes included reduced transcriptional machinery, increased neurogenesis and neuropeptide production, and dysregulated cell structure and signaling genes.



- Brain-related transcriptional changes are dynamic and plastic during readaptation from exposure to spaceflight-like conditions.
- While these exposures may lead to long-term neurological consequences, the active processes of neuroplasticity and repair are engaged to maintain neural homeostasis.

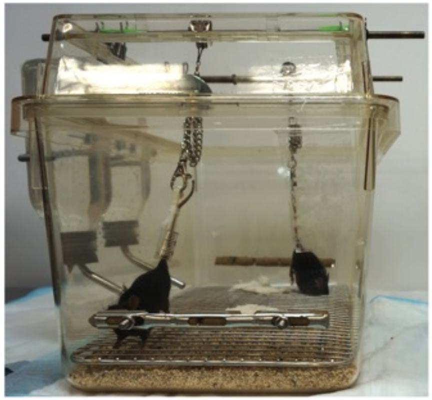
Space Biology Sponsored Publication

Tahimic CGT, Paul AM, Schreurs A, Torres S, Rubinstein L, Steczina S, Lowe M, Bhattacharya S, Alwood JS, Ronca A, Globus RK. Influence of social isolation during prolonged simulated weightlessness by hindlimb unloading. Front in Phys. (Accepted). doi: 10.3389/fphys.2019.01147.

- HU animals in both single and social housing displayed expected musculoskeletal deficits
- Some immune and HPA axis responses differentially impacted by social environment:
    - Decreased CD4+ T cells in single but not social housed HU mice; seen in immune suppressed humans
    - Adrenal weights higher in single housed mice (indicates stress response)
    - Refinement made to standard NASA Ames HU model allows for social housing in pairs

Dr. Ruth Globus, Ph.D
NASA Ames Research Center

(B) Social housing HU



(A) Single housing HU

RODENT RESEARCH ON ISS

Spaceflight aboard ISS and the Space Shuttle (STS-135), and hind limb unloading results in similar arthritic responses in male and female mice

Jeffrey Willey, PhD
Wake Forest School of Medicine

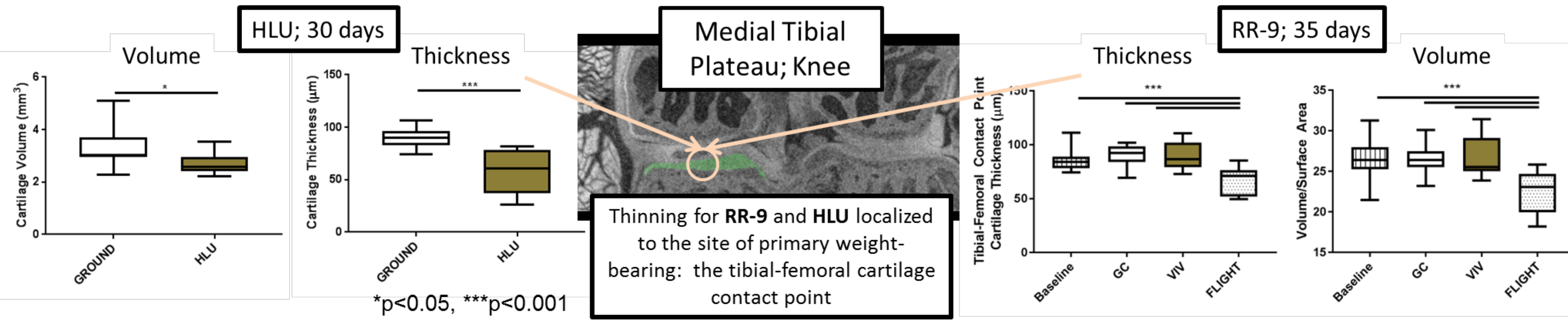
Damage to the articular cartilage and menisci that maintain the health of mouse knees were assessed after:

~35 days aboard ISS (**RR-9; male).** NASA NNX15AB50G

13 days aboard Space Shuttle Atlantis (**STS-135; female).** NASA Ames BSP

30 days of hind limb unloading (**HLU, male and female).** NASA NNX15AB50G

Note: RR-9 involved huts

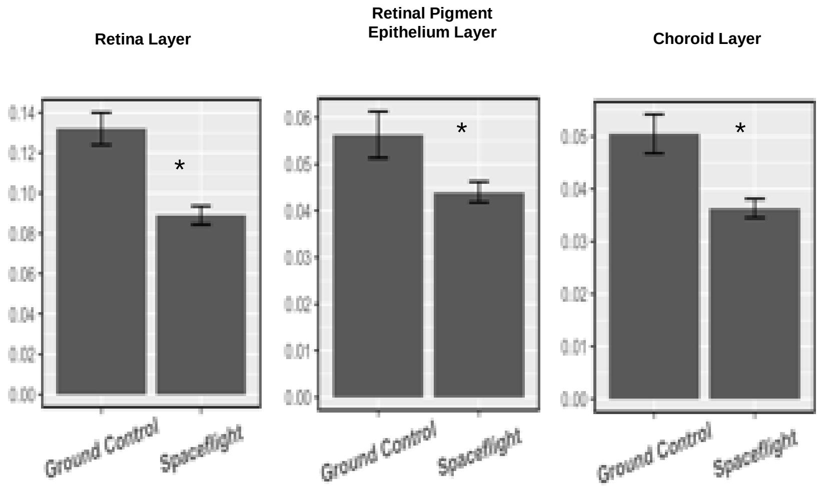


*p<0.05, ***p<0.001

- Articular cartilage lost volume (**RR-9, HLU**); thinning occurred at area of highest weight bearing
- Menisci lost volume (**RR-9, HLU**) and lost similar matrix proteins (**RR-9, HLU, STS-135**), across sexes
- Pathways indicating increased oxidative stress, decreased matrix formation, and arthritis identified within knee and hip soft tissues from FLIGHT and HLU mice (decreased EIF2, NRF2, mTOR; **RR-9, HLU**).
- Reduced weight-bearing in mice from spaceflight and HLU resulted arthritic knee and hip responses, occurring spatially at similar locations in the knee and with similar, catabolic molecular pathways

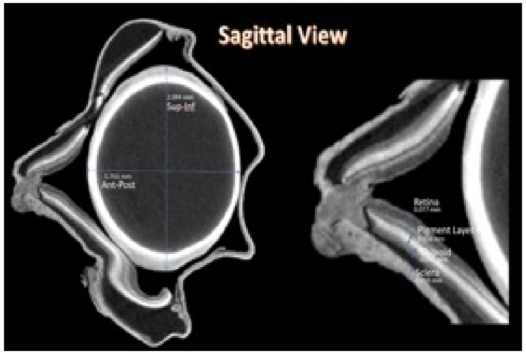
Space Biology Sponsored Publication

Overbey EG, da Silveira WA, Stanbouly S, Nishiyama NC, Torres GR, Pecaut MJ, Zawieja D, Wang C, Willey J, Delp M, Hardiman G, Mao XW. Spaceflight influences gene expression, photoreceptor integrity, and oxidative stress-related damage in the murine retina. Sci Rep. 2019 Sep 16;9(1):13304. doi: 10.1038/s41598-019-49453-x.



Dr. Xiao Mao, MD, Loma Linda School of Medicine

- Retinal performance may decrease over extended periods of spaceflight and cause visual impairment.
- This data will also be useful for understanding other spaceflight related health concerns, such as disruption of circadian rhythm and accelerated aging.



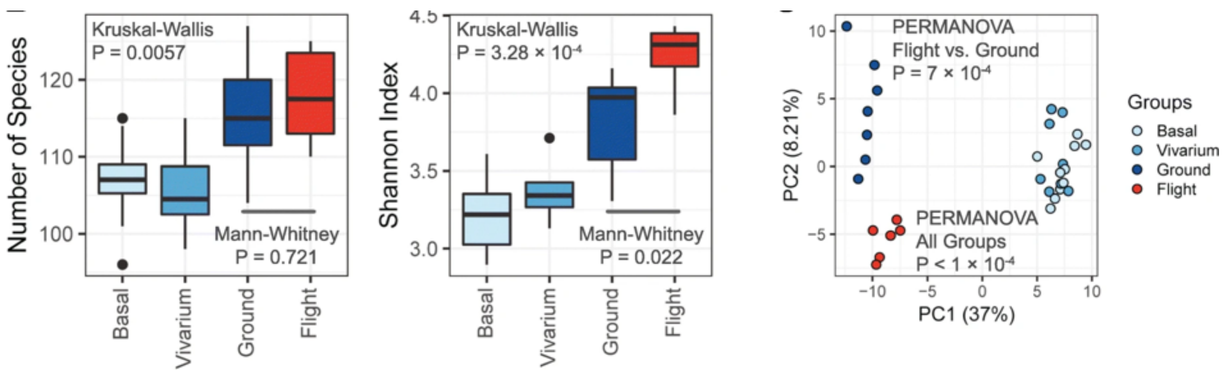
Sagittal view of of a ground control mouse.

Space Biology Sponsored Publication

Jiang P, Green SJ, Chlipala GE, Turek FW, Vitaterna MH. Reproducible changes in the gut microbiome suggest a shift in microbial and host metabolism during spaceflight. Microbiome. 2019 Aug 9;7(1):113. doi: 10.1186/s40168-019-0724-4.

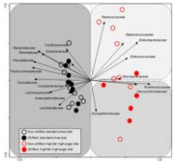
Dr. Martha Vitaterna, Ph.D
Northwestern University

- Spaceflight environment elevated microbial diversity and altered the gut microbial community structure
- Effects of spaceflight on the gut microbiome were reproducible.



- The effect of spaceflight on the gut microbiome appears to be attributed to specific space environmental factors, likely microgravity, rather than radiation.
- Taxon abundance was significantly altered by spaceflight.
- Identified host genes expressed in the RR1 liver (obtained through publicly available GeneLab database) that were concordantly altered with the inferred gut microbial gene content

Rodent Research-7 Biospecimen Sharing Program Overview

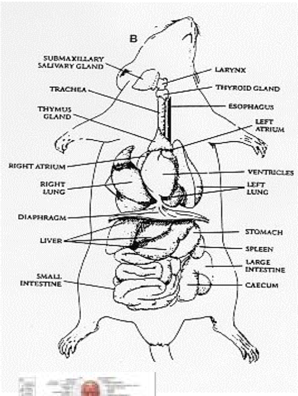


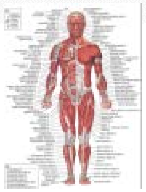
The Rodent Research (RR)-7 Biospecimen Sharing Program (BSP) dissections were successfully completed in February 2019. The Fred Turek lab (Center for Sleep and Circadian Biology at Northwestern University) hosted the BSP team during the historically cold, Polar Vortex that hit the Midwest.

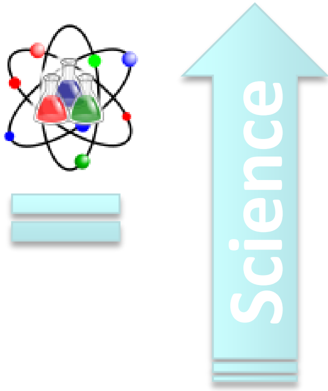
As part of a directed-BSP, the Principal Investigator (PI) and BSP teams performed an integrated dissection to collect over **20 tissues** each from 80 RNAlater-preserved, frozen carcasses (~1800 vials). 6 tissue types were collected by the PI team for further analysis related to the primary investigation, 12 tissue types were stored in ALSDA, and 2 tissue types were provided to GeneLab.

PI: Dr. Fred Turek (Northwestern Univ.)

Co-Is: Dr. Martha Vitaterna (Northwestern Univ.), Dr. Stefan Green (Univ. of Illinois- Chicago), Dr. Ali Keshavarzian (Rush Univ. Medical Center), Dr. Christopher Forsyth (Rush Univ. Medical Center)







SCHEDULE	ACTIVITY
Mar. 2018	✓ BSP Tissue List identified
Jan. 17, 2019	✓ On-orbit carcasses from SpX16 shipped to PI lab
Jan. 28-29, 2019	✓ Integrated practice dissection with PI & BSP teams
Jan. 31- Feb 6, '19	✓ Frozen carcass BSP dissections completed
Feb. 12, 2019	✓ Sample shipment received at ARC & stored in ALSDA

Picture: Members of PI and BSP teams at Northwestern University in Evanston, Illinois.

Space Biology Rodent Research on ISS

PI	Year Solicited	Title	RR	Flight
Turek	2014	Effects of Spaceflight on Gastrointestinal Microbiota in Mice: Mechanisms and Impact on Multi-System Physiology	RR-7	SpX-15 2018
Delp	2012	Simulated Microgravity-Induced Systemic Inflammation and Its Impact on Circulatory Function and Structure	RR-9	SpX-12 2017
Willey	2014	Space flight environment induces remodeling of vascular network and glia-vascular communication in mouse retina	RR-9	SpX-12 2017
Mao	2014	Space flight environment induces remodeling of vascular network and glia-vascular communication in mouse retina* Flight 1	RR-9	SpX-12 2017
Pecaut	2012	Impact of Spaceflight on Primary and Secondary Antibody Responses	RR-12	NG-11 2019
Mao	2014	Space flight environment induces remodeling of vascular network and glia-vascular communication in mouse retina* Flight 2	RR-18	SpX- 21 2020
Almeida	2014	Role of P21/CDKN1a Pathway in Microgravity-Induced Bone Tissue Regenerative Arrest - A Spaceflight Study of Transgenic P21/CDKN1a Null Mice in Microgravity	RR-10	NG-14 2020
Taylor (replaced Robbins)	2014	Vascular Health in Space: MicroRNAs in Microgravity	RR-11	TBD
Robling	2014	Foundational In Vivo Experiments on Osteocyte Biology in Space	RR-15	TBD
Tahimic (Globus retired)	2014	Free Radical Theory	TBD	TBD
Christenson (Tash retired)	2014	Female reproductive health: Space flight induced ovarian and estrogen signaling dysfunction, adaptation, and recovery	TBD	TBD

MICROBIOLOGY/MOBE STUDIES

Study of the presence of microbial populations on the International Space Station (ISS)

Crystal Jaing, Ph.D.
Lawrence Berkeley National Laboratories

International Space Station – Microbial Observatory of Pathogenic Virus, Bacteria, and Fungi (ISS-MOP) Project (Grant# 80NSSC18K0113)
Spaceflight study that will generate the microbial censuses associated with various ISS modules using traditional culture-based methods and state-of-the art molecular techniques. The objectives are to measure presence of viral and select bacterial and fungal pathogens and correlate their presence on crew, including periods when the crew changes to different astronauts.

Specific Aims:

1. Collect samples from ISS surfaces and air and from the crew to perform culture-based analyses of viruses and microbial pathogens associated with the ISS habitat.
2. Investigate the culture-independent molecular viral community and select microbial pathogens associated with ISS habitat
3. Establish an ISS-MOP data repository

Specimens: Multiple microorganisms.

Publications

Urbaniak C, Sielaff AC, Frey KG, Allen JE, Singh N, **Jaing C**, Wheeler K, Venkateswaran K. Detection of antimicrobial resistance genes associated with the International Space Station environmental surfaces. 2018 Sci Rep. 2018 16;8(1):814.

Venkateswaran K, Checinska Sielaff A, Ratnayake S, Pope RK, Blank TE, Stepanov VG, Fox GE, van Tongeren SP, Torres C, Allen J, **Jaing C**, Pierson D, Perry J, Koren S, Phillippy A, Klubnik J, Treangen TJ, Rosovitz MJ, Bergman NH. Draft genome sequences from a novel clade of Bacillus cereus Sensu Lato strains, isolated from the International Space Station. 2017 Genome Announc. 10;5(32):e00680-17

Be NA, Avila-Herrera A, Allen JE, Singh N, Checinska Sielaff A, **Jaing C**, Venkateswaran K. Whole metagenome profiles of particulates collected from the International Space Station. 2017 Microbiome 1;5(1):111

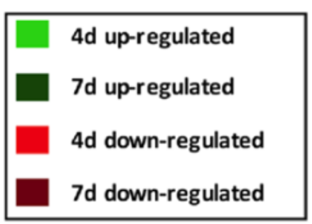
Space Biology Sponsored Publication

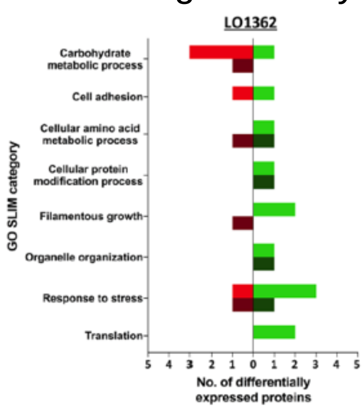
Romsdahl J, Blachowicz A, Chiang AJ, Chiang Y, Masonjones S, Yaegashi J, Countryman S, Karouia F, Klakum M, Stajich JE, Venkateswaran K, <u>Wang CCC</u>. International Space Station conditions alter genomics, proteomics, and metabolomics in *Aspergillus nidulans*

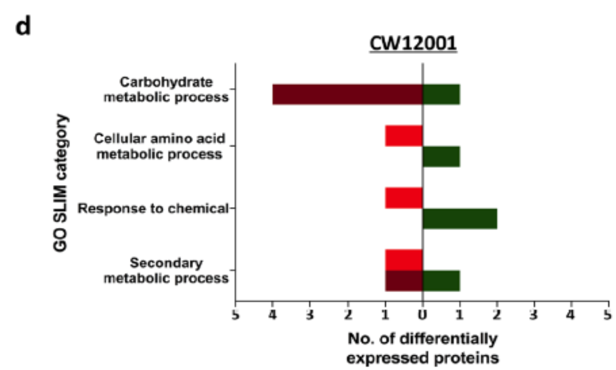
Appl Microbiol Biotechnol. 2018 Dec 12;103:1363-1377. doi:10.1007/s00253-018-9525-0.

- Small proportion of proteins are differentially expressed in ISS-grown *A. nidulans* samples, which emphasizes potential of *A. nidulans* as a therapeutic production host during long-term space travel.

- Spaceflight increased production of asperthecin, an anthraquinone pigment, in mutant *A. nidulans* strains, supporting previous studies that pigment production is a key adaptive response of fungi and may serve as a protective mechanism from radiation.



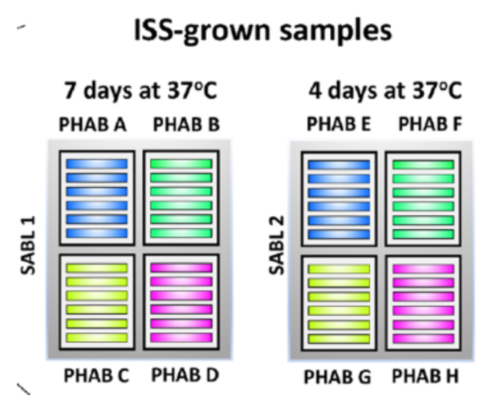




- Whole genome sequencing revealed that specific genetic missense mutations are involved in the adaptive mechanisms of fungi.

- Differential expression of proteins involved in oxidative stress response, carbohydrate metabolic processes, and secondary metabolite biosynthesis were observed in *A. nidulans*.

Dr. Clay C. C. Wang, Ph.D., University of Southern California



Study of the fundamental understanding of microorganisms

John Hogan, Ph.D.
NASA Ames Research Center

Investigating the Physiology and Fitness of an Exoelectrogenic Microorganism Under Microgravity Conditions (Grant# internal)

Spaceflight study to investigate the effects of spaceflight and microgravity on the physiology of a model exoelectrogen, Shewanella oneidensis MR-1, using a combination of genetic, biochemical, and "omic" approaches. The study seeks to gain further understanding of how the space environment affects MR-1 extracellular electron transport and biofilm development, molecular processes crucial for microbial-assisted Bio-Electrochemical Systems (BES).

Specific Aims:

1. Measure S. oneidensis MR-1 extracellular electron transport (EET) rates and determine the EET mechanism(s) impacted by microgravity using biochemical and genetic approaches.
2. Characterize and quantify S. oneidensis MR-1 biofilm development under microgravity conditions using confocal laser scanning microscopy (CLSM)
3. Identify other key molecular and genetic components for biofilm formation and extracellular electron transport that are affected by microgravity by using genome-wide gene expression and mutant fitness profiling

Specimens: *Shewanella oneidensis*

"Spaceflight and simulated microgravity conditions increase virulence of *Serratia marcescens* in the *Drosophila melanogaster* infection model." Rachel Gilbert, Medaya Torres, Rachel Clemens, Shannon Hateley, Ravikumar Hosamani, William Wade, Sharmila Bhattacharya. *Nature Microgravity, accepted 09/2019 pending minor revisions.*

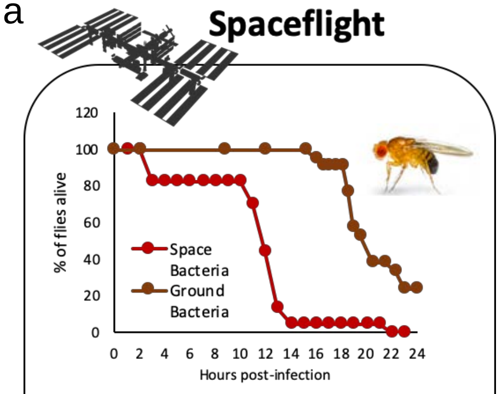
A human bacterial pathogen (*Serratia marcescens*) grown on the International Space Station for 5 days killed fruit flies significantly faster than bacteria that was grown on the ground (Figure a).

This increase in virulence was abrogated when the same spaceflight-grown bacteria was brought back to Earth and allowed to grow under normal ground conditions.

This reversible increase in virulence could be recapitulated in the ground-based rotating wall vessel (RWV) device, which keeps the cells in constant freefall in a liquid media and simulates a microgravity environment (Figure b).

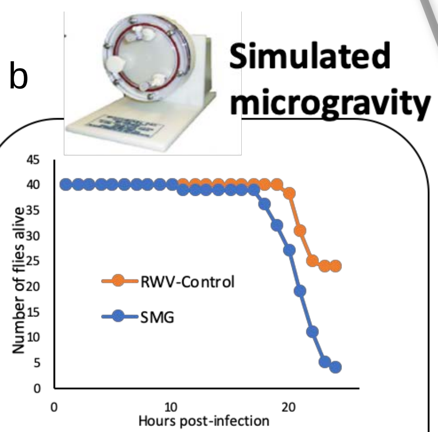
This ground RWV model allowed us to dissect the underlying molecular pathways and to discover a novel mechanism by which microgravity-altered changes in nutrient metabolism are causing the transient increase in growth and virulence of the bacteria. This will be published in a follow-up paper.

a



Spaceflight-exposed *Serratia marcescens* killed flies faster than ground control bacteria (P<0.0001)

b



Ground-based studies with simulated microgravity (SMG) reared *Serratia marcescens* also killed flies faster than control (P=0.0002)

Sharmila Bhattacharya
NASA Ames Rsch Center

Rachel Gilbert

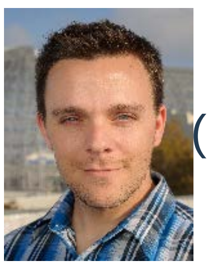
Discovery of mechanisms underlying increased bacterial virulence of *Serratia marcescens* in altered gravity conditions using *Drosophila* host model

Microbiology of the Built Environment (MoBE) Fellows

- Selected from Sloan/NASA NRA NNH16ZTT001N-MOBE
- 2-year fellowships

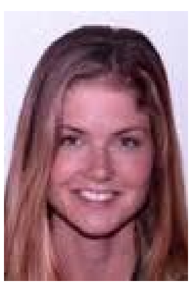
Noelle Bryan, Ph.D.
(M. Zubar/C. Carr, MIT)
Start Date: 9/13/17
Complete

Genomic and functional analysis of biofilm morphotypes of International Space Station isolated Staphylococcus epidermidis and their pathogenicity in *Caenorhabditis elegans*



Michael Lee, Ph.D.
(C. Everroad, NASA ARC)
Start Date: 9/13/18
2nd Year

Microbial evolution and transmission aboard the ISS: inferring mutation rates, assessing pangenomes, and tracking microbiome transmission between astronauts and the space-based built environment



Aubrie O'Rourke,Ph.D.
(C. Dupont, J. Craig Venter Institute)
Start Date: 6/6/17
Complete

Virulence and Drug Resistance of *Burkholderia* species Isolated from ISS Potable Water Systems

PLANT BIOLOGY RESEARCH

PI: Rob Ferl
Univ. of Florida

Cosmic Ray Exposure Sequencing Science (CRESS)
Califar,Tucker, Cromie, Sng, Schmitz, Callaham, Barbazuk, Paul, Ferl "Approaches for Surveying Cosmic Radiation Damage in Large Populations of *Arabidopsis thaliana* Seeds – Antarctic Balloons and Particle Beams." *Gravitation and Space Research, Volume 6 (2) December 2018.*

- Proof of concept experiment to assess genomic impact of space radiation on seeds.
- Secondary payload for December 2016 high-altitude (36-40 km), 30 day south polar balloon flight carrying the BACCUS (Boron and Carbon Cosmic Rays in the Upper Stratosphere) experiment.
- Studied biological effects of Galactic Cosmic Radiation (GCR), - High-Z and Energy (HZE) ions
- Used *Arabidopsis thaliana* seeds and compared to a simulation of GCR at Brookhaven National Laboratory (BNL) and to lab control seeds.
- CRESS was designed to 1U CubeSat specifications ($10cm^3$, ≤1.33 kg), 1 atm internal pressure, containing 580,000 seeds and 12 CR-39 Solid-State Nuclear Track Detectors (SSNTDs).
- Exposed BNL and ANT Generation 1 (M_0) seeds showed significantly reduced germination rates & elevated somatic mutation rates compared to non-irradiated controls - BNL mutation rate higher than ANT.
- Plant genomic DNA with distinct aberrant phenotypes was evaluated with whole-genome sequencing, which revealed an array of structural genome variants in the M_0 and Generation 2 (M_1) plants.
- This was the 1st whole-genome characterization of space-irradiated seeds & demonstrated efficiency & efficacy of Antarctic long-duration balloons for the study of space radiation effects on eukaryotes.

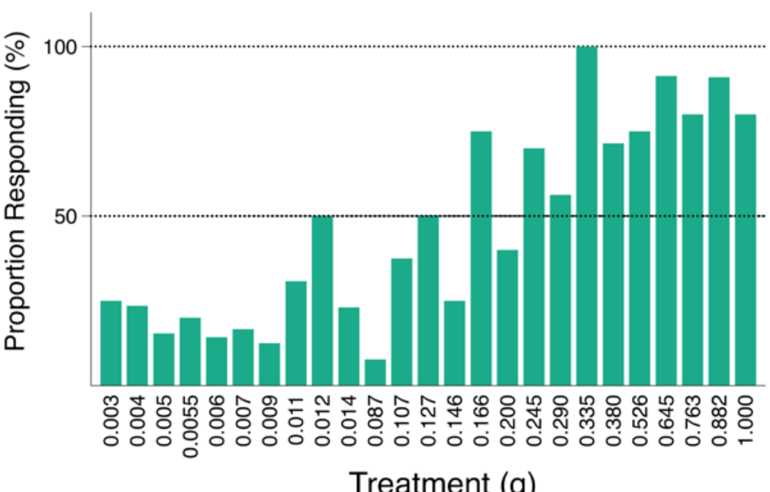
Characterizing Plant Gravity Perception Systems – ISS experiment conducted January through April 2018

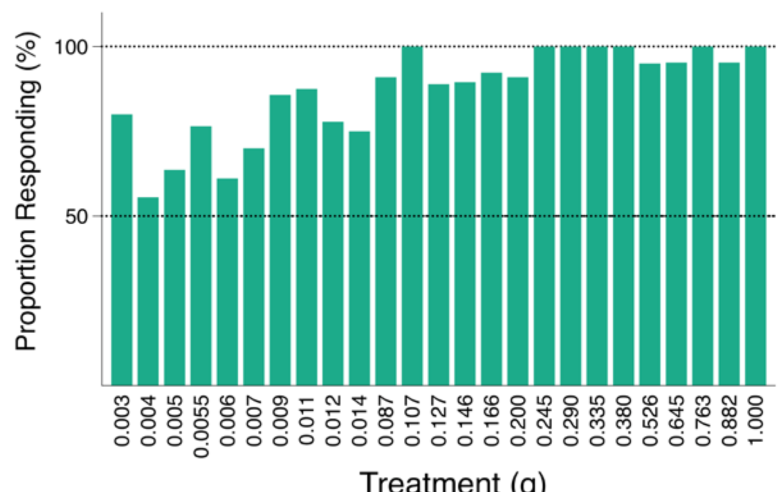
- Overall Objective: Test the effects of gravity as a continuous variable on plant growth using wild-type and starchless mutants, which lack a key component of gravity sensing.
- Used the EMCS centrifuge on the ISS to apply a range of accelerations from 0.003 g up to 1.0 g while collecting images for morphometric analyses; plant tissue was frozen at -80º C on board and returned for analysis of gene expression changes across continuum.

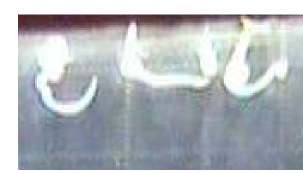
Key Findings: Majority of WT roots responded to ≥ 0.003 g of acceleration, while starchless mutants showed >50% response rate only at or above 0.2 g. Statolith sedimentation system is key for maximum gravity perception.

In Progress: RNAseq study with organ-level resolution across treatment range to identify genes differentially regulated by gravity.

Chris Wolverton, Ph.D.
Professor of Botany,
Ohio Wesleyan Univ.



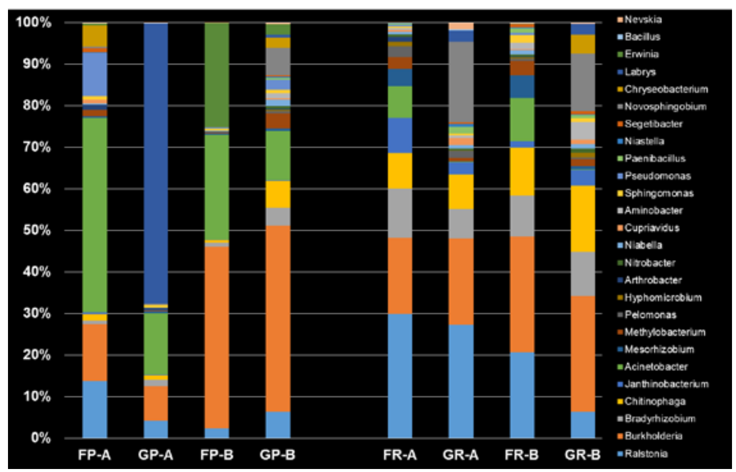




Space Biology Sponsored Publication

Khodadad, C.L.M., Hummerick, M.E., Spencer, L.E., Dixit, A.R., Richards, J.T., Romeyn, M.W., Smith, T.M., Wheeler, R.M., Massa, G.D. “Microbiological and nutritional analysis of lettuce crops grown on ISS indicates a safe and nutritious supplement to astronauts’ diet” Frontiers in Plant Sci. Submitted, 2019.

- *In situ* production of safe, nutritious food to supplement the packaged diet may be needed for astronaut health on future deep space exploration missions, and ISS is a testbed to demonstrate this capability.
- Veggie plant growth chambers have been tested on ISS with leafy crops.
- Findings: Red romaine lettuce was successfully grown in three tests in Veggie with two different harvest methods, and yields were comparable to growth on Earth.
- Human pathogens were not detected in flight grown plants, but there were some differences in total microbial counts and community structure between tests and environments.
- Some measured nutrients showed different levels between tests, but anthocyanins and antioxidant capacity were the same.



- Leafy vegetable crops, like red romaine lettuce and red Russian kale, are good candidates to supplement astronaut diets with fresh nutritious food.

Dr. Gioia Massa, NASA, KSC

COLLABORATIONS AND PARTNERSHIPS

Space Biology and HRP

Active Space Biology and HRP jointly funded projects:

"Pick-and-Eat Salad-Crop Productivity, Nutritional Value, and Acceptability to Supplement the ISS Food System"; Giola Massa (NASA Kennedy Space Center), Co-Is: Grace Douglas, Raymond Wheeler, *et al.*

"The Integrated Impact of Diet on Human Immune Response, the Gut Microbiota, and Nutritional Status During Adaptation to Spaceflight"; Grace Douglas (NASA Ames Research Center), Team: Brian Crucian, Hernan Lorenzi, *et al*.

"International Space Station – Microbial Observatory of Pathogenic Virus, Bacteria, and Fungi (ISS-MOP) Project"; Crystal Jaing (Lawrence Livermore) Team: Satish Mehta, Kasthuri Venkateswaran, *et al.*,

"Partial-Gravity Dose Response: Roles of vestibular input & sex in response to AG" - Charles Fuller (University Of California, Davis) Team: Josh Alwood, Tana Hoban-Higgins, April Ronca

"Musculoskeletal response to partial-gravity analog in rats: structural, functional & molecular alterations" - Seward Rutkove (Beth Israel Deconess) Teml: Bouxsein

Space Biology and ISS National Lab

- Potential future collaboration with SB and through the Rodent Mission of Unusual Specimen Quantity (RMOUS-Q)
- Biospecimen sharing as possible from CASIS or NASA missions
- Data sharing through GeneLab

ISS U.S. NATIONAL LABORATORY

Exploration and Pioneering Scientific Discovery
Space Biology: Partnerships and Collaborations

- **International Space Life Sciences Working Group**: Forum for developing life science collaborations between NASA, CSA, ESA (CNES, DLR, ASI) and JAXA

JAXA

- Tissue sharing from JAXA MHU-1 and MHU-2 missions
- Joint publication from tissue sharing collaboration - first NASA-JAXA collaboration for rodent tissue sharing and is promoted under the “Japan-U.S. Open Platform Partnership Program (JP-US OP3)”
- Space Biology and HRP released a joint solicitation for ISS rodent studies using the JAXA habitats and centrifuge
- Expose mammals (mice) to 0,0.25,0.5, 0.75 *g*
- Discussion on MHU tissue sharing opportunities continues

Russia/IBMP

- Enabled through the US/Russian Joint Working Group and ISS partner agreements
- 30+ yr collaboration with the Russian Institute of Biomedical Problems
- On-going tissue sharing in exchange for crew time on ISS
- U.S. participation on Bion-M2 mission (free flyer biosatellite)
  - 30 day biosatellite mission NET 2022 w/ C57bl
  - Joint U.S. and Russian Science
  - NASA NRA solicited U.S. PIs for mission

Kaitlin O'Connell-Rodwell
SCORPIO-V
HNu Photonics, Maui

Effect of Microgravity on Neuronal Cytoskeletal and Intracellular Trafficking
(Grant# 80NSSC19K0715)

This ISS flight investigation will quantify the effect of microgravity on neuron microtubule organization and neurite outgrowth during differentiation. In addition, it will quantify the impacts to intracellular vesicle trafficking and microtubule polymerization on neuronal structure and intracellular communication. This study will collect data on a minute to hourly basis to analyze the dynamics of neuron differentiation and neuron structure and function in microgravity.

Specific Aims:

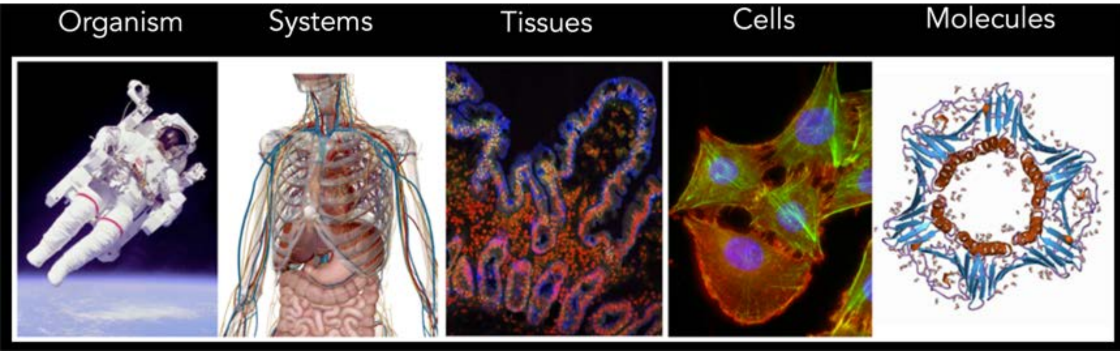
1. Quantify the effect of microgravity on neuron microtubule organization and neurite outgrowth during differentiation
2. Quantify the effects of microgravity on intracellular vesicle trafficking within terminally differentiated neurons
3. Characterize formation and intracellular trafficking with terminally differentiated neurons in microgravity

- Bioengineering company-based biologist whose NASA ROSBio-submitted proposal passed peer review and was awarded by the Space Biology Program
- Represents a seminal scientific interaction between NASA Space Biology and a commercial company to address important fundamental biological questions of common interest

Image credit: Wellcome Library, London

NEW RESEARCH CAPABILITIES

Space Biology / Space Biosciences Collaborative



- **The NASA Ames Biosciences Collaborative Laboratory** (BCL, Building N288), completed in 2019, brings together technical competencies in four lab quadrants dedicated to **Chemistry, Microbiology, Cellular Biology, and Organismal Biology** to advance interdisciplinary research across the biological sciences and support NASA's mission of leading in space exploration and conducting world class space biology, astrobiology, and bioengineering research
- **The BCL will co-locate Ames researchers across multiple scientific and engineering disciplines as the basis of an open and collaborative community**. This community will consist of Investigators funded through proposals or projects, including principal investigators, project scientists, and post-doctoral or other fellows, as well as technicians, graduate students, interns, and other collaborators. The BCL is dedicated to catalyzing interdisciplinary research between the biosciences and engineering fields
- **Space Biology Equipped the BCL with state-of-the-art research equipment for:** Genomics, Bioinformatics and nucleic acid Sequencing, led by GeneLab, PCR Thermal Cyclers, Nucleic Acid Analyzers, Confocal Microscopy, Fluorescence-activated Cell Sorting (FACS), High-Throughput and High-Content Cell Imaging Systems, High Resolution Biomedical 3D printing and Prototyping, Environmental SEM and Elemental Analysis, Micro-Computed Tomography, Radiobiology, Cell and Tissue Biology Culture, Histology and Tissue Processing, Ultra Freezers, Refrigerators, Autoclaves, Water

New capabilities at NASA Ames: DNA sequencing

- The NASA Space Biology GeneLab project operates a sequencing laboratory.
- Keystones are an Illumina NovaSeq 6000, capable of sequencing 400 transcriptomes every 48 hours, and an expert staff dedicated to implementing superior SOPs.
- A variety of support hardware are also utilized to support nucleic acid extraction, library preparation, and QC/QA.
- Developed standardized methods and protocols for sample processing and sequencing to enable higher fidelity data for comparisons and tp reduce the risk for data bias.

Nucleic acid isolation

Library prep

Quality control

DNA sequencing

45

Space Biology / microCT

The Bruker **SkyScan 1272 MicroCt** Instrument was acquired with Space Biology, HRP, and PI Grant funds to support ground and flight studies of bone and structural biology in response to spaceflight factors including microgravity, and radiation. The CT instrument has become a strong catalyst for new research projects and grants, leading to important new discoveries at nanoscale resolution previously not achievable.

Work supported includes:

Space Radiation Effects on the Skeleton (Shirazi-Fard, Alwood, and Globus)

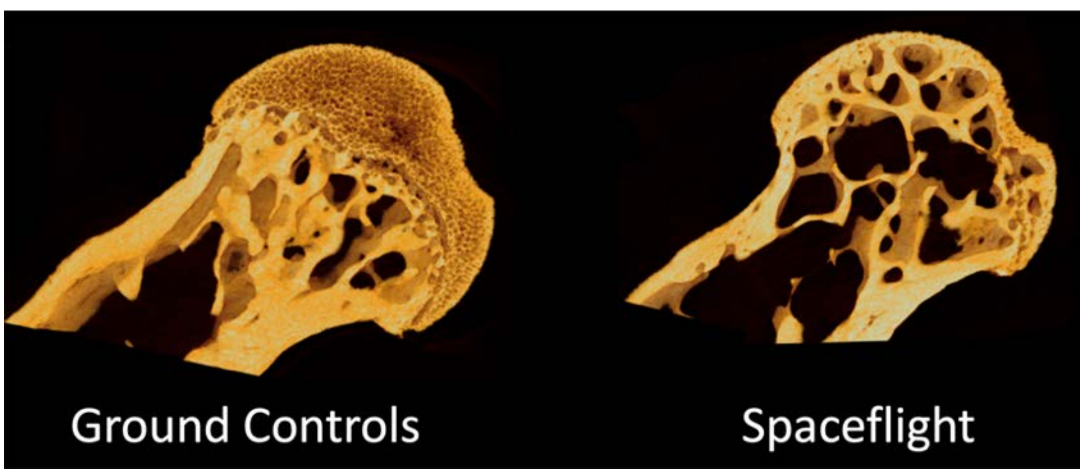
RR-10 Mission ground studies of HU and p21 null mice (Almeida, and Blaber)

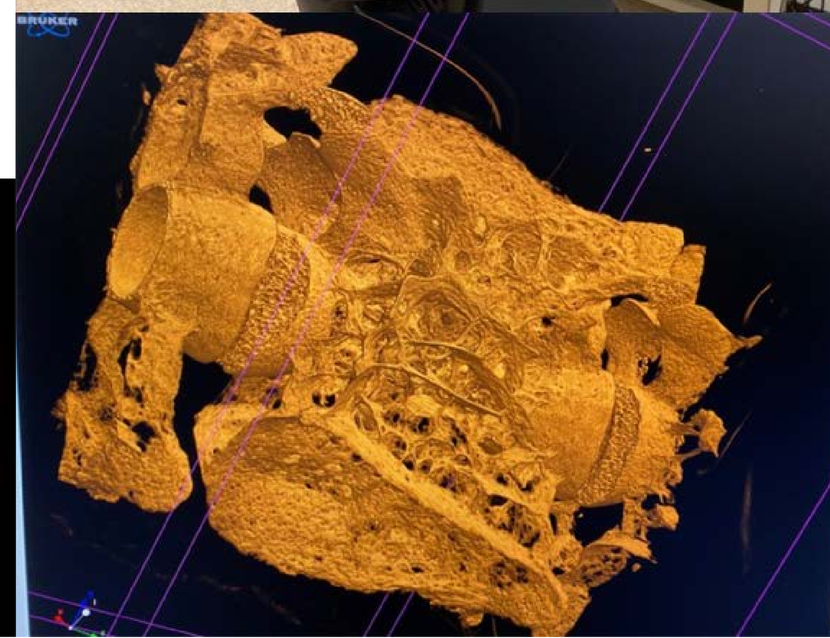
Rodent Habitat Alternative enrichment Studies (Shirazi-Fard, Globus, and Almeida)

Nutritional Countermeasures for Bone Loss in Microgravity Globus, and Schreurs)

Bion M1 Femoral Head cartilage in microgravity (Almeida, and Blaber)

Microgravity Effects on Foton M3 Newt Tail Vertebrae (Almeida, Juran)





Space Biology / Single Cell Sequencing

Space Biology purchased two 10X Genomics Chromium Controllers for single cell/nuclei sequencing purposes. This technology enables study of RNA expression with resolution at the individual cell level.

Figure 1: Chromium controller

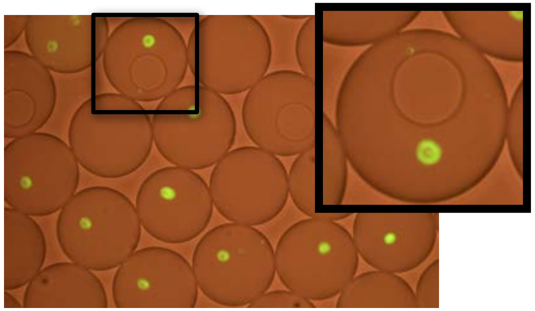


Figure 2: Gem encapsulation. Each droplet is its own reaction tube and within a single droplet is a single cell and a bead containing all the reagents needed for reaction.

Presentations

Juran, C.M., Zvirblyte, J., and **Almeida, E.A.C.** The Role of Gravity Mechanotransduction in Regulating Stem Cell Tissue Regenerative Potential at the Single Cell Expressome Level. International Symposium Stem Cells & Organoids in Development & Disease, 2019 Amsterdam, Netherlands.

Juran, C.M., Zvirblyte, J., and Almeida, E.A.C. The omics of stem cell based regeneration: A pilot single cell RNA-seq study of Mechanotransduction. Keynote address at NextGen Stem Cell Conference (NextGen) 2019, Saratoga Springs, NY.

Estimated Number of Cells
23,181

Post-Normalization Mean Reads per Cell
27,918

Median Genes per Cell
2,326

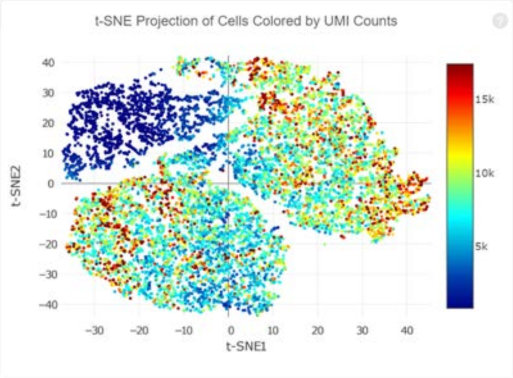


Figure 3: Preliminary data output from the single cell sequencing pipeline CellRanger showing RNA density per cell. This data is from the embyoid body study in progress.

Single cell resolution studies in progress include:

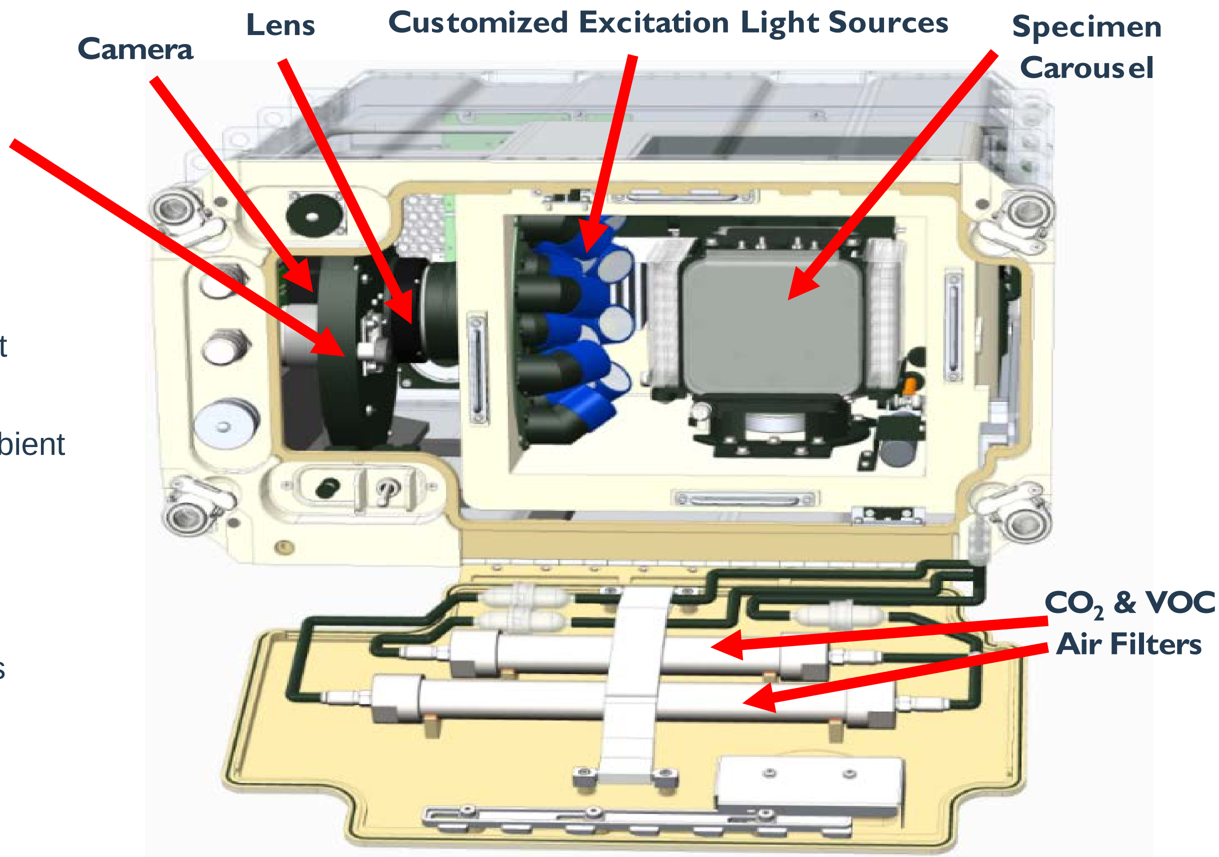
1. Gravity loading influences on pre-implantation embryoid body transcriptome
2. Role of CDKN1a/p21 in managing proliferative and differentiative response of bone marrow stem cells during osteogenesis.
3. How age effects transcriptomics recovery during recovery period after spaceflight exposure: RRRM-2 samples analyzed in conjunction with GeneLab
4. Spaceflight disruption of the regenerative immune loop: single cell RNA-seq analysis of splenocytes, peripheral blood mononuclear cells, loaded femur marrow and minimally loaded humerus marrow.

Next Steps

1. Eduardo Almedia (PI) and Liz Blaber's rodent research flight RR-10 was approved to include the live cell freezing sample storage for application of the first single cell sequencing spaceflight experiment.
2. Liz Blaber (PI), Cassie Juran and Eduardo Almeida were awarded the Bion-2 award titled: "Single cell analysis of bone marrow progenitor and differentiated progeny populations in response to long-duration spaceflight"

Spectrum: Multispectral Fluorescent Imager (NG-12: 11/19)

- Images *in vivo* reporter genes using fluorescent proteins incorporated into model organisms.
- Accommodates 10 cm x 10 cm Petri plates, multi-well culture plates, & other custom containers.
- Capable of capturing high-resolution images with dissection scope level magnification.
- Data collection, storage & downlink retrieval for near-real time evaluation by the investigator team.



Schematic displaying front view of Spectrum.

Internal Environmental Conditions

- Programmable Temperature: 18-37C
- Programmable Light Cycles
- Relative Humidity: Ambient ISS environment
- Ethylene scrubbing (< 25 ppb)
- CO_2 control (between 400 ppm and ISS-ambient levels in units of 100 ppm)
- Airflow to prevent condensation that would interfere with imaging.
- Lighting (at 10 cm beneath the light cap)
  - Broad-spectrum white light 0-100 mmoles (400-750 nm)
  - Darkness: <1 µmoles
  - Red light: 0-100 µmoles (630-660 nm)
  - Blue light: 0-50 µmoles (400-500 nm)
  - Green light 0-30 µmoles (520-530 nm)

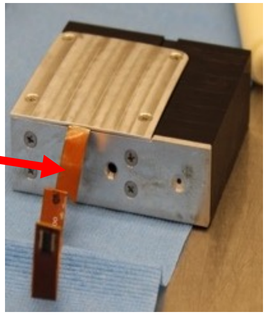
Biological Research In Canisters Light Emitting Diodes (BRIC-LED; SpX-12: 8/17)

BRIC-LED Hardware Objective: Provide discrete illumination to biological specimens contained in 60mm Petri dishes that are subjected to a microgravity environment.

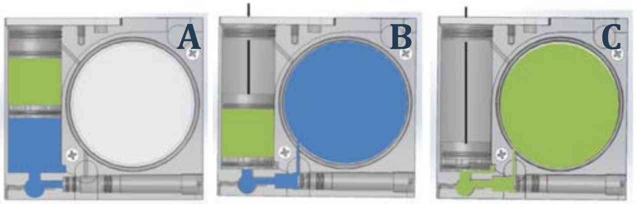
Petri Dish Fixation Units

Single or Dual Fluid Injections: 17mL total fluid volume for injection of Growth media and/or liquid treatments/preservatives/fixatives.

- N=36 60mm Petri dishes available per mission
- Capable of >150 μmoles of light
- >10% intensity resolution control for each wavelength
- 4 discrete LEDs types currently ranging from 430-750nm (blue, red, white and far-red)
- Customizable wavelengths
- Programmable lighting schedule with 1 sec resolution
- >70% light uniformity when using 4 discrete wavelengths
- Light tight from external sources



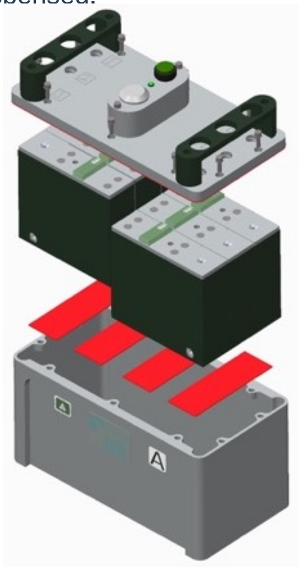
BRIC-LED PDFU Lid showing LEDs.

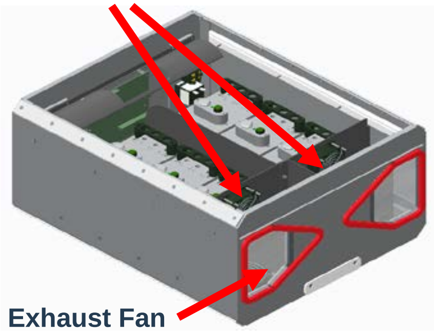


A. Pre-Actuation: Filled with 2 Fluids. B. 1st Actuation: Fluid 1 Dispensed. C. 2nd Actuation: Fluid 2 Dispensed.

Locker & Tray

- Resides in US Lab on ISS
- Holds 6 BRIC Canisters
- Canisters Travel up/down
- KSC Ground Station controlled
- Commanding start/stop of expt.
- Real time telemetry of Tray and Canister temperatures & LED status
- Forced air cooling to reject heat
  - 1.5C between Canisters
  - 3C from EXPRESS AAA air
- Internal Canister Pressure Logged
- Temperature Sensors on Canister Lid Boards
- One 3-Axis Accelerometer





Passive Orbital Nutrient Delivery System (PONDS)

Veg-PONDS-01: SpX-14 (4/18); Veg-PONDS-02: NG-11 (4/19); Veg-PONDS-03: NG-13 (2/20)

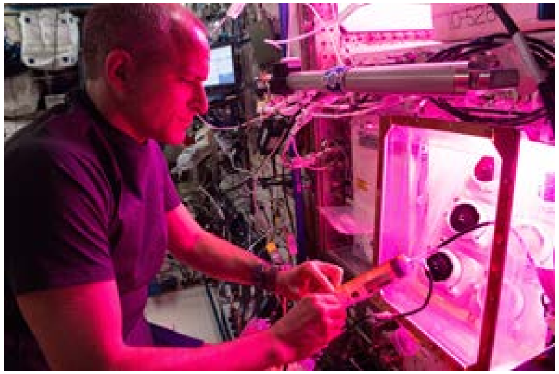
PONDS is a new plant growth approach that contains both an area for a contained plant growth substrate and a reservoir for water and/or plant nutrient solutions. It was developed to fit under the Veggie light cap and replace the current Reservoir/Pillow Nutrient Delivery System used within Veggie on ISS. The system provides more reliable water delivery to seeds for germination (while avoiding overwatering), and fulfills the requirement to transport water from the reservoir for improved plant growth while providing adequate nutrients and aeration to the root zone under both 1g and microgravity conditions.

KSC Prototype → Ground Studies

Flight Unit Development Team:
Techshot and Tupperware

Status:

- Veg-PONDS-01 ISS Tech Demo completed 5/18
- Veg-PONDS-02 ISS Tech Demo completed 5/19
- Veg-PONDS-03 launch targeting NG-13 (2/20)



Veg-PONDS-02 ISS plant growth initiation in Veggie

Veg-PONDS-02 Fluid Test on ISS

RAPID FREEZE CHARACTERIZATION TESTS OF THE GLOVEBOX FREEZER

Validate the freezing capability of the Rapid Freeze using Rodent Tissues

- Characterize the thermal performance of the freezers over multiple days of carcass processing and freezing
- Characterize the freezing rate profile of whole and partial carcasses
- Assess the resulting tissue quality after freezing
- Data will be used to inform future investigators & determine operational constraints that should be applied to Rapid Freeze system use.

Ground Control Unit

Test Conditions

Glovebox freezer tissues vs liquid nitrogen controls

- with thermocouples for freezing profiles
- without thermocouples for tissue quality assessment

Whole carcasses in Ground Control Unit vs liquid nitrogen vs slow freeze

Partial carcasses in Ground Control Unit vs liquid nitrogen vs slow freeze

Glovebox Freezer qual unit

Preliminary results

- Glovebox freezer cryo-chamber increased by only +3.6˚C after multiple days of carcass freezing
- Glovebox freezer cryo-chamber increased by only +10C after multiple days of carcass freezing
- Learned that using a temperature-controlled room will improve the temperature cycling of the ground control unit to improve hardware cool down time.
- Glovebox Freezer test complete 8/14/2019; Cryochiller test complete 8/22/2019; GeneLab RNAseq estimated for 4/2020; Submitted for publication by end of FY2020

Exploration Garden in Support of Plant Research Beyond LEO

Objective:

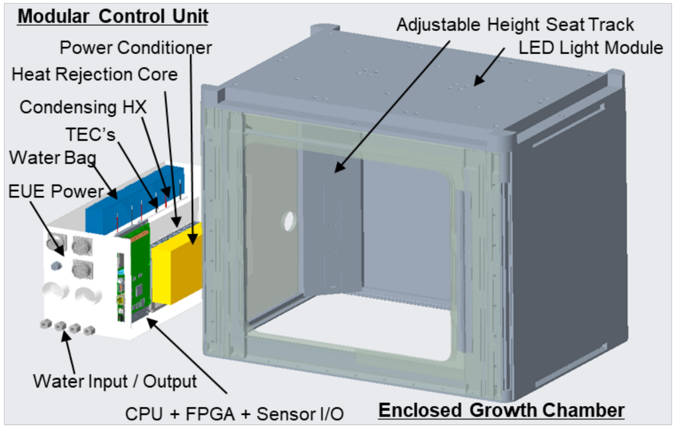
- Identify, evaluate, develop and deploy a suite of modest research hardware for investigation into the effects of the deep space environment on seeds, seed germination and the growth and development of small plants at the Gateway. Hardware will include an "Exploration Garden" plant growth system designed to accommodate both model and candidate crop plants as well as a selection of plant research hardware based on existing or re-purposed BRIC canisters.

Relevance/Impact:

- Understanding the combined effects of microgravity, the radiation environment and absence of a magnetic field at the Gateway on seeds and the biological processes associated with early plant growth will provide valuable information for the selection of potential crops and hardware systems necessary to support sustainable supplemental food production for long duration spaceflight beyond LEO. Enable research into the microbiome of the vehicle-plant interface and develop strategies to maintain a healthy vehicle environment.

Project Development Approach:

- Specifies the development of an "Exploration Garden" flight unit for demonstration on the ISS prior to the development of ground and flight versions in support of Gateway operations.
- Evaluate existing hardware for Gateway science requirements.



Operational Concept

Launch – Pre-planted root modules, stowed in logistics module

Installation – Gateway vehicle interface & water fill – crew tended

Experiment Sequence

Tele-operated experiment initiation – ground control

Plant harvest, cleaning and replanting – crew tended

Data acquisition – Via imagery telemetry; growth-area, plant health

CEV Sample Return - Gas, water or tissue samples

Expanding research capabilities on ISS

Dedicated Life Sciences Glovebox
JAXA H-IIB, 9/22/18

Techshot Multi-Use Variable-g Platform (centrifuge systems for Plants, invertebrates, Cells, Microbes)
SpaceX CRS 14,4/2/18

NASA Advanced Plant Habitat

(OA-7 & SpaceX CRS-11: 5/17 & 6/17)

NASA Bioculture System
SpaceX CRS-13,5/15/17

Enabling Rodent Research on ISS

Rodent Habitat

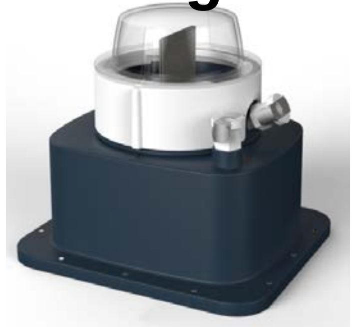
JAXA Mouse Habitat Unit
0xg to 1xg

Techshot Dexascan

ISS Rodent Research Handbook: www.nasa.gov/sites/default/files/atoms/files/np-2015-03-016-jsc_rodent-iss-mini-book_detail-508.pdf

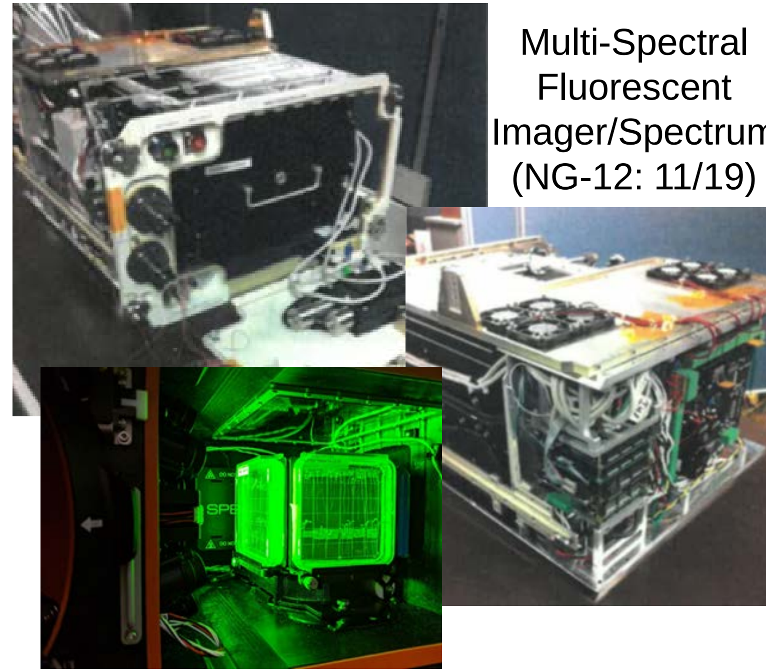
Expanding research capabilities on ISS

Two Veggie Units On-Orbit



PONDS
Tech Demos
(SpX-14: 4/18)
(NG-11: 4/19)
NG-13: 2/20)

APEX Plant Growth Chamber



Multi-Spectral
Fluorescent
Imager/Spectrum
(NG-12: 11/19)

BRIC-LED
(SpX-12: 8/17)
On-Orbit

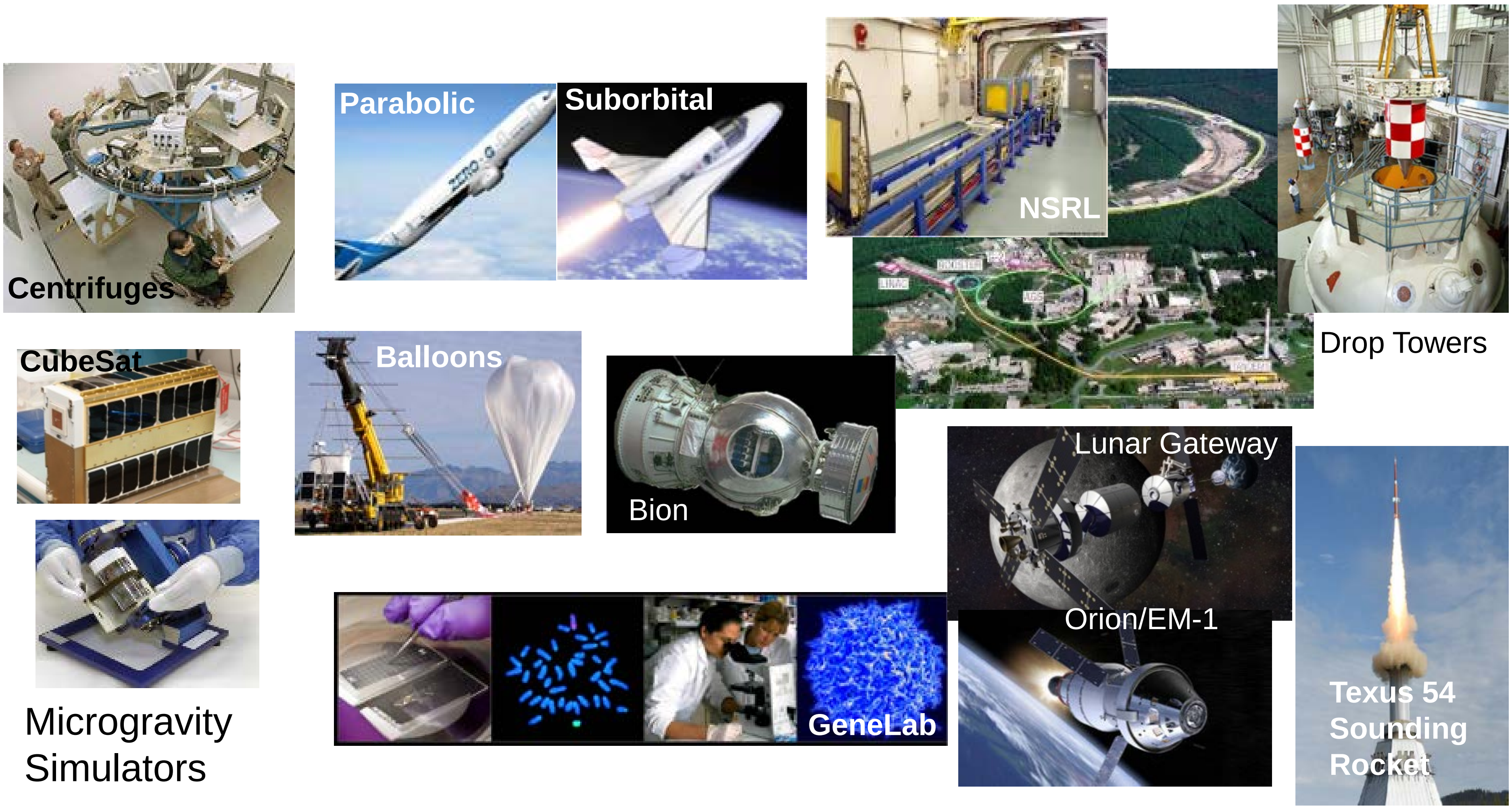
Wetlab-2 qRT-PCR on ISS
(On-Orbit)

MinION
(On-Orbit)

Alternative Platforms for Space Life Sciences

Objective:

Provide capabilities and opportunities to support NASA-selected life science investigations (and technical demonstrations) to meet recommendations of the NRC Decadal Report and Space Biology Plan with appropriate platforms.



SOLICITATION ACTIVITY

Recent Space Biology Solicitation Activity

OMNIBUS - NRA NNH18ZTT001N Research Opportunities in Space Biology (ROSBio) - 2018 Released: July 21, 2018, active through July 31, 2023)

– Covers all basic and applied Space Biology research and technology
– Specific research/funding opportunities are announced through Appendices soliciting proposals to address specific Space Biology's objectives

EM1 MISSION - NRA NNH18ZTT001N-EM1 Appendix A: "Orion Exploration Mission-1 Research Pathfinder for Beyond Low Earth Orbit Space Biology Investigations"
- Released: July 20, 2018

- Solicited experiments to be flown in BRIC hardware on the Orion Exploration Mission 1 (EM-1), a 2020 unmanned lunar orbital flight
- Two-step submission and review process used to solicit proposals to this Appendix
- **16** Step-1 proposals were submitted by the August 17, 2008 deadline.
- 12 Investigators who submitted Step-1 Proposals were invited to submit complete Step-2 proposals, due on November 30, 2018
- 4 Projects were selected for definition/implementation

Orion Space Biology Pathfinder to the Moon and Back

Value to SLPS and Orion

1)*Paves the way* for biology research beyond LEO with critical sample return-to-Earth capability.

2)*Provides critical and unique data* about life beyond LEO for the first time in over 40 years

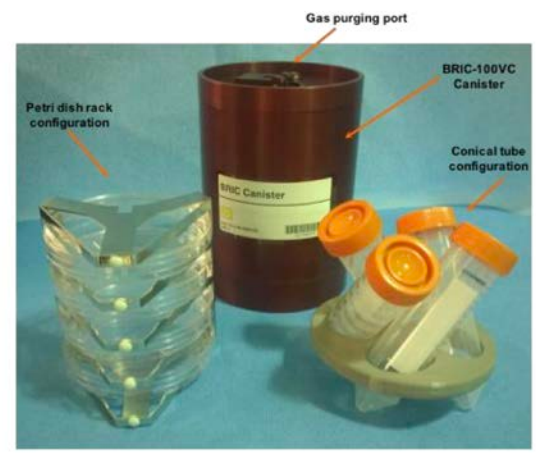
3)*Answers questions* about sustaining life beyond LEO for long periods

Leverages existing small payload hardware systems that require no power.

Collaborate with Orion to support payload without interfering with Orion EM-1 mission objectives.

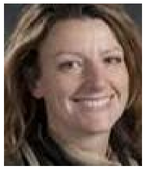
Science Approach

- Specimen return for analysis
- Radiation dosimeters
- Minimum of a 45-day Cis-Lunar orbit space environment exposure
- Samples in the crew compartment



BRIC-100VC with a petri dish rack insert (left) capable of holding four 100 mm petri dishes, and a 50 mL conical tube holder (right)

Selections for NRA NNH18ZTT001N-EM1 Appendix A: "Orion Exploration Mission-1 Research Pathfinder for Beyond Low Earth Orbit Space Biology Investigations"



- Federica Brandizzi, PhD, Michigan State "*Life beyond Earth: Effect of space flight on seeds with improved nutritional value*" Characterize how spaceflight effects plants seed nutrient stores to gain new knowledge to help increase the nutritional value of plants grown in spaceflight

- Timothy Hammond, PhD, Institute For Medical Research. "*Fuel to Mars*" Will study photosynthetic algae, Chlamydomonas reinhardtii, to identify important genes that contribute to its survival in deep space

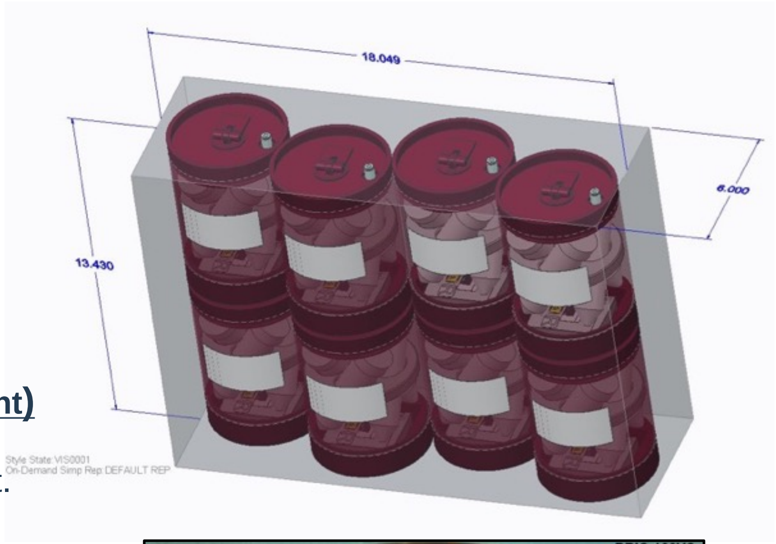
- Zheng Wang, PhD, Naval Research Laboratory "*Investigating Roles of Melanin and DNA Repair on Adaptation and Survivability of Fungi in Deep Space*" Will use fungus Aspergillus nidulans to study radioprotective effects of melanin & DNA damage response

- Luis Zea, PhD, Univ. Colorado, Boulder "*Multi-Generational Genome-Wide Yeast Fitness Profiling Beyond and Below Earth's van Allen Belts*" Will use yeast to identify genes that help organisms adapt to conditions of both deep spaceflight on the EM-1 mission, and of Low Earth Orbit on ISS

Exploration Mission-1 (EM-1): Payload Concept

SLPSRA Path Forward Plan

- Release Appendix listing Payload Constraints and requesting science community to propose to use a diverse group of model organisms (cells, plants, microorganisms, invertebrates) within the provided capabilities (BRIC 100VCs, others).
- Peer Review Submitted Proposals and select scientifically meritorious proposals (Score ≥ 70) for Technical Feasibility and Programmatic Relevance.
- Implement EM-1 Payload Experiments.

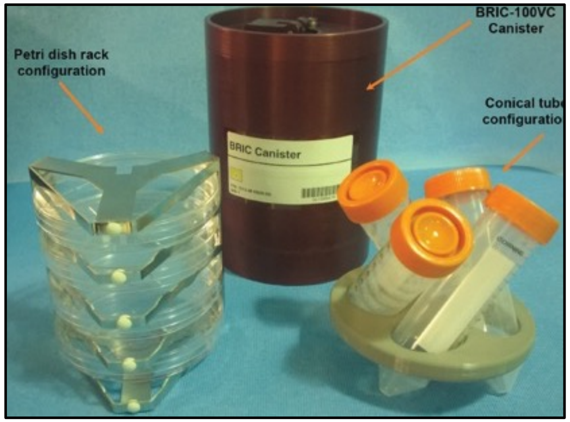


Mission Flight Plan

- 21-42 day mission duration.
- Payload turn over at L-7 to 30 days (up to 65 days due to launch delays).
- Vehicle return, recovery, and transport to KSC 2-5 days
- Payload Retrieval and Science De-integration 3-5 days
- Internal crew compartment temp predictions: 60-95°F
- Payload shall meet all requirements during and after exposure to thermal range of 49-143°F (MPCV.SBP.007)

Passive Payload (replaces Mass Simulator in Orion Crew Compartment)

- Housing can contain space for several experiments.
- Containment container will be mounted to an interface plate for attachment.
- Interface and payload must meet Orion requirements.
- Multiple proposals may be awarded 3 year grants (TBD).
- Allowable payload dimensions and CG location are baselined.



Concept of Operations Under Consideration

- Fly 6-8 BRIC-100VC canisters with dosimeters and data loggers.
- Petri dishes or other biology containment options (conical tubes, etc.) loaded into autoclaved canisters.
- Canisters flushed with PI-specified gas mixtures (e.g. 5% CO_2).
- Canisters installed into Orion, launch, recovery, post-flight analysis.

Recent Space Biology Solicitation Activity

ANNUAL SPACE BIOLOGY SOLICITATION NRA NNH18ZTT001N-FG Appendix B: "Solicitation of Proposals for Flight and Ground Space Biology Research: Released: December 7, 2018

4 Research Topics Specific to this Appendix:

- **Microbiology studies** that will produce new understanding to augment and expand our knowledge of the Microbiology of the Built Environment (MoBE) in Space and suggest how to manipulate and control it in the closed environment of exploration spacecraft.
- **Plant Biology studies in support of Human Space Exploration** making use of the capabilities of the VEGGIE and Advanced Plant Habitat) on ISS to study environmental effects on plant growth and interactions with microbes and fungi. Proposed studies should answer fundamental questions about how plants adapt to spaceflight and provide new understanding of how to grow plants in space that will enable human space exploration.
- **Animal Biology Studies (Vertebrate & Invertebrate) in Support of Human Space Exploration to** Increase understanding of basic mechanisms animals use to adapt and/or acclimate to spaceflight and to alterations in gravity in general. Basic knowledge gained may accelerate solutions to biomedical problems affecting human space exploration and human health on Earth.
- **Validation of Microgravity Analogs:** Studies designed to compare results and validity of microgravity "simulators" in parallel with flight and ground-based studies..

Awards were made to 15 investigators from 14 different institutions to conduct flight and ground-based research projects. Six of there awards were to investigators who are new to Space Biology. Total value of awards = $9M (Selected 15/71 submitted Step-2 proposals)

Selections for NRA NNH16ZTT001N-FG Appendix B: “Proposals for Flight and Ground Space Biology Research”

Awards for Microbiology Studies:

Jack Gilbert, Ph.D., University of California, San Diego
Quantifying selection for pathogenicity and antibiotic resistance in bacteria and fungi on the ISS – a microbial tracking study

Janet Jansson, Ph.D., Pacific Northwest National Laboratory, Richland, Washington
Dynamics of microbiomes in space (DynaMoS)

Jiseon Yang, Ph.D., Arizona State University, Phoenix
Microbial social behavior and heritable genetic or epigenetic changes affected by the spaceflight environment: Understanding the evolution of microbial interactions during spaceflight **Was MoBE Post-Doc**

Clay Wang, Ph.D., University of Southern California, Los Angeles
Characterization of ISS microorganisms that assist in the decomposition of complex organic matter during spaceflight

Selections for NRA NNH16ZTT001N-FG Appendix B: "Proposals for Flight and Ground Space Biology Research" (Cont.)

Awards for Plant Biology Studies:

Robert Ferl, Ph.D., University of Florida, Gainesville
The role of Ca2+ signaling during the early events of plant adaptation to spaceflight

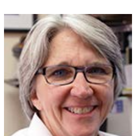
Anjali Iyer-Pascuzzi, Ph.D., Purdue University, West Lafayette, Indiana
Effect of spaceflight and simulated microgravity on plant defense responses

Christer Jansson, Ph.D., Pacific Northwest National Laboratory, Richland, Washington
C4 Photosynthesis in Space (C4Space)

Norman Lewis, Ph.D., Washington State University, Pullman
Dissecting beneficial plant-microbe interactions and their efficacy in the ISS spaceflight environment, a model study

Gioia Massa, Ph.D., NASA's Kennedy Space Center, Florida
Spaceflight microbiome of a food crop grown using different substrate moisture levels

Patrick Masson, Ph.D., University of Wisconsin, Madison
Can polyamines mitigate plant stress response under microgravity conditions?



Sarah Wyatt, Ph.D., Ohio University, Athens
Spaceflight alters post-transcriptional regulation

Selections for NRA NNH16ZTT001N-FG Appendix B: “Proposals for Flight and Ground Space Biology Research” (Cont.)

Awards for Animal Biology Studies:

Michael Delp, Ph.D., Florida State University, Tallahassee
Effects of simulated microgravity and partial unloading on organ systems of the body

Foteini Mourkioti, Ph.D., University of Pennsylvania, Philadelphia
Telomere length regulation in muscle atrophy

Seward Rutkove, Ph.D., Beth Isreal Deaconess, Boston
Approaching gravity as a continuum: musculoskeletal effects of fractional reloading

Ritesh Tandon, Ph.D., University of Mississippi Medical Center, Jackson
Effect of space radiation on cytomegalovirus reactivation and lytic replication

Recent Space Biology Solicitation Activity

- **NRA NNH18ZTT001N-PT: Appendix C: Development of Microgravity Food Production: Plant Watering, Volume Management, and Novel Plant Research on the International Space Station"** Released: June 07, 2019
  - Represents a collaboration between NASA SLPS and NASA's Advanced Exploration Systems (AES)
  - Solicited R&D projects for the development of more reliable water and nutrient delivery approaches/concepts for growing edible pick-and-eat plants in spaceflight, that will eventually lead to new hardware. Proposals could focus on developing:
    1. Edible Plant Water and Nutrient Delivery Systems for spaceflight
    2. Edible Plant Spacing Systems that will optimize the use of plant-growing volume in space
  - Ultimate goal of Appendix will be procuring the development of a reliable space-ready plant water and nutrient delivery system in three distinct phases.
    - Phase-A: solicits key components and experiments (**Current Appendix**)
    - Phase-B: prototype development and system investigations
    - Phase-C: demonstration on the ISS.

34 Step-1 proposals were submitted, of which 29 were invited for the submission of Step-2 proposals. 22 proposals will be reviewed for Scientific/Technical merit at the end of CY 2019.

Upcoming Space Biology Solicitation Activities

ANNUAL SPACE BIOLOGY SOLICITATION NRA NNH18ZTT001N Appendix D: "Solicitation of Proposals for Flight and Ground Space Biology Research: Planned Release: Oct, 30, 2019

The three Research Topics specific to this Appendix:

- **Microbiology studies** that will produce new understanding of how microbiological organisms and/or communities acclimate to, evolve, and/or behave in the spaceflight environment. Topical areas include Microbiomes of the Built Environment (MoBE); biofilm formation; and mechanisms responsive to and governing phenotypic changes in microbial biology.
- **Plant Biology studies** making use of the ISS capabilities. Proposed studies should answer fundamental questions about how plants adapt to spaceflight and provide new understanding of how to grow plants in space that will enable human space exploration.
- **Animal Biology (vertebrate and invertebrate)** experiments that lead to the characterization of organ systems, behavioral adaptations, and the underlying cellular and molecular mechanisms of phenotypic changes within tissues and between physiological systems.

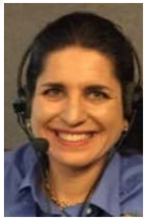
This Appendix will solicit the following 6 studies types:

- ISS Spaceflight or Ground-Based Studies
- Special Focus- Validation of Ground Microgravity Analogs
- Special Focus-Parabolic and/or Suborbital Flight Studies
- Special Focus-Antarctic Balloon Studies (Secondary Payloads Only)
- New Space Biology Investigations
- Space Biology Post-Doctoral Fellowships

RECENT AND UPCOMING ISS FLIGHT ACTIVITY

Completed Flight Missions in 2019

SpaceX CRS-16
12/05/18 - 1/13/19



Gioia Massa, Ph.D.
(Kennedy Space Center)
VEG-04A
Pick-and-Eat Salad Crop Productivity, Nutritional Value, and Acceptability to Supplement the ISS Food System

Simon Gilroy, Ph.D.
(Univ. of Wisconsin, Madison)
APEX-05 LMM Reflight Spaceflight-Induced Hypoxic-ROS Signaling

Rad-Dorm Process Demo

NG-11
4/17/19

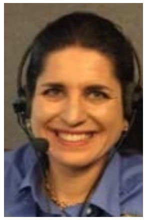
Michael Pecaut, Ph.D.
(Loma Linda University)
RR-12 Impact of Spaceflight on Primary and Secondary Antibody Responses

Veg-PONDS-02 Tech Demo

SpaceX CRS-17
5/4/19 – 6/3/19

Sheila Nielsen, Ph.D.
(Montana State University)
Micro-14 Characterizing the effects of spaceflight on the *Candida albicans* adaption process

SpaceX CRS-18
7/25/19 – 8/27/19



Gioia Massa, Ph.D.
(Kennedy Space Center)
VEG-04B
Pick-and-Eat Salad Crop Productivity, Nutritional Value, and Acceptability to Supplement the ISS Food System

Craig Everroad, Ph.D.
(Ames Research Center)
MVP-Cell-02 Experimental Evolution of *Bacillus subtilis* Populations in Space; Mutation, Selection and Population Dynamics

Bruce Hammer, Ph.D.
(Loma Linda University)
Micro-15 Study of Mammalian Pluripotent Stem Cells in Microgravity

Upcoming Flight Missions for 2019 - 2020

NG CRS-12
NET Launch 11/2/19

Spectrum Hardware

SpaceX CRS-19
NET Launch 12/4/19

Spectrum-001 Tech Demo

NG CRS-13
NET Launch 2/7/20

Karl Hasenstein, Ph.D.
(Univ of LA, Lafayette)
PH-02
Nutrition and Growth Parameters of Space-Grown Plants

Veg-PONDS-03 Tech Demo

SpaceX CRS-20
NET Launch 3/1/20

Simon Gilroy, Ph.D.
(Univ. of Wisconsin, Madison)
BRIC-LED-002
BRIC: Exploring Spaceflight-Linked Changes in Plant Defense Capabilities

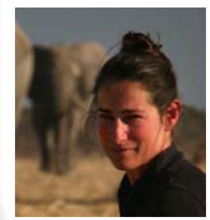
SpaceX CRS-21
NET Launch 8/5/20

Marcela Rojas-Pierce, Ph.D.
(North Carolina State Univ.)
BRIC-24
Membrane Contacts in Plant Gravity Perception

Xiao Wen Mao, Ph.D.
(Loma Linda University)
RR-18
Space flight environment induces remodeling of vascular network and glia-vascular communication in mouse retina

SpaceX CRS-21
NET Launch 8/5/20

Thomas Boothby, Ph.D.
(University of Wyoming)
CS-04 Using Water Bears to Identify Biological Countermeasures to Stress During Multigenerational Spaceflight



Caitlin O'Connell, Ph.D.
(Hnu Photonics, LLC)
MANTIS The Effect of Microgravity on Neuronal Cytoskeletal and Intercellular Trafficking

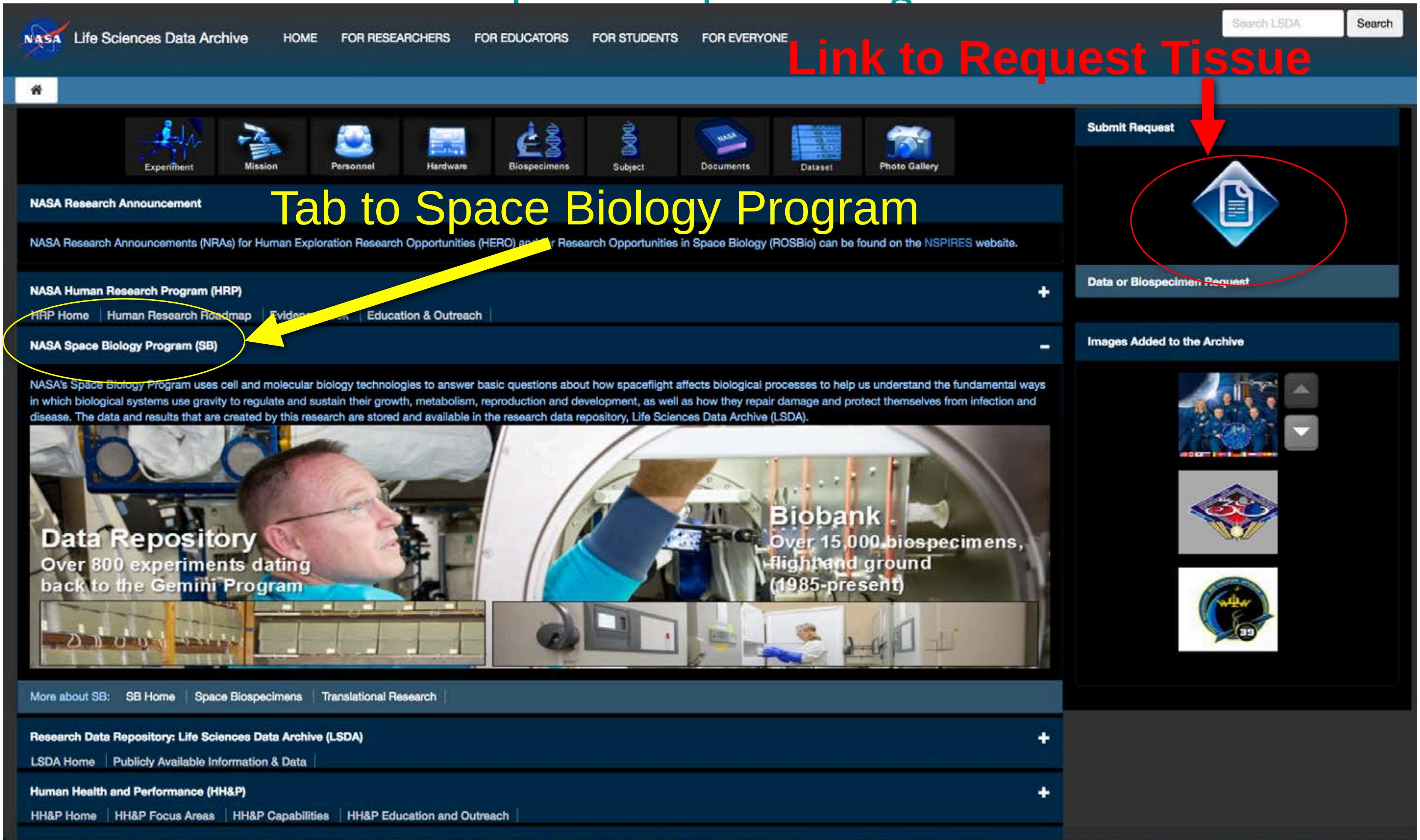
NG-14
NET Launch Oct. 2020

Eduardo Almeioda, Ph.D.
(NASA Ames Research Center)
RR-10
P21 and Bone Tissue Regeneration

ARCHIVING AND GENELAB

Life Sciences Data Archive

https://lsda.jsc.nasa.gov/



GeneLab Status Update

- 213 data sets available world-wide
- 39 publications linked to a dataset; 11 additional publications derived from GeneLab-produced datasets - Latest publications at https://genelab.nasa.gov/publications
- Sample processing: sequenced tissues from 7 missions/experiments, 19 tissues, over 700 samples, 15 new datasets
- Established the Analysis Working Groups (AWG), ~120 scientists participated
  - NASA, NIH, academia, industry, and international institutes
  - Achieved consensus on the pipelines to generate higher-order data
  - Generated publications
  - Next AWG meeting on November 19, 2019 in Denver, CO
- Established the Visualization Working Groups, which completed Version 1 of tools, and will released the Visualization Portal on Oct. 1, 2019
- GeneLab Data Systems Release 3.2, 3.2.1, 3.2.2, 3.2.3, 3.3
  - Implemented data versioning (requirement to be officially listed in mainstream science journals: e.g. Nature)
  - Added dataset citation feature
  - Updated genelab.nasa.gov and GLDS user interface
  - Release 3.3 (Internal) – Repository UI upgrades, visualization integration links

THE NEXT GENERATION OF SPACE BIOLOGISTS

Training and Internship Opportunities

NASA Student Internships : https://intern.nasa.gov

NASA Post-Doctoral Program (NPP) https://npp.usra.edu

Provides early-career and more senior scientists the opportunity to share in NASA's mission

Fellows work on 1 to 3 year assignments at NASA centers and institutes

Fellows contribute to our national scientific exploration, confirm NASA's leadership in fundamental research, and complement the efforts of NASA's partners in the national science community.

Space Life Sciences Training Program (SLSTP)
https://www.nasa.gov/ames/research/space-life-sciences-training-program

- Trains next generation of scientists and engineers to enable NASA to meet future space life sciences research and engineering challenges
- Students conduct hands-on research, attend science lectures, develop professional & project management skills, perform a team project & submit an abstract to present at a scientific or engineering meeting
- Students attend the American Society for Gravitational and Space Research to present posters and lightning talks
- 2019 Class: 10 students (2 Juniors, 5 Seniors, and 3 recent graduates) chosen from 68 applications, and 2 staffers recruited from previous cohort of Research Associates

Training and Internship Opportunities at ARC

<u>GeneLab for High Schools (GL4HS)</u>

https://www.nasa.gov/ames/genelab-for-high-schools

- Introduce and teach high school students about system biology, bioinformatics, ‘omics data and how it relates to biological research, with a specific focus on space based research and the GeneLab Data System
- Provides an opportunity for selected students to develop their educational and scientific skills including leadership, team-work, data analysis, problem solving, and independent learning through training modules, networking and competition
- Provide tools for teachers to translate both Space Biology research and bioinformatics skills into their classrooms
- 2019 Class: 8 Juniors and 7 Seniors from 12 different schools (3 out of state and one student from San Diego)

Training and Internship Opportunities at KSC

NASA/Fairchild Tropical Botanical Garden Challenge Collaboration – Growing Beyond Earth

KSC touches several thousand middle and high school students who are collecting data on crop production for NASA's space crop projects. Students 1) assist NASA scientists in testing edible plant varieties for growth in ISS Veggie and Advanced Plant Habitat; 2) conduct experiments to help NASA select plants to grow on ISS; and 3) test new crops and different growing techniques including harvest methods, fertilizer levels, photoperiod, and interaction effects of different species. Miami's Fairchild Tropical Botanic Garden provides each participating school equipment and materials that mimic Veggie. Each school receives a Fairchild-designed mini botany lab that mimics the ISS plant growing environment including a growth chamber, LED lighting, watering system, pots, soil, fertilizers and seeds.

Fairchild and NASA scientists train teachers to experiment with growing food plants following special research protocols for schools to follow each academic year to explore different variables and asking various research questions. As plants grow, students share data and observations online with NASA scientists, who use the data to select potential candidates for ISS and future space exploration

- **2018-2019 School Year:** 173 teams of school students participated from schools in Florida (Miami and West Palm Beach areas), Puerto Rico, Colorado and Ohio; students either looked at the growth of a variety of crops or focused on two crops and looked at the neighbor effect of two species growing in close proximity; students tested different plant arrangements and monitored the plant growth environment, how plants grew over time, and yields of total and edible mass at harvest; data were compiled and results shared via twitter at @growbeyondearth. This year's projects culminated in a student symposium on 4/27/19 in Miami, where top teams presented research posters and received awards.
- **2019-2020 School Year:** Teacher kickoff events held 9/21/19 in West Palm Beach and 9/28/19 at Fairchild Tropical Botanic Garden in Miami, FL.
- **Weblinks:** https://www.fairchildgarden.org/Science-Conservation-/Growing-Beyond-EarthNasa-and-Fairchild and https://sites.google.com/site/growingbeyondearth/home

Training and Internship Opportunities

X-Hab – eXploration Systems and Habitation
Charles Quincy- charles.d.quincy@nasa.gov
Full challenge solicitation: https://www.spacegrant.org/xhab

With the forward progress of NASA's new Space Launch System and emphasis on future deep space missions, the breadth of technology required to successfully complete such long duration flights is inadequate. The challenges that exist – from producing food on the journey to effectively recycling breathable air to utilizing every last resource available – create opportunities for groundbreaking innovation. The X-Hab challenge series represents one of several platforms NASA leverages to solicit external solutions to complex challenges to keep humans healthy and productive in deep space and on the journey to Mars. With the X-Hab program NASA KSC is working with undergraduate and graduate students in engineering design courses to develop new concepts, hardware and methodology for future space crop production. In 2019 two Space Life Science X-Hab teams completed work on issues related to space crop production, one from Ohio State University (OSU) and a second from University of Southern Alabama. KSC researchers advised these teams as they worked on their projects through the 2018-2019 school year.

Three new X-Hab 2020 Academic Innovation Challenge Space Life Science projects were selected:

- **Ohio State University, Wooster (PI Peter Ling): *Volume Optimization for Food Production During Deep Space Exploration***: Students will improve the efficiency of plant growing volume for space missions by developing dual-usage solutions for space as well as day-to-day application for vertical farming in urban environments.
- **Auburn University, Alabama (PI David Beale): *Volume Optimization for Food Production During Deep Space Exploration:*** Students will design, build and test an electromechanical plant growth chamber that can automatically sense, actuate and control functions to maintain growth during a long duration Mars mission.
- **University of Miami, Coral Gables, Florida (PI Victoria Coverstone): *Volume Optimization for Food Production During Deep Space Exploration:*** Students will optimize available volume for food production and define the food production volume requirements for deep space missions. They will develop and analyze viable solutions and provide a functional prototype.

Training and Internship Opportunities

INSPIRE - ENGAGE - EDUCATE - EMPLOY
The Next Generation of Explorers

NASA Internships, Fellowships, and Scholarships (NIFS) https://intern.nasa.gov/

NASA internships & fellowships leverage NASA's missions & programs to enhance and increase capability, diversity and size of the nation's future science, technology, engineering and mathematics (STEM) workforce. Internships are available from high school to graduate level. Internships provide students with the opportunity to participate in either research or other experiential learning, under the guidance of a mentor at NASA.

In the 2018-2019 school year, the KSC Life Sciences Office hosted 16 interns:

- Jess Bunchek, Purdue University, Vegetable Production Systems Component Tests
- Lane Diesa, North Carolina State University, New Crop Testing
- Joseph Emhof, Penn State, Plant and Rhizobacteria Symbioses to Enhance Crops
- Michael Gildersleeve, Cornell University, Ventilation and the Impact from the Movement of Air on Radish and Lettuce Growth and Metabolic Processes
- Ben Greaves, University of Michigan, Development of an Integrated Plant Growth System
- Savannah Hollingsworth, University of Arkansas, The Effects of Radiation on Tomato Seed Development
- Audrey Lee, Columbia, Changes in Biological Organisms Cultured under Simulated Microgravity Conditions
- Aishwarya Nambiar, Georgetown University, Effects of Simulated Microgravity on Cell Nuclear Morphology and Gene Expression
- Daphne Onsay, Michigan State University, Plant Growth in Amended Martian Regolith Simulant
- James Rieser, Purdue University, Plant Growth Research for Food Production
- Steven Russell, North Dakota State, Microbial Adaptation to Microgravity and Effects on Antibiotic Resistance
- Pia Sen, University of Texas at Dallas, Study of TK6 Lymphoblastoids
- Tait Sorenson, University of Central Florida, Mechanical Design Analysis for Rapid Freezing Hardware for Biological Samples in Microgravity
- Joseph Taylor, North Carolina State University, Russian Crop Testing and Root Crop Growth Systems
- William Thomas, University of Texas at Austin
- Jacob Torres, Purdue University, Water and Nutrient Delivery Testbed for Food Production

From Graduate Student to University Professor
Dr. Elizabeth A. Blaber

11/2013: Ph.D., University of New South Wales, Australia
NASA Ames Research Center (ARC)
NASA Graduate Advisor- Dr. Eduardo Almeida

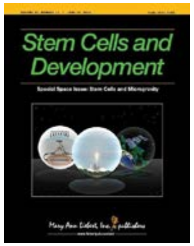
12/2013 to 11/2016: NASA Post-Doctoral Program Fellow

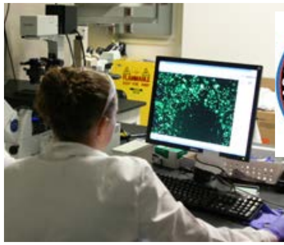
2016 to 2019: USRA Research Scientist (NASA ARC)

2019 to Current: Assistant Professor
Rensselaer Polytechnic Institute, NY

Accomplishment Highlights:

- 37 publications, which includes 20 first authorships
- With Dr. Eduardo Almeida: discovered linkage between bone loss and stem cell altered function in spaceflight, including identifying CDKn1a/p21as the central factor
- PI for upcoming ISS and Bion-M2 Space Biology-sponsored missions (peer reviewed)
- Co-I on three space shuttle spaceflight investigations
- Co-I on IBMP/NASA Bion-M2 science mission
- PI and Co-I on multiple NASA Space Biology Grants (2 NASA PI grant awards)
- Developed the NASA Space Biology GeneLab for High School Program
- NASA Early Career Public Achievement Medal; Australian Individual Innovation Award; VSSEC/NASA Australian Space Prize; ASGSB Tom Scott Student Award; ASGSR Thora Halstead Young Investigator Award





HOW DOES ALL THIS GOOD STUFF HAPPEN??

Space Biology Organization

Space Biology NASA Headquarters

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- *Kevin Sato - Acting Deputy Program Scientist – kevin.y.sato@nasa.gov*
- ***Programmatic Support:***
  - *Anthony Hickey*
  - *Linda Timucin*

NASA Ames Research Center

- Acting Project Manager: **Diana Ly – diana.c.ly@nasa.gov**
- Acting Project Scientist: **Marianne Sowa– marianne.sowa@nasa.gov**

NASA Kennedy Space Center

- Life Science Utilization Manager: **Bryan Onate– bryan.g.onate@nasa.gov**
- ISS Research Office Chief Scientist: **Howard Levine – howard.g.levine@nasa.gov**

PI's, co-I's, Post-docs, Students, Lab Techs, Admins

Space Biology Approach

Priorities:

- Continue prioritizing enabling exploration and pioneering scientific discovery
- Maximize utilization of ISS
- Leverage on partnerships and shared funding to increase reach of science
- Execute the highest quality science possible to enable agency goals and objectives

Executing Science

- Continue annual NRA cadence – annual call and focused NRAs as required
- Find better balance between ISS and other platforms for spaceflight
- Continue balance of ground vs. flight within portfolio
  - This allows for promoting ground experiments to flight if promising ground data is generated
- Follow select for definition funding profile followed by release of majority of funds when grant is selected for flight
- Formulate tangible path forward for rodent research flight payload execution

Largest Challenges

- Crew time availability
- Cold stowage availability
- Balance new start BLEO and Lunar Investments with ISS Utilization

TRENDS, FORECASTING SCIENCE DIRECTIONS IN THE COMMUNITY

FORECASTING SCIENCE NEEDS - Space Biology Strategy

Enable exploration and pioneer scientific discovery in flat budget environment:

- Align with NASA's needs without diluting NASA's ability to fund fundamental research for new knowledge
- Increased funding of individual grants reduces the number of awards while there is interest from a larger applicant pool
- Expanding to other platforms without additional resources reduces ability to fund science robustly
- Use synergies with other government agencies and international partners

Develop alternative platform science pipeline while emphasizing ISS research

- ISS crew time is at an all time high
- Pursuing partnerships and collaborations

Maintain an annual NRA cadence

- Balancing flight and ground portfolio

Maintain Outreach to Future Space Biology Scientists - Student opportunities

- High school, undergraduate, and graduate student opportunities
- Post-doctoral and new investigation researcher program

CHALLENGES OF INTEREST FOR THE DECADAL SURVEY

Programmatic Challenges

Crew Time:

- Unknowns for commercial crew mean crew time challenges that are feast or famine scenarios.
- Famine = 9 hours crew time total for SLPSRA across an ISS increment.
- Working closely with ISS to enable execution of science – this is especially challenging for ISS experiments with late load and experiment initiation requirements due to the biology

Cold Stowage:

- Many of the cold stowage assets on orbit are at capacity – very limited space for new experiments
- Working flexibilities with PIs where ever possible – many challenges for rodent, cell and plant payloads that require cold stowage for a large amount of sample biomass

Funding:

- Migrating to an approach that tracks obligations to grants vs. when funding is actually costed.
    - Allows for greater flexibility for PIs
    - Protects key PI staff and personnel in times of crew time uncertainty
- Significant requests for grant augmentations flowing in – trying to balance augmentation requests with our ability to fund and implement new awards

Beyond LEO:

- Working to balance budget for ISS activities with Beyond LEO activities

PROBLEMS IMPLEMENTING Space Biology Rodent Research

- 10 Rodent Investigators were selected for definition from 3 NRA solicitations from 2012-2014
  - (2012 SB NRA, 2014 ILSRA, 2014 Space Biology NRA)
- Delp, Willey and Moa were integrated, and investigators made concessions in order to fly
- Pecaut cancelled and brought back due to HRP need for the data. Only completed 2 of 3 aims
- No Space Biology Rodent Flight Research solicitation since 2014 – Additional costs of delays - $5M

PI	Year Solicited	Title	RR	Flight
Turek	2014	Effects of Spaceflight on Gastrointestinal Microbiota in Mice: Mechanisms and Impact on Multi-System Physiology	RR-7	SpX-15 2018
Delp	2012	Simulated Microgravity-Induced Systemic Inflammation and Its Impact on Circulatory Function and Structure	RR-9	SpX-12 2017
Willey	2014	Space flight environment induces remodeling of vascular network and glia-vascular communication in mouse retina	RR-9	SpX-12 2017
Mao	2014	Space flight environment induces remodeling of vascular network and glia-vascular communication in mouse retina* Flight 1	RR-9	SpX-12 2017
Pecaut	2012	Impact of Spaceflight on Primary and Secondary Antibody Responses	RR-12	NG-11 2019
Mao	2014	Space flight environment induces remodeling of vascular network and glia-vascular communication in mouse retina* Flight 2	RR-18	SpX- 21 2020
Almeida	2014	Role of P21/CDKN1a Pathway in Microgravity-Induced Bone Tissue Regenerative Arrest - A Spaceflight Study of Transgenic P21/CDKN1a Null Mice in Microgravity	RR-10	NG-14 2020
Taylor (replaced Robbins)	2014	Vascular Health in Space: MicroRNAs in Microgravity	RR-11	TBD
Robling	2014	Foundational In Vivo Experiments on Osteocyte Biology in Space	RR-15	TBD
Tahimic (Globus retired)	2014	Free Radical Theory	TBD	TBD
Christenson (Tash retired)	2014	Female reproductive health: Space flight induced ovarian and estrogen signaling dysfunction, adaptation, and recovery	TBD	TBD

Standard Housing: Hut/No-Hut ISS Flight Test

Rodent Housing Background

- First 5 missions had no added animal enrichment or sleep arrangements.
- A hut (similar to an standard igloo) was added for the 6th rodent research mission to comply with Flight-IACUC requirements for housing released in 12/2018
- Some results obtained since have differed significantly from decades of published results on the physiological consequences of spaceflight
- Space Biology in collaboration with HRP, ISS and CASIS is directing development and implementation of a flight study to study these inconsistencies and improve the rodent research evidence base

SB convened a Science Definition Team (SDT) on July 8, 2019 including NASA scientists and other SMEs to discuss an RR mission to address concerns of the Flight IACUC regarding animal welfare and PI community regarding possible impacts of the hut and answer the following questions:

- Does use of a sleeping/huddling hut provide measureable improvement in mouse health & welfare?
- Will the use of the hut alter NASA's understanding of the effect of spaceflight on physiology?

Study Objective: The mission goal is to measure nesting Hut effects on various physiological systems, while maintaining animal health and welfare requirements. Health and science measures will be combined to measure and document animal well-being.

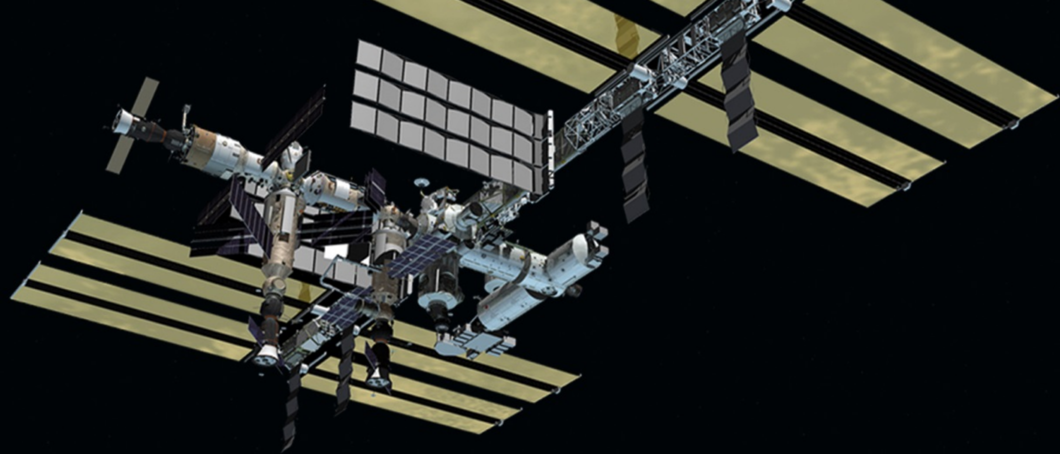
Outcome measures – SB will facilitate a study design review by non-advocate peer review :

- Detailed analyses of animal behavior via video collection and identifying individual animals
- Determine body temperature and relationship to circadian rhythm, rest-activity cycles, and metabolism using data loggers
- Measurement of body mass as an indication of overall animal health and well being.
- Analyses of cancellous bone microarchitecture and muscle atrophy, immune function, cardiovascular structure and function, and physiological indicators of stress

OPEN QUESTIONS

- Strategy for Maximizing Scientific Research through Potential future collaboration with partners on the Rodent Mission of Unusual Specimen Quantity (RMOUS-Q)?
- What emphasis should NASA give to Rodent Research on ISS given the crew time and Flight ACUC issues?
- How to balance research approaches and platforms in a flat budget environment? What research in what environment
- What can we do to make the Pull-push approach work better??
- What new hardware capabilities are needed to enable NASA to maintain cutting-edge science in the upcoming decade?

National Aeronautics and Space Administration



Space Biology

Nicki Rayl, David Tomko, Kevin Sato, Anthony Hickey, Howard Levine, Bryan Onate, Marianne Sowa, and Diana Ly are the main contributors to preparing this presentation. I am grateful to all of them, as well as those who are unnamed here. Thank you!

QUESTIONS?

CBPSS at NAS October 23, 2018

Year	Solicitation Number	Description	Step 1s/ NOIs	Proposals Received	Proposals Selected	Selection (%)
2018	NNH18ZTT001N-PT	Appendix C: Development of Microgravity Food Production: Plant Watering, Volume Management, and Novel Plant Research on the International Space Station	34	22	TBD	TBD
2018	NNH18ZTT001N-FG	Appendix B: Solicitation of Proposals for Flight and Ground Space Biology Research	100	71	15	21%
2018	NNH18ZTT001N-EM1	Appendix A: Orion Exploration Mission-1 Research Pathfinder for Beyond Low Earth Orbit Space Biology InvestigationsZ	16	12	4	33%
2017	NNH16ZTT001N-FG	Appendix G: Solicitation of Proposals for Flight and Ground Space Biology Research	141	108	29	27%
2017	NNH16ZTT001N-AB	Appendix F: Solicitation of Proposals to Conduct Research on Antarctic Balloon Flights	12	9	1	11%
2017	NNH16ZTT001N-PS	Appendix E: Solicitation of Proposals to Conduct Research In Parabolic and Suborbital Flights	16	11	1	9%
2017	NNH16ZTT001N-MS	Appendix D: Solicitation of Proposals to Conduct Research Using Microgravity Simulation Devices	53	43	13	30%
2017	NNH16ZTT001N-BION	Appendix C: Solicitation of Proposals for Possible Inclusion in a Russian Bion-M2 Mission	35	17	9	53%
2017	NNH16ZTT001N-MOBE	Research Opportunities for Post-Doctoral Fellowships in Space Biology to Study the Microbiome of the ISS as a Built Environment: Using ISS as a Microbiological Observatory	14	12	3	25%
2016	NNH16ZTT001N-GL	Appendix A: GeneLab Innovation Awards for Translational Systems Biology and Informatics Research Using the GeneLab Data System	45	34	6	18%
2014	NNH14ZTT002N	Research Opportunities for Flight Experiments in Space Biology (ILSRA)	45	38	10	26%
2014	NNH14ZTT001N	Spaceflight Research Opportunities in Space Biology	112	92	26	28%
2012	NNH12ZTT002N	Research Opportunities in Space Biology	116	100	31	31%
2011	NNH11ZTT002N	Research Opportunities in Space Biology	52	52	15	29%
2008	NNH08ZDA009O-SCMAFSB	Small Complete Missions of Opportunity in Astrobiology and Fundamental Space Biology	10	6	2	33%
2009	NNH09ZTT004N	Research Opportunities for Flight Experiments in Space Life Sciences: Biological Research In Canisters for Arabidopsis thaliana	0	4	4	100%
2009	NNH09ZTT003N	Research Opportunities in Space Life Sciences: Fundamental Space Biology - Animal Physiology	25	25	5	20%
2009	NNH09ZTT002N	Research Opportunities for Flight Experiments in Space Life Sciences (ILSRA 2009)	13	11	6	55%
2008	NNH08ZTT003N	Research Opportunities for Fundamental Space Biology Investigations in Microbial, Plant and Cell Biology	71	69	17	25%
2007	NNH07ZTT001N	Research Opportunities for Space Flight Experiments: BION-M1 Project	43	33	10	30%
2004		Research Opportunities for Flight Experiments in Space Biology (ILSRA 2004)	154	148	12	8%
2003		Fundamental Space Biology NRA 03	120	118	28	24%
2001		Fundamental Space Biology 2001	119	100	28	28%
2001		STS-107 Biospecimen Sharon Plan	0	8	3	38%
2000		Life Sciences Intl Flight NRA (ISLRA 2000)	172	115	11	10%
2000		Fundamental Space Biology NRA 2000	217	157	31	20%
Total		Total (2000-2018)	1735	1415	320	23%

Going forward

International Space Life Sciences Working Group (ISLSWG) Plant Workshop Planning

Initiated and Coordinated by NASA

Topics to be included:

- Plant and Soil Microbiome
- Microgreen Research
- Improving Photosynthetic Efficiency

Potential International Co-sponsors/Partners

- Italian Space Agency (ASI)
- French Space Agency (CNES)
- German Space Agency (DLR)

Venue and Date - TBD

- U.S., Italy, France or Germany
- Within the next 12-18 months
- Proceedings published as a peer-reviewed journal supplement
- Coordinated with a Major Plant Biology Association meeting

Space Biology RESEARCH FOR HUMAN EXPLORATION

National Aeronautics and Space Administration

NASA

GROUND-BASED RESEARCH HIGHLIGHT

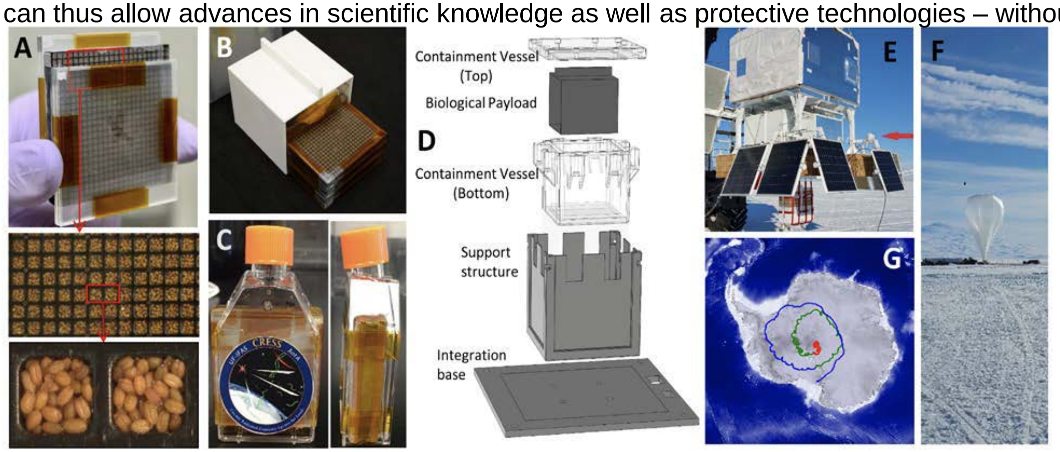
Approaches for Surveying Cosmic Radiation Damage in Large Populations of *Arabidopsis thali*ana Seeds – Antarctic Balloons and Particle Beams." B. Califar, R. Tucker, J. Cromie, N. Sng, R.A. Schmitz, J.A. Callaham, B. Barbazuk, A-L. Paul, and R.J. Ferl. *Gravitation and Space Research, Volume 6 (2) December 2018.*

Background: The Cosmic Ray Exposure Sequencing Science (CRESS) 1U CubeSat was the 1st whole-genome characterization of space-irradiated seeds & demonstrated the efficiency & efficacy of **Antarctic (ANT)** long-duration balloons for the study of **space radiation effects on eukaryotes** using *Arabidopsis thaliana* seeds. It was designed as a secondary payload for a **high-altitude (36-40 km), 30 day south polar balloon flight** and compared to a simulation of Galactic Cosmic Rays (GCR) at NASA's Space Radiation Lab (NSRL) at the **Brookhaven National Laboratory (BNL)**. CRESS maintained 1 atm internal pressure, and carried an internal cargo of **580,000 seeds** and twelve Solid-State Nuclear Track Detectors.

Germination: Exposed BNL & ANT Generation 1 (M_0) seeds showed significantly reduced germination rates of 76.4% & 82.5%, respectively (**statistically indistinguishable from each other**) vs 98% for the controls. There were **no significant effects of radiation on germination in the next generation of seeds**, compared to that of the control group.

Mutations: Exposed BNL and ANT **Generation 1 (M_0)** seeds showed **significantly elevated somatic mutation rates** (& **developmental aberrations**) when compared to non-irradiated controls, with the **BNL mutation rate being significantly higher than that of ANT** (demonstrating significant differences between these radiation sources). There was decreased germination and viability of M_0 seed due to mutations. Mutant phenotypes in the ANT and BNL M_0 were likely to have occurred due to somatic mutations in the seed embryo, which resulted in the death or delayed growth of certain plant organs. **In the M_0 BNL and ANT test groups, the type of mutations and their frequencies differed**. The discolorations observed in the BNL M_0 were most prevalent near the shoot apex. Deficiencies of cotyledon development were also seen to be enriched in the BNL M_0 seedlings. However, the ANT M_0 also contained a subset of abnormally-colored and stunted mutants. Dwarf architecture was statistically significant in its enrichment within the M_0 BNL test group. **BNL Generation 2 (M_1)** seeds had a significantly higher mutation rate than the non-irradiated control plants (averaging 6 aberrations per 100 germinated seeds), which was significantly lower than that observed in the M_0 BNL seeds. There were therefore heritable mutations obtained in the BNL M_1 seeds that were the result of radiation damage to the M_0 BNL seeds.

Conclusions: These results show that long duration Antarctic balloon flights (low dose & diverse particle types) provide exposure to enough ionizing radiation to realistically emulate, not just simulate, deep space radiation exposure for the purposes of gathering data on genomic impacts, and therefore provide insights on the biological effects of ionizing radiation on eukaryotes relevant to space exploration. The further development of Antarctic balloon-based biological payloads can thus allow advances in scientific knowledge as well as protective technologies – without requiring travel beyond the Earth's magnetic field.



Payload Design for BNL Exposure & ANT High-Altitude Balloon Flight. (A) A seed tray flanked by Solid-State Nuclear Track Detector sheets. Each bin contained ≈ 250 *A. thaliana* seeds. (B) The custom 3D-printed holder used in the Antarctica payload. (C) The flask containing the biology irradiated at the NASA Space Radiation Lab (NSRL). (D) Diagram of the individual components and configuration of the ANT payload for the balloon flight. (E) The ANT payload (red arrow) integrated onto the primary payload. (F) Balloon being inflated. (G) Path of the payload over Antarctica, where blue indicates earlier time points and red indicates time points at the end of the flight.