Space Biology

National Academy of Science Project: A Midterm Assessment of Implementation of the Decadal Survey on Life and Physical Sciences Research at NASA

February 7, 2017 3:15 – 4:15 PM

Space Life and Physical Sciences Division
Human Exploration and Operations Mission Directorate
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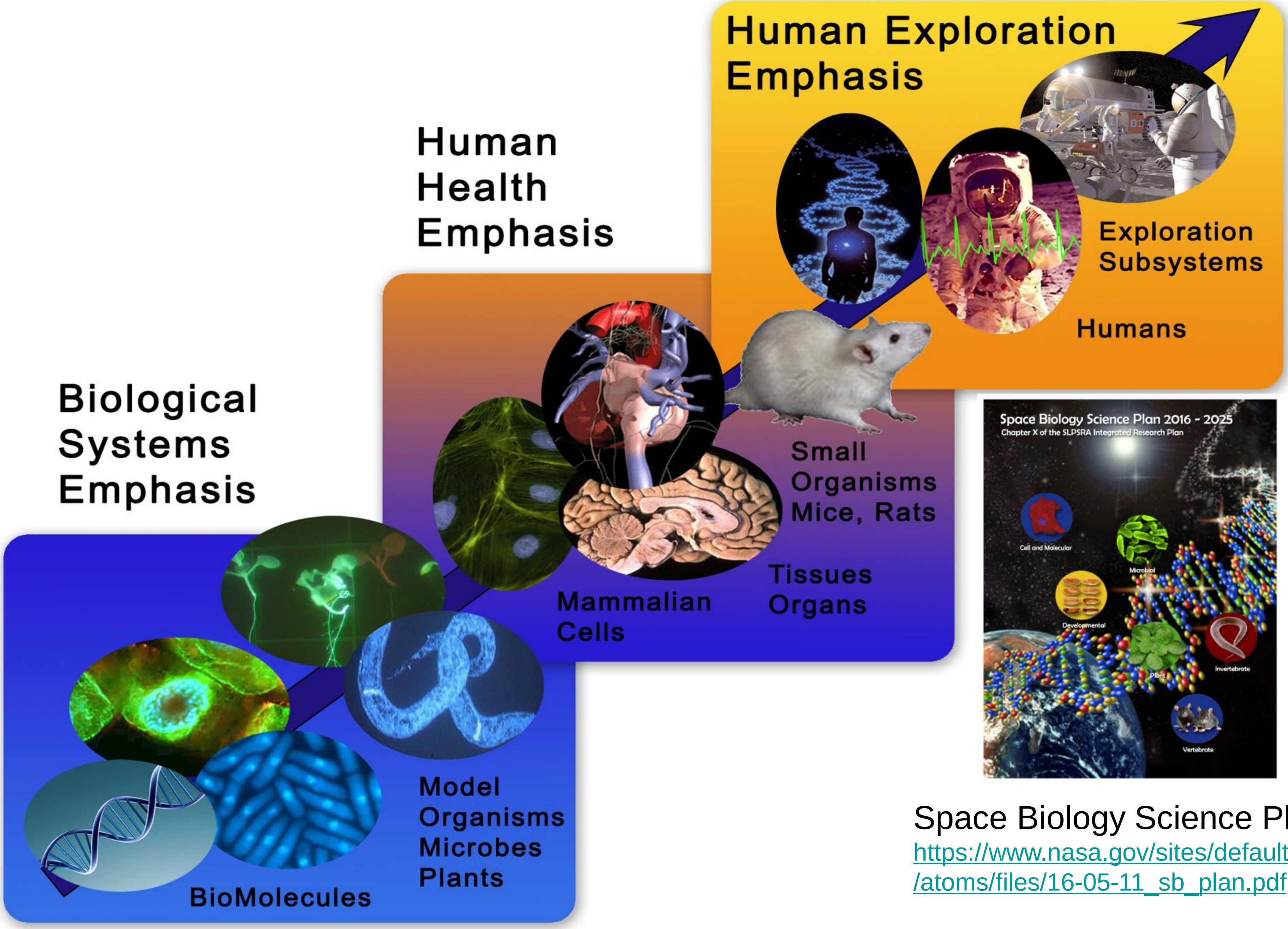
(With A Hickey, E Keller, H Levine, K Sato, N Rayl, E Taylor)

Research for Human Exploration

Space Life Sciences Research Continuum



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

What is Translational Research by Design?

- 2011 NRC Decadal Survey Recommended Horizontal Integration and Vertical Translation within the NASA Life Sciences Portfolio
  - **Horizontal Translation:** increase integration of efforts within each level of research, toward conduct of cross-disciplinary, focused translational research and development with applications to human space risks and related areas supporting human space exploration.
  - **Vertical Translation:** Research teams to create "meaningful interactions among basic, preclinical, and clinical scientists to translate fundamental discoveries into improvements in health and well-being of crew members in space and their re-adaptation to gravity."
- **For Space Biology and HRP management** - Identify a knowledge gap that basic research can potentially fill, and solicit research to fill that gap.
  - This can either be "pull", where the program with the gap asks for the research or "push", where the basic science program sees a potential solution to an applied problem and solicits research to fill it.
  - Either way, the solicitation of the fundamental research is "**designed**" to fill a particular gap, or answer a particular question.

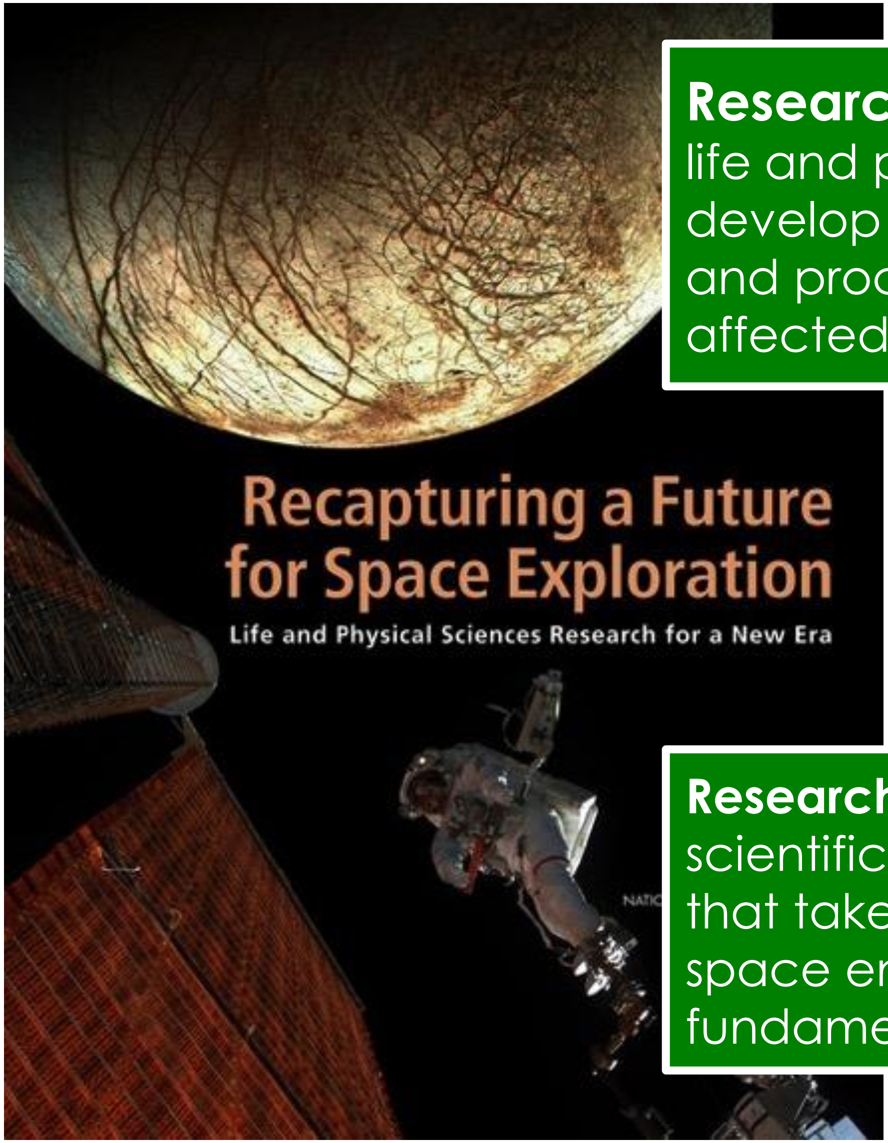
Space Biology and Human Research Program

Basic Research

Space Biology (SB)	Synergies	Human Research Program (HRP)
How does life respond, adapt, develop, interact and evolve in spaceflight across gravity levels? • Microbiology • Cell & Molecular Biology • Plant and Animal Biology • Developmental, Reproductive & Evolutionary Biology • Systems Biology & Omics (GeneLab) ***Science exploring the unknown***	**SB** and **HRP** coordinate to define research priorities and identify opportunities to facilitate countermeasure development o Cell, Tissue & Animal Studies o Immunology o Wound healing & fracture repair o Radiation/Microgravity interactions o Oxidative Stress and Damage o Microbe-Host Interactions o Visual Impairment Syndrome o Artificial Gravity/ Gravity as a Continuum	**Identify, characterize, and mitigate human health & performance risks in spaceflight** • Exercise Countermeasures • Physiological Countermeasures • Space Radiation Biology • Behavioral Health and Performance • Space Human Factors and Habitability • Exploration Medical Capability ***Science addressing known risks***

Medical Operations

Portfolio Guided by Decadal Survey



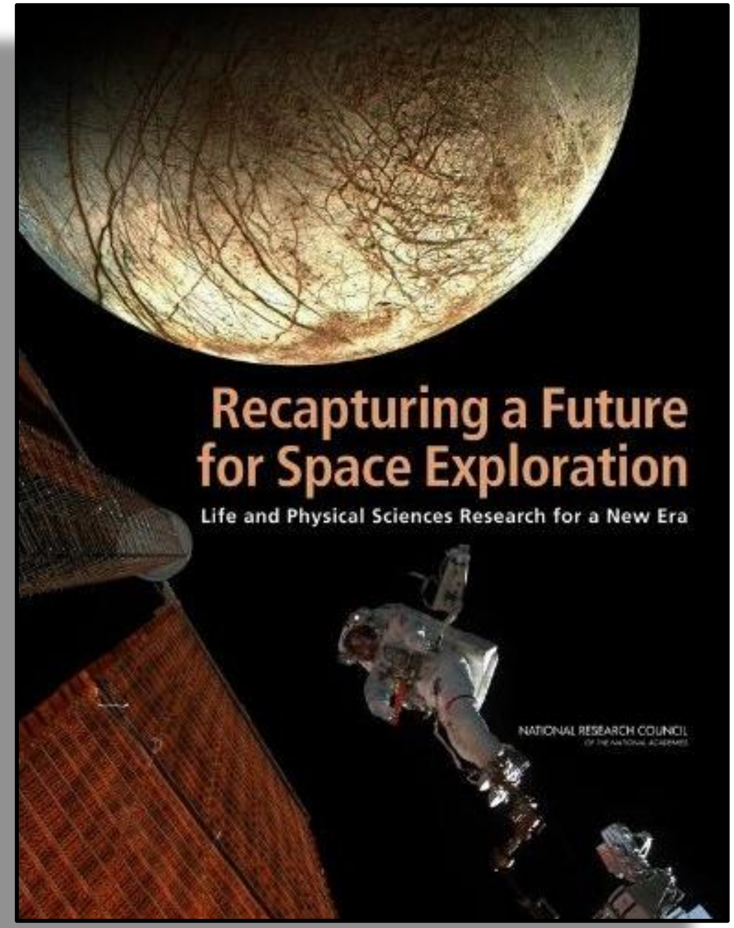
Research that enables space exploration: life and physical sciences research needed to develop advanced exploration technologies and processes, particularly those profoundly affected by operation in a space environment.

SLPS' purpose is two-fold:
Enable exploration and Pioneer scientific discovery

Research enabled by access to space: scientific research in life and physical sciences that takes advantage of unique aspects of the space environment to significantly advance fundamental scientific understanding

Space Life Sciences Recommendations for 2010-2020: Using the 2011 NRC Decadal Study

- Plant and Microbial Biology
  - ☐ Multigenerational studies
  - ☐ Responses to spaceflight
  - 🚶 Plants and microbes in closed-loop life support
- Animal and Human Physiology
  - ☐ Bone and muscle studies
  - ✖ Drug/countermeasure evaluation
  - ☐ Vascular and interstitial pressure changes during spaceflight
  - ✖ Orthostatic intolerance
  - ✖ Deposition of aerosols in lung
  - ☐ T-cell and immune system studies
  - ☐ Multi-generation and early development
- Cross-Cutting Issues for Humans in Space
  - 🏃 Artificial-G as a countermeasure
  - 🏃 Animal studies to assess radiation risks
  - ✖ Cellular studies to define biomarkers for radiation toxicity
  - 🚶 Understanding sex differences in adaption to spaceflight

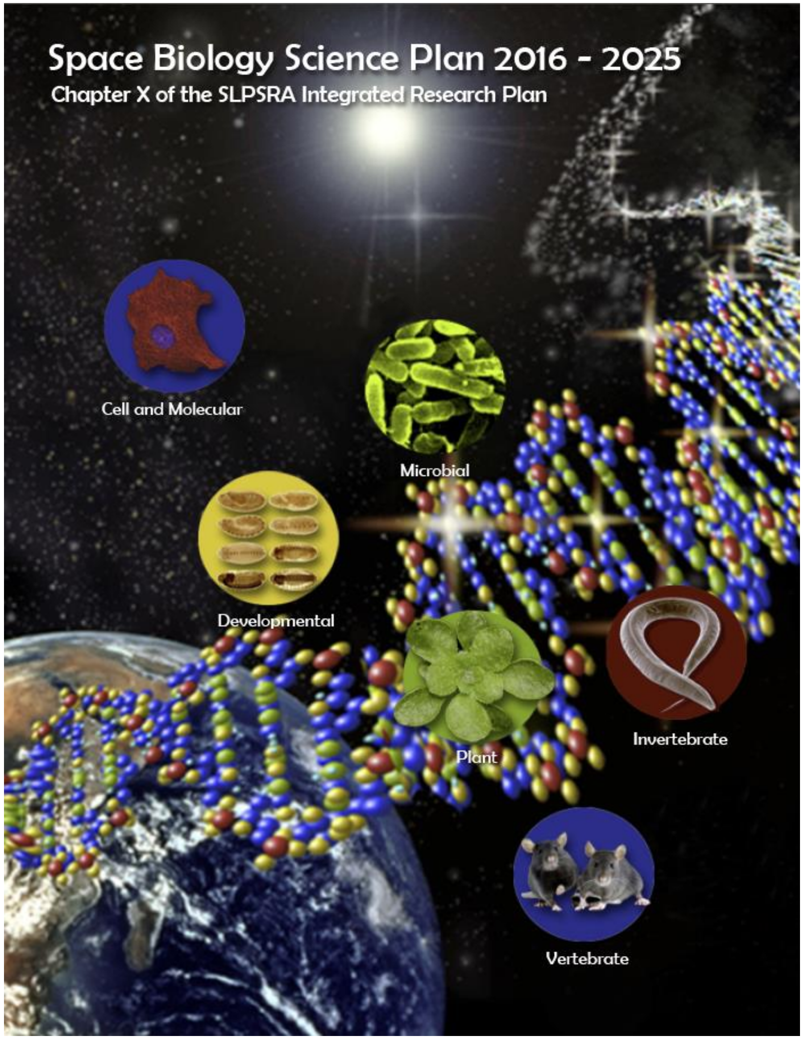


http://www.nap.edu/catalog.php?record_id=12944

Space Biology Elements and Strategic Plans

Vision and Goals

- Create new knowledge of how different gravity levels affect biological systems important to human space exploration
- Build Links between Space Biology and Human Research
- Perform translational research by design from DNA and RNA to clinical medicine
- Leverage & amplify Space Biology findings using state-of-the art omics, molecular/systems biology tools, & open access GeneLab data base
- Train and inspire a new generation of Space Biologists



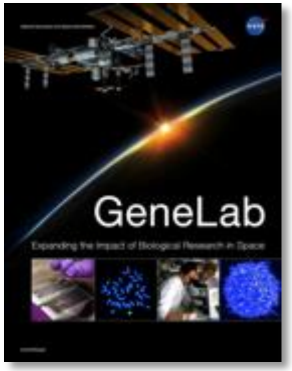
https://www.nasa.gov/sites/default/files/atoms/files/16-05-11_sb_plan.pdf

Implementation Strategies

- Scientific Investigations
  - PI-led Individual Projects
  - Team-led
    - Solicited Science Integration Teams
    - Co-Principal Investigator Teams

- Disseminate results
  - Publication in peer reviewed journals
  - Timely data deposition
    - With space flight metadata
    - With unique analysis tools
  - Supported by GeneLab, Physical Science Informatics, MaterialsLab, etc.

- Partner to ensure value and increase capability
  - Exploration customers (AES, HRP, STMD)
  - Potential adopters (CASIS, NIH, NIST, NSF, OGAs; international partners; industry)



Space Biology: Agenda

Topics to be covered today

- Status
- Planning
- Research findings/Accomplishments
- Genelab Update

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Space Biology Ground Research Status - I

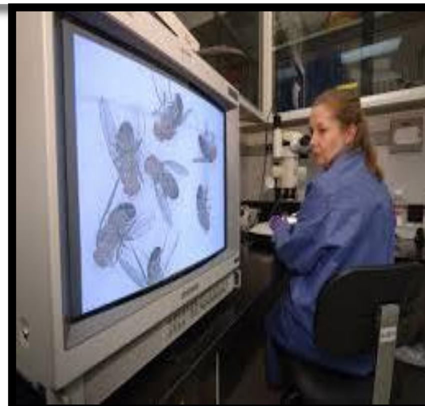
Current Ground Grant Status:

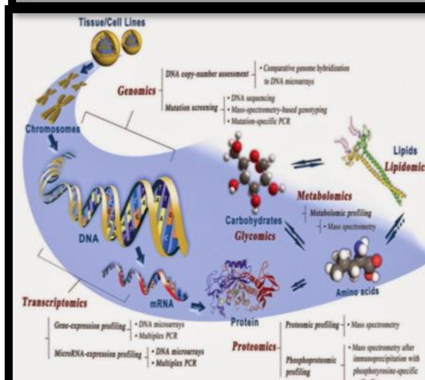
• 16 Ground NRA Space Biology grants

PI Last Name	Project Title
Bloomfield	Iron Overload and Oxidative Damage: Regulators of Bone Homeostasis in the Space Environment
Delp	Disuse Osteopenia: A Potential Vascular Coupling Mechanism--NNX14AQ57G
Foster	Impact of Microgravity on the Cell-Cell Interactions between a Mutualistic Bacterium and Its Animal Host
Haswell	Mechanosensitive Ion Channels in Plants: Genetic, Computational and Systems-Level Approaches to Underst
Hoffman	Gravity-Induced Plasticity in Mammalian Utricular Hair Cells: Intrinsic or Multisensory?
Hogan	Pre-Treatment Approaches for Improving the Response of Bone to Disuse and Recovery
Judex	Genes that Predict the Loss of Bone during Weightlessness
Lawler	Redox Regulation of nNOS Translocation and Muscle Atrophy During Mechanical Unloading
Mao	Role of Oxidative Stress in Mediating the Effects ofCombined Exposure to Simulated Microgravity and Radiatic
Mehta	Determination of Roles of Microgravity and Ionizing Radiation on the Reactivation of Epstein-Barr Virus
Monshausen	Defining the Role of the Receptor Like Kinase FERONIA in Plant Architecture Development Under Mechanical L
Rojas-Pierce	The Role of Vacuole Membrane Fusion in Plant Gravity Perception
Sabanayagam	Epigenetic and Protein Expression Pattern Profiling of Caenorhabditis elegans Exposed to Time-Varying Gravita
Sams	Integration of Mechanotransduction and T-cell Activation Thresholds: Understanding of the Effects of Mechani
Ferl	Hypobaric Plant Biology - Molecular Responses of Arabidopsis to the Low Atmospheric Pressures of Spaceflight
Roux	Rapid Signaling Changes Induced by Gravity in Cells of the Fern Ceratopteris richardii

• 2 Space Biology/HRP Artificial Gravity grants

- 15-15 NASA AG-0013 "Partial-Gravity Dose Response: Roles of vestibular input & sex in response to AG" - Charles Fuller (University Of California, Davis) Team Alwood, Hoban-Higgins, Ronca
- 15-15 NASA AG-0005 "Musculoskeletal response to partial-gravity analog in rats: structural, functional & molecular alterations" - Seward Rutkove (BETH ISRAEL DEACONESS) Co-I Bouxsein





Space Biology Planning: Gravity as A Continuum Example – Body mass and bone density

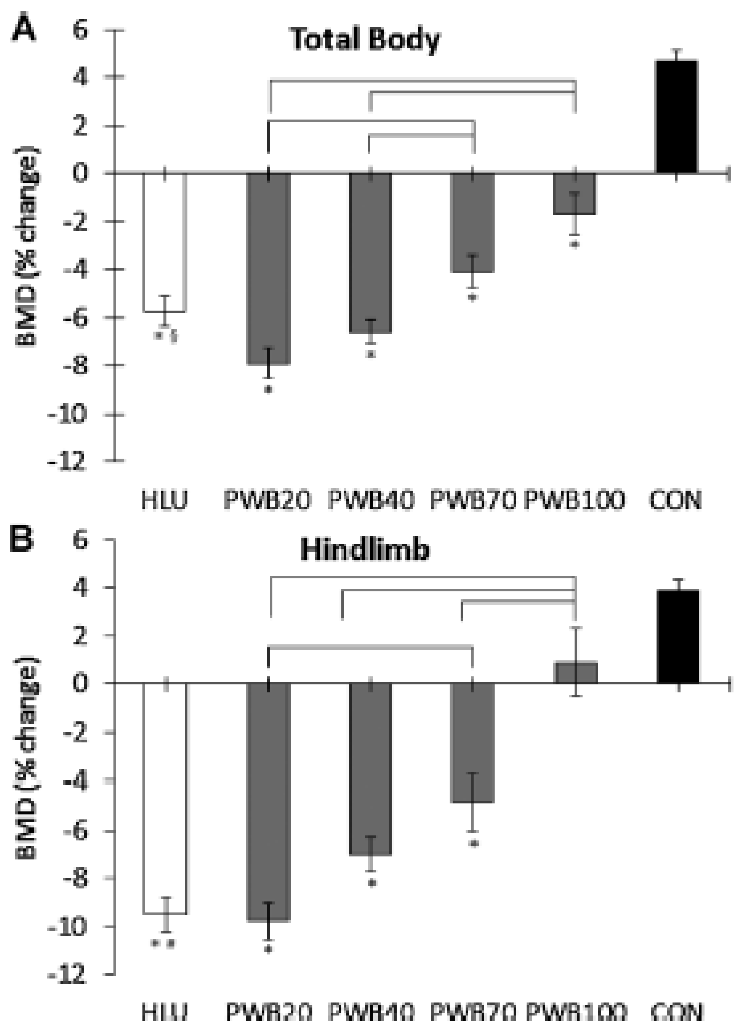


Fig. 5. Percent change in (*A*) total body and (*B*) hindlimb bone mineral density from baseline to day 21 (mean ± SEM). * = Different from CON ($p < 0.05$), § = HLU different from PWB20, PWB100 ($p < 0.05$), # = HLU different from PWB70, PWB100 ($p < 0.05$), brackets = pairwise differences between PWB groups (ANOVA with Scheffé post hoc, $p < 0.05$).

Partial Reductions in Mechanical Loading Yield Proportional Changes in Bone Density, Bone Architecture, and Muscle Mass - Rachel Ellman, Jordan Spatz, Alison Cloutier , Rupert Palme, Blaine A Christiansen, and Mary L Bouxsein Journal of Bone and Mineral Research, Vol. 28, No. 4, April 2013, pp 875–885

Space Biology Ground Research Status - II

6 GeneLab Innovation grants awarded October 2016

PI Last Name	Organization	Title	Experimental or Tool Development	Model System	NEW SB PI or Early Career Investigator?	Center Mgmt
Allen	University Of Florida, Gainsville	Microgravity effects on co-cultured vascular cells types	Experimental	Cocultures of endothelial and circulating stem cells	Yes (Young Investigator)	ARC
Gilroy	University Of Wisconsin, Madison	Genelab: Revealing Spaceflight- and Gravity-Response Networks in Plants	Experimental and Tool Development	Arabidopsis	No	KSC
Keller	University Of Wisconsin, Madison	Omics data mining of the ISS Aspergillus fumigatus strains in elucidating virulence characteristics	Experimental	Aspergillus	Yes (New)	ARC
Meyerowitz	Cal Tech	Using GeneLab Data to identify novel gravity sensory components in Arabidopsis	Experimental	Arabidopsis	Yes	KSC
Nicholson	University Of Florida, Gainsville	Comparative evaluation of microbial transcriptomic responses to spaceflight stress: elucidating underlying molecular mechanisms	Experimental and Tool Development	B. subtillis and other bacteial strains	No	KSC
Porada	Wake Forest University	Effects of microgravity on the risks of space radiation-induced leukemogenesis	Experimental	Human Hematopoetic Stem Cells	Yes (New)	ARC

Space Biology Flight Research Status
Partnership and Collaboration with Physical Sciences

Characterization of Biofilm Formation, Growth, & Gene Expression on Different Materials & Environmental Conditions in Microgravity – PI Luis Zea (Univ Colorado)
Characterize impact of long duration spaceflight on Biofilm Mass, Thickness, Morphology, & microbial gene expression of Uropathogenic *Pseudomonas aeruginosa* and *Penicillium chrysogenum* to form and maintain biofilms on different surfaces commonly used in spacecraft, cellulose, aluminum 6061, titanium alloy, polycarbonates, silicon, stainless steel and carbon fibers. Biofilm formation and risk of causing equipment malfunction and human illnesses is a problem that needs to be addressed to enable safe long-term human space exploration. Experiments will elucidate biomechanical & transcriptomic mechanisms involved in formation of "column-and canopy" biofilm architecture observed in space & assess gene expression associated with conferring resistance to oxidative stress, acidity, & antimicrobials on microorganisms in biofilms.

Polymicrobial biofilm growth & control during spaceflight - Robert McLean (Texas State - San Marcos) Microorganisms grow as surface-adherent biofilm communities in spaceflight. Organisms in biofilms are resistant to traditional antimicrobial chemicals & can foul water treatment filters. Biofilms on ISS include many species, but studies to date have only tested single-species biofilms. Experiments will characterize how two bacterial species (*E. coli* and *P. aeruginosa*) interact, and how interactions impact biofilm's ability to adhere to surface, antimicrobial resistance, & ability to degrade a metallic surface. Characterize biological mechanisms by which these microbes interact with each other, such as changes in quorum-sensing and other secreted proteins. Transcriptomic analyses & changes in microbial gene expression will provide insights into effects of spaceflight on microbial physiology.

Space Biology - Pioneering Scientific Discovery Reference Mission Team Experiments

Effects of Spaceflight on Gastrointestinal Microbiota in Mice: Mechanisms & Impact on Multi-System Physiology. Lead - Fred Turek, (Northwestern) Team members - Green, S (Univ Ill), Keshavarzian, A (Rush Univ Med Ctr), Forsyth, C (Rush Univ Med Ctr), Vitaterna, M (Northwestern)
Examine potential role(s) of disruption of microbial communities in gut (dysbiosis) in mammalian adjustment to space environment. Challenges to mammalian physiological homeostasis & immune, inflammatory, & metabolic changes in spaceflight may be attributable in part to dysbiosis. A series of experiments in mice will define mechanisms by which time in space, diet, & host genotype interact to impact the gut microbiota, & how dysbiosis relates to gene expression (by RNA-seq) & physiology in serum, colon, ileum, spleen, liver, & fat as well as the sleep/wake cycle & feeding behavior.

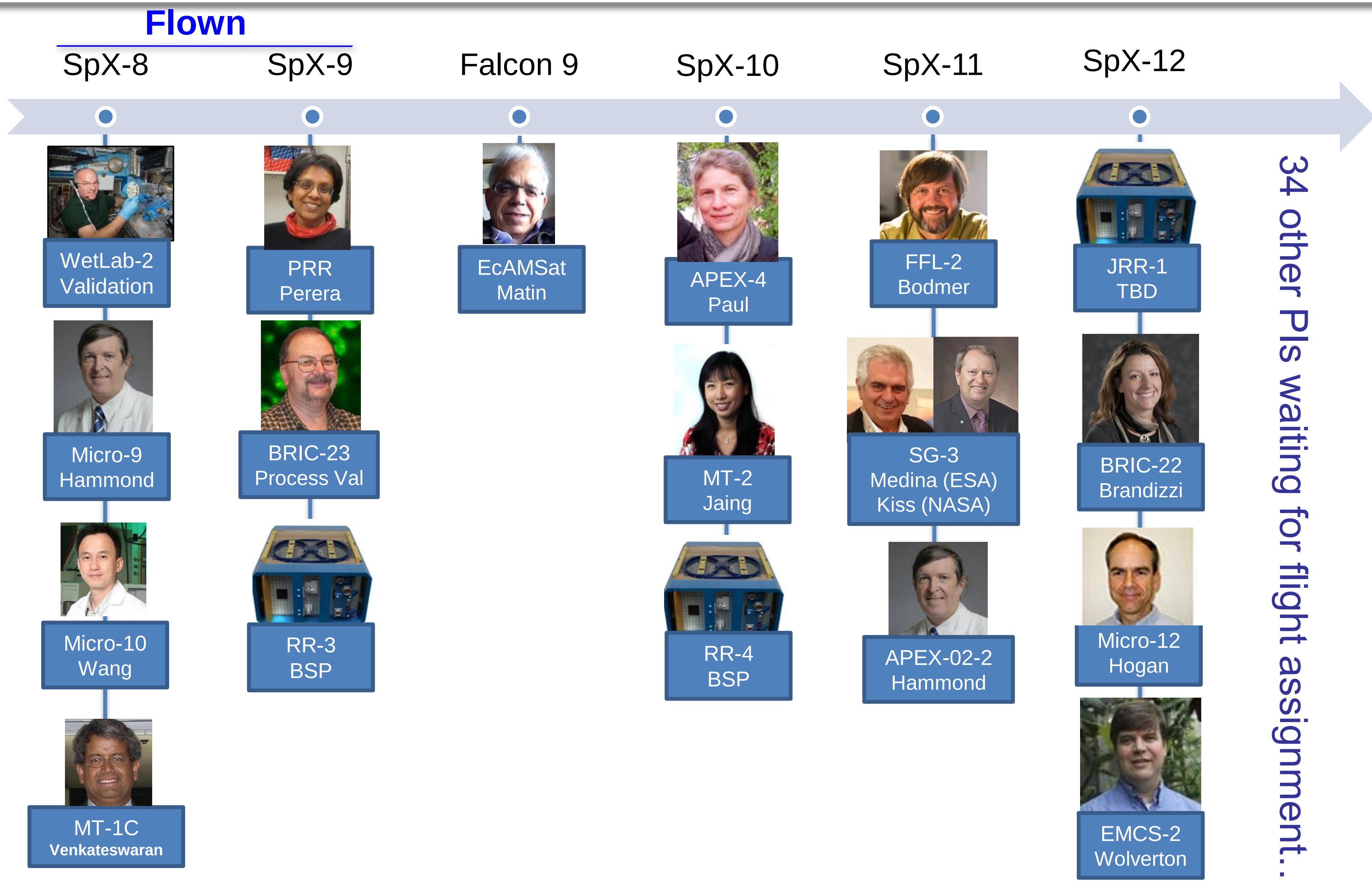
ISS Microbial Observatory of Pathogenic Virus, Bacteria, & Fungi (ISS-MOP) project. Lead - Crystal Jaing, (Lawrence Livermore) Team members - Mehta, S (Wyle), Pierson, D (NASA JSC), Smith, D (NASA ARC), Venkateswaran, K (NASA JPL) In this ISS-MOP project a cultivation-based assay (Aim 1) provides a valid estimation of microbial presence, but offers a limited assessment of the phylogenetic/pathogenic diversity & physiological breadth of the microbial population present. So, the team will use novel & comprehensive LLMDA microarray specifically designed to elucidate the pathogenic viral & microbial diversity profiles of complex samples to at least the species level (Aim 2). In addition, they will characterize virulence & antibiotic resistance mechanisms associated with these ISS pathogens by developing a new Virulence & Antibiotic Resistance gene array & analyze the samples on this array. The final deliverable of the proposed project to NASA will be the ISS-MOP dataset, a compilation of all of the genomic sequences & genetic information of viruses & microbes encountered on & within the ISS habitat (Aim 3).

Space Biology - Pioneering Scientific Discovery Reference Mission Team Experiments

Integrated Omics Guided Approach to Lignification & Gravitational Responses: The Final Frontier Lead - Norman Lewis (Washington State) Team members - Davin, L (Washington State), Hanson, D (Univ NM), Lipton, M (Battelle Memorial Inst), Sayre, R (NM Consortium), Starkenburg, S (Los Alamos Natl Lab) Arabidopsis multi-omics study (metabolomics, transcriptomics, proteomics, phenomics) melded with integrated computational biology (ICB) approach. Plant lines include mutants with different lignin amounts & lines enhanced in C assimilation capacity. Will investigate gene/metabolic network relationships & adaptations resulting from varying lignin & C assimilation levels on photosynthesis, C allocation, water use efficiency (WUE), plant growth/development, vasculature performance, auxin transport & gravitational adaptations. integrated omics analyses of factors controlling lignin will provide new insights into global effects on plant biological processes at both 1g & in micro-g.

High dimensional biology to understand the functional response of Salmonella to long-term multigenerational growth in the chronic stress of microgravity Lead - Cheryl Nickerson, Arizona State Team members - Abu-Ali, G (Harvard) Barrila, J (Arizona State), Huttenhower, C (Harvard), Ott, M (NASA JSC), Travisano, M (U Minnesota) Investigators propose that long-term growth in spaceflight culture will induce heritable genomic & epigenetic changes in micro-organisms with adverse phenotypic outcomes that could have a profound impact on risk to crew health & vehicle system performance. They hypothesize that long-term multigenerational culture of,S. Typhimurium in flight will result in stress-induced mutations & genomic instability as reflected in genomic, epigenetic, transcriptomic & select phenotypic characteristics changes (including virulence) that reflect a central role for Hfq as a global regulator of these heritable responses. They will characterize the impact of long term spaceflight culture (~300 generations) of an S. Typhimurium wild type & hfq mutant on genomic, epigenetic, transcriptomic, virulence & pathogenesis-related characteristics.

Space Biology Upcoming Payloads Status



Space Biology Upcoming Payloads Status

- *Non-ISS Free Flyer: EcAMSat NET January 2017 (Formosat-5/SHERPA)*

- SpaceX-10 (January 2017)

  APEX-04 (PI: Paul) PI/GeneLab collaborative experiment with *Arabidopsis* will be the first spaceflight experiment to directly investigate how the plant methylome contributes to space adaptation.

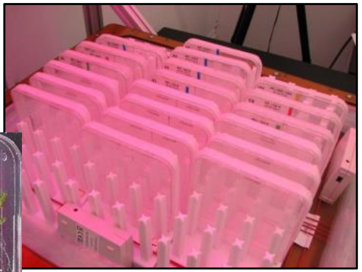
  MT-2 (PI:Jaing) Pre-position kits

- SpaceX-11 (March 2017)

  Fruit Fly Lab-2 (PI: Bodmer) PI/translational GeneLab collaborative study of cardiac genes in the fruit fly that relate to human heart health and arrhythmia

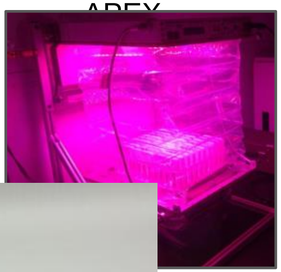
  Seedling Growth-3 (PIs: Kiss & Medina) an ESA-led experiment with NASA and ESA PIs using the EMCS to study gravity and light sensing and control in *Arabidopsis*

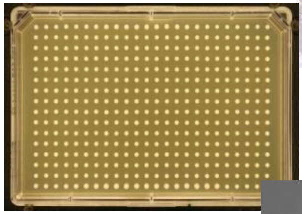
  APEX-02-02 (PI:Hammond) APEX-02R research investigates *S. cerevisiae* growth and physiological responses to the multiple stimuli encountered in spaceflight environments.

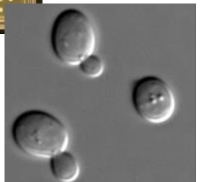


COTS *Arabidopsis* plate holder for

Arabidopsis after 12 days growth.

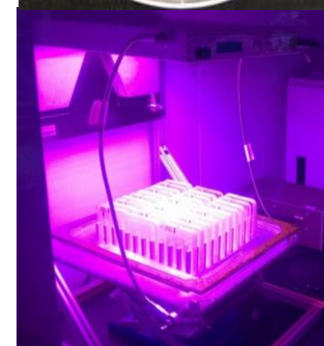


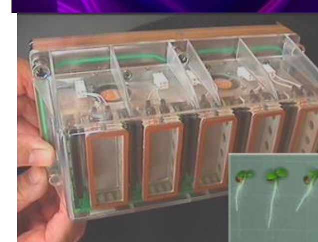




Space Biology Upcoming Payloads Status

- SpaceX-12 (June 2017)
  - JRR-1 (US and Russian PI Team) First ISS based collaborative US/Russian Rodent Research Payload (Space Biology team led by Delp and including Mao and Willey)
  - BRIC-22 (PI:Brandizzi) Expand upon results from the BRIC-18 spaceflight experiment pertaining to the regulation of stress responses in plants exposed to microgravity conditions by the AtIRE1 protein (a master regulator of transcription).
  - Micro-12 (PI:Hogan) : Investigating the Physiology and Fitness of an Exoelectrogenic Microorganism Under Microgravity Conditions
  - BRIC-24 LED SDT Mission and Hardware Validation
  - Veggie SN0001
- SpaceX-13 (September 2017)
  - APEX-05 (PI:Gilroy) This experiment will determine how responses seen on orbit can be explained by the development of long-term hypoxia linked to the microgravity environment as compared to plants grown on the ground
  - Plant Gravity Perception (PI: Wolverton) Characterize the primary statolith-based gravity sensing mechanism, including the threshold acceleration force responsible for activation and capacity for modulating growth regulation.
  - Plant Habitat-01 (PI:Lewis) Multi-omics team based investigation to modulate lignin content and vascular apparatus performance by manipulation of the arogenate dehydratase multi-gene family.





Space Biology NRAs: 2011-2016

	Solicitation Number	Description	Step 1s/NOIs processed	Proposals received	Proposals Selected	Selection %
2017		Appendix F: "Space Biology Experiment aboard the BION M2 Biosatellite"				
2017		Appendix E: Microgravity Simulation Research				
2017		Appendix C: Parabolic Flight and Suborbital				
2017		Appendix D: Balloons				
2016	SLOANMOBE2016	Sloan ISS MoBE Postdoctoral Fellowship - To Study Microbiome of ISS as a Built Environment: Using ISS as a Microbiological Observatory	6	5	TBA	TBA
2016	NNNH16ZTT001N-MoBE	Appendix B: Post-Doctoral Fellowships in Space Biology to Study Microbiome of ISS as a Built Environment: Using ISS as a Microbiological Observatory	14	12	TBA	TBA
2016	NNNH16ZTT001N-GL	Appendix A: GeneLab Innovation Awards for Translational Systems Biology and Informatics Research Using the GeneLab Data System	45	34	6	18%
2016	NNH16ZTT001N	Research Opportunities in Space Biology (ROSBio) - 2016	NA	NA	NA	NA
2014	NNH14ZTT002N	Research Opportunities for Flight Experiments in Space Biology (ILSRA 2014)	45	38	15	25%
2014	NNH14ZTT001N	Spaceflight Research Opportunities in Space Biology	112	92	26	28%
2012	NNH12ZTT001N	Research Opportunities in Space Biology	116	100	31	31%
2011	NNH11ZTT002N	Research Opportunities in Space Biology	52	52	15	29%
2011	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%

Space Biology: Recent/Current Solicitations

- **NRA NNH16ZTT001N Research Opportunities in Space Biology (ROSBio)** (Release 3/24/16)
  - ROSBio-2016 is an Omnibus Research Announcement that covers all aspects of basic and applied research and technology supporting Space Biology
  - Specific research/funding opportunities announced through Appendices that solicit proposals for experiments to address Space Biology's primary objectives
  - Appendices will be released until Dec. 31st, 2017

- **NRA NNH16ZTT001N-GL Appendix A: "GeneLab Innovation Awards for Translational Systems Biology & Informatics Research Using GeneLab Data System"** (Release 3/24/16)
  - Solicited research projects to: 1) perform ground-based experiments to test novel hypotheses derived from analysis of data in GeneLab Database, or 2) develop novel computational tools to enhance usability & value of GeneLab. **Total value ~$1.4M**
  - Selected 6/32 proposals: 4 New and 2 Veteran Space Biology Investigators

- **NRA NNH16ZTT001N-MoBE Appendix B: "Research Opportunities for Post-Doctoral Fellowships in Space Biology to Study Microbiome of ISS as a Built Environment"** (Release 9/15/16 - Close 11/30/16)
  - Solicits research from post-doctoral fellows to conduct studies to characterize microbial populations isolated from ISS
  - Released in parallel with Sloan Foundation solicitation for same purpose
  - NASA and Sloan expect to grant 2 awards each with a duration of 2 years

Space Biology: Agenda

Topics to be covered today

- Status
- Planning
- Research findings/Accomplishments
- Genelab Update

Space Biology Research Planning

GREAT UNKNOWN

BioSentinel

Understand biological processes that support human health and safety in exploration beyond LEO - moon or Mars or ??

Near Earth Objects

Mars
36 million mi

Moon
240,000 mi

BECOMING KNOWN

LITTLE KNOWN

ISS & free flyers in LEO
180-300 mi

62 mi

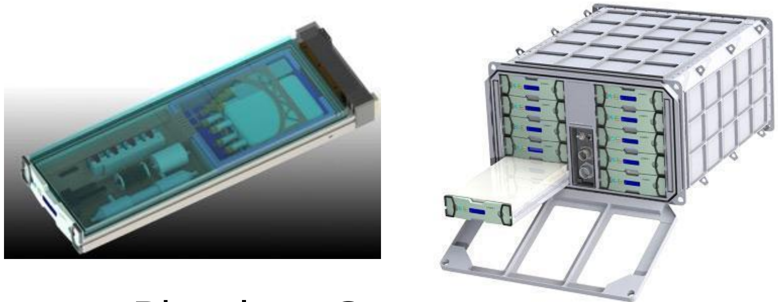
Mission Duration

Minutes- 12.5 Days

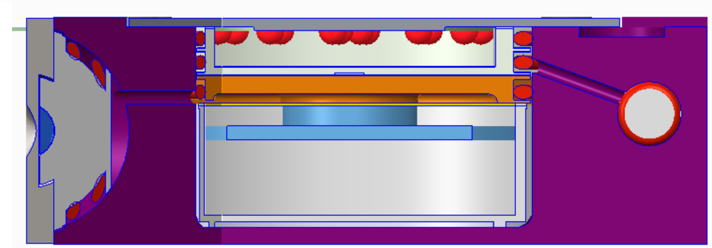
12 Months

3 Years

Space Biology Research Planning
New Hardware to Enable Research

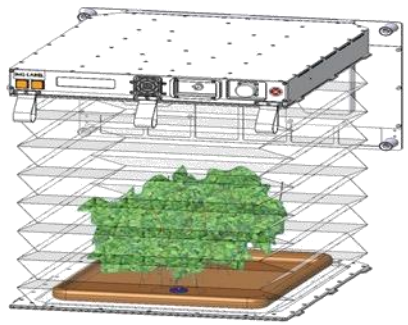


Bioculture System
(SpaceX-13: Sept 2017)



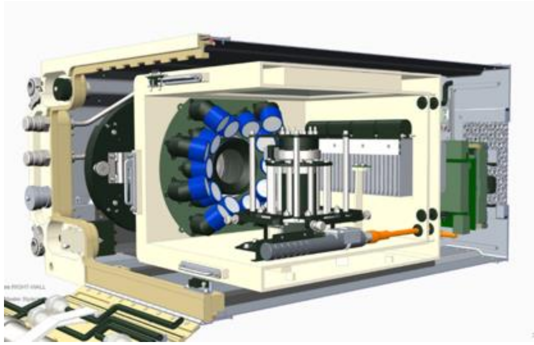
BRIC LED
(SpaceX-12: 6/1/17)

Veggie Unit #1 on ISS,
Veggie Unit #2 arrives on
SpaceX-12 (6/1/17)



WetLab 2
(On ISS, ready for use)

Advanced Plant Habitat
(OA-7: Feb 2017)

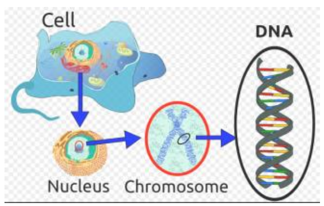


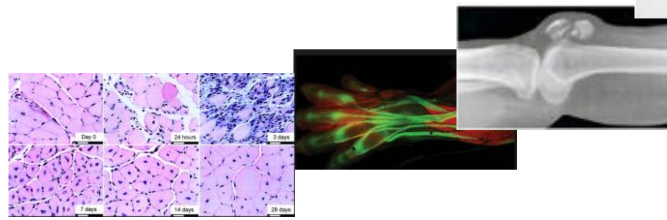
MultiSpectral Fluorescent Imager/Spectrum
(SpaceX-15: April 2018)

Space Biology Planning: Gravity as A Continuum

The Gravity Dose Response Curve: Threshold or Continuum?

- Hypothesis: Gravity induces biological responses at the gene expression, cellular, systems and whole organism level
- The dose response curve of any of these responses is not fully characterized





- It is not known if responses are a continuum or are based on reaching thresholds
- Its is not known if responses require continuous or intermittent exposures
- It is not known if the sensitivity/dose response changes during development

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

<u>Ground-based Centrifuges</u>

<u>In-flight Centrifuges (EMCS, KUBIK, JAXA mouse centrifuge, Free-flyers)</u>

<u>Parabolic Flight</u>

<u>Ground-Based Fractional G Simulators (Clinostats, RPMs, HARVs, etc)</u>

<u>Partial Unloading in Animals and Humans</u>

Space Biology Planning: Upcoming Solicitations

<u>Early 2017</u>

- **NRA NNH16ZTT001N Appendix C: "Space Biology Research Using Parabolic and Suborbital Flight Campaigns"** Solicit research to use parabolic or suborbital flight to conduct investigate biological effects of shortn micro- or partial-gravity on bio-systems
- **NRA NNH16ZTT001N Appendix D: "Space Biology Research Using Antarctic Balloon Flight Campaigns"** Solicits proposals to use balloon campaigns to investigate biological effects of long duration space radiation exposure on living organisms
- **NRA NNH16ZTT001N Appendix E: "Space Biology Research Using Microgravity Simulation Devices"** Solicits research to use microgravity simulators to test specific hypotheses regarding effects of altered gravity on biological systems
- **NRA NNH16ZTT001N Appendix F: "Space Biology Experiments On the Bion M2 Mission"** TBD after further discussions with our Russian Collaborators
- **NRA NNH16ZTT001N Appendix: "Plant and Microbial Biology Research"** Solicits flight & ground plant & microbe research to answer Space Biology questions & maximize ISS use

<u>Mid to Late 2017</u>

- **NRA NNH16ZTT001N Appendix: "Space Biology Ames Life Science Data Archive Biospecimen Sharing"**
- **NRA NNH16ZTT001N Appendix: "Space Biology Beyond Low Earth Orbit (Orion EM-1)"**
- **NRA NNH16ZTT001N Appendix: "Gravity as a Continuum"**

<u>Joint between HRP and SB in preparation</u>

- Effects of hindlimb unloading and space radiation in rats (at NSRL)
- Flight experiments using the JAXA Mouse centrifuge

Space Biology: Agenda

Topics to be covered today

- Status
- Planning
- Research findings/Accomplishments
- Genelab Update

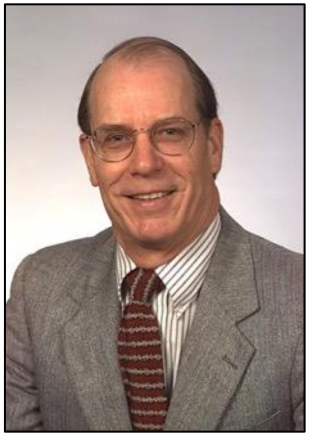
Space Biology Research Accomplishments 2010-2016

	Decadal Survey Recommendations	# Grants	# Publications
P1	ISS Microbial Observatory: long-term, multigenerational studies	4	**22**
P2: Microbial	Microbe growth and physiology	28	**49**
P2: Plant	Plant growth and physiology	41	**146**
P3	Roles of microbe-plant systems in long-term advanced life support systems	4	**13**
AH2	Preservation/reversibility of bone structure and strength	20	**57**
AH3	Bone loss studies of genetically altered mice	10	**19**
AH4	Osteoporosis drug tests in animal models	2	**2**
AH5	Underlying mechanisms regulating skeletal muscle protein balance	4	**14**
AH7	Daily recruitment of flexor and extensor muscles of the neck, trunk, arms, and legs	3	**6**
AH8	Basic mechanisms, vascular/interstitial pressures (Starling forces)	8	**14**
AH9	Microgravity and partial g (3/8 or 1/6 g) enabling levels of work capacity.	0	**0**
AH10	Integrative mechanisms of orthostatic intolerance (both 1 g and 3/8 g)	0	**0**
AH14	Mechanism(s) of changes in the immune system; multiple organ systems	13	**31**
AH15	Perform mouse studies of immunization and challenge on the ISS	4	**8**
AH16	Multigenerational studies; develop rodent breeding habitat for space	3	**2**
CC2	Is artificial gravity needed? Establish dose-response relationships	8	**3**
CC8	Expand animal use for radiation research	3	**13**
CC10	Gender Differences	12	**8**

Space Biology Research Accomplishments: Microbial Tracking Experiment MT-1 Identifies New Bacterial Species Aboard the ISS and Confirms Fungal Virulence

Decadal Survey Recommendation: P1

Researchers on the Microbial Tracking-1 experiment announce the discovery of a new species of bacteria sampled from two different locations aboard the International Space Station. *Enterobacter piersonii* have been named after Dr. Duane Pierson (at right) at Johnson Space Center to commemorate his many years of service to NASA in the field of microbiology. The Space Biology-funded flight investigation involved researchers from NASA's Jet Propulsion Laboratory and is led by Dr. Kasthuri Venkateswaran. These eight strains of bacteria are most closely related to E. cloacae – a known, opportunistic human pathogen. As a member of the genus Enterobacter these newly identified strains are also likely to be resistant to drugs and more work is needed to study them in a model organism host.



Duane Pierson

Unique Fungus Discovered on the International Space Station by Microbial Tracking-1 Investigators Found to be More Virulent than Known Clinical Strains in a Zebrafish Model. Analysis of samples from the first of the three flights found that a unique strain of the fungus *Aspergillus fumigatus* was collected from the ISS. This fungus was characterized and compared to well-established strains isolated in experiments in Earth laboratories. Assessment of the fungal growth characteristics, secondary metabolite production, and susceptibility to chemical stresses revealed no differences between the ISS and clinical strains that would suggest special adaptation to life aboard the International Space Station. Virulence of the unique fungus was assessed using a zebrafish model, which revealed that the ISS isolates were significantly more lethal compared to clinical strains.

Kasthuri Venkateswaran

Space Biology Research Accomplishments:

Spaceflight Promotes Biofilm Formation by *Pseudomonas aeruginosa*

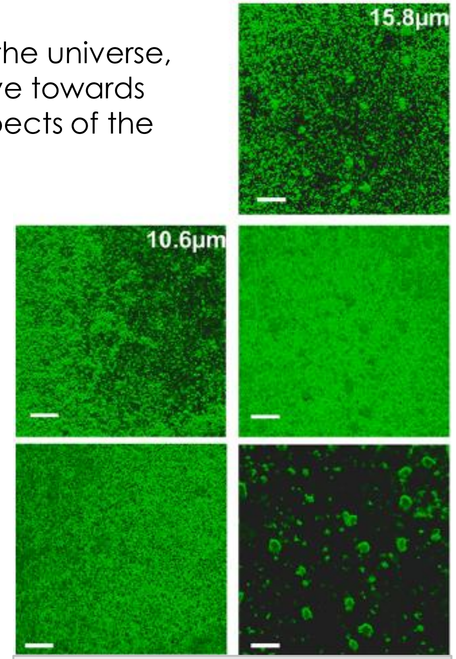
Decadal Survey Recommendation: P1/2

Cynthia Collins

Manned space missions conducted over the past 50 years have expanded our knowledge of the universe, and have led to the identification of a range of challenges that must be addressed as we move towards the next phase of human space exploration. A key concern is how microgravity and other aspects of the spaceflight environment affect bacterial growth, physiology, and virulence.

Surface-associated bacterial communities, known as biofilms, were abundant on the Mir station and continue to be a challenge on the ISS. The health and safety hazards linked to the development of biofilms are of particular concern due to the suppression of immune function observed during spaceflight. While planktonic cultures of microbes have indicated that spaceflight can lead to increases in growth and virulence, the effects of spaceflight on biofilm development and physiology remain unclear. To address this issue, *Pseudomonas aeruginosa* was cultured during two Space Shuttle missions: STS-132 and STS-135, and the biofilms formed during spaceflight were characterized.

Spaceflight was observed to increase the number of viable cells, biofilm biomass, and thickness relative to normal gravity controls. Moreover, the biofilms formed during spaceflight exhibited a column-and-canopy structure that has not been observed on earth. These findings represent the first evidence that spaceflight affects community-level behaviors of bacteria and highlight the importance of understanding how both harmful and beneficial human-microbe interactions may
be altered during spaceflight.



Above: Confocal laser scanning micrographs of 3-day-old biofilms formed during normal gravity and spaceflight culture conditions.

Kim W., Tengra F., Young Z., Shong J., Marchand N., Chan H., Pangule R., Parra M., Dordick J., Plawsky J., Collins C. Spaceflight Promotes Biofilm Formation by Pseudomonas aeruginosa. PLoS One 8(4): e62437. doi:10.1371/journal.phone.0062437. April 29, 2013.

Wayne Nicholson

Space Biology Research Accomplishments: First GeneLab Reference Mission Test

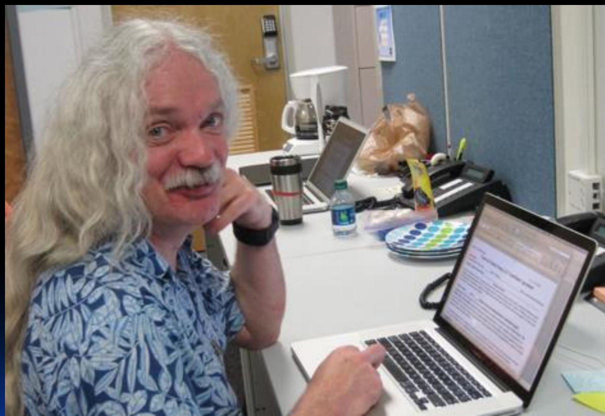
Decadal Survey Recommendation: P1/2;
"A strategy that would benefit … these research approaches would be the creation of robust databases that could be used by extramural scientists to address research questions." Page 392

GeneLab

Open Science for Exploration

First GeneLab Reference Mission Test

Kennedy Space Center developed and flew the first experiment designed to test the cradle-to-grave process for collecting spaceflight reference mission data for the NASA GeneLab Data System. The BRIC-23 experiment, titled **GeneLab Process Verification Test Using Microorganisms *Bacillus subtilis* and *Staphylococcus aureus***, was designed by KSC Project Scientist Dr. Howard Levine and Science Consultant Dr. Wayne Nicholson, University of Florida. The microorganisms were allowed to dry on Petri dishes, integrated into the BRIC-PDFU hardware, and flown to the ISS on SpaceX-9. The ISS crew initiated growth of the organisms by injecting nutrient solution into the Petri dishes. After the organisms reached the desired growth stage, they were frozen at -80°C then returned to Earth on SpaceX-9 for GeneLab "omics" analyses.



Space Biology Research Accomplishments:

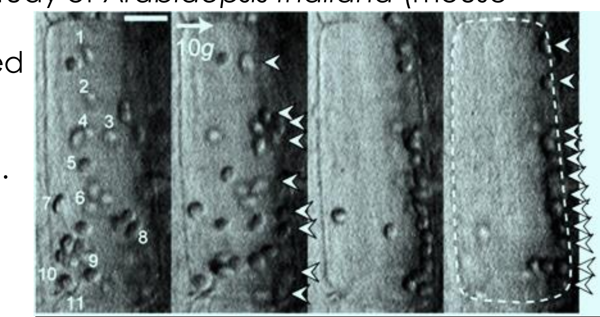
Plant Biologist Demonstrates Gravity Sensing Mechanisms in Plants

Decadal Survey Recommendation: P2

Simon Gilroy

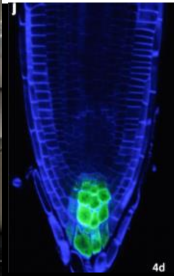
Space Biology researcher Simon Gilroy of the University of Madison-Wisconsin successfully demonstrated a long-held theory about the behavior of plants; how they "sense" gravity. The "starch-statolith hypothesis" was proposed over 100 years ago, which posits that the sedimentation of high-density starch-filled cellular organelles in cells in the plant shoots and roots is important for gravisensing of each organ. Through Dr. Gilroy's careful study of *Arabidopsis thaliana* (mouse ear cress) seedlings under a microscope inside of a centrifuge he has shown that starch-filled granules called amyloplasts settling along the walls of highly-specialized statoliths in the plant shoots signal the plant to change direction. Results of the investigation demonstrated the magnitude of the plant responses to changes in the gravity vector appears to be dependent on the number of settled amyloplasts.

In the present study, Gilroy's team constructed a new centrifuge microscope in which all the optical devices and the specimen were mounted on a single motor and rotated providing for effectively continuous visualization and illumination. The centrifuge microscope allowed imaging of the thick, less-transparent inflorescence stem specimen (approximately 0.5 mm thick). This equipment was used to investigate gravitropism and amyloplast movement induced by hypergravity conditions in wild type and gravitropic mutants that have normal starch content in amyloplasts but do not respond to gravity at 1g. By statistically correlating gravitropism and amyloplast movement they helped define the amyloplast movement/state required for gravisensing and provide support for the starch-statolith hypothesis of plant gravisensing.



Above: Microscopic image of Arabidopsis shoots under 10g hypergravity conditions. The gravity vector is aligned from left to right. Note the number and position of the amyloplasts (small ellipses) within the statolith in this time sequence; the starch grains have migrated towards the wall on the terminal end of the gravity vector, which then signals the cell.

Source article: Toyota, M., Ikeda, N., Sawai-Toyota, S., Kato, T., Gilroy, S., Tasaka, M., & Morita, M. T. (2013). Amyloplast displacement is necessary for gravisensing in Arabidopsis shoots as revealed by a centrifuge microscope. *Plant J, 76*(4), 648-660. doi:10.1111/tpj.12324



Space Biology Research Accomplishments:

Glow-in-the-dark Science: Fluorescent genes help botanists visualize gravity sensing in space grown plants

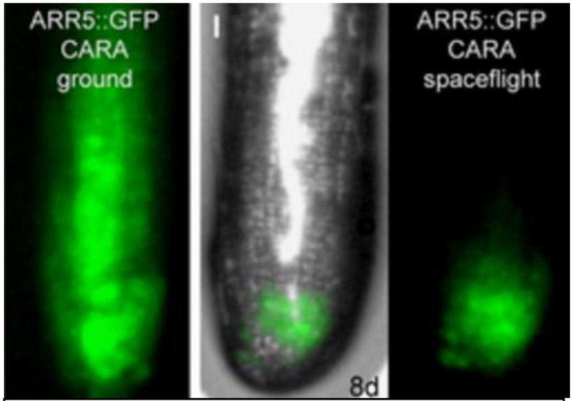
Decadal Survey Recommendation: P2

Anna Lisa Paul Robert Ferl

Space Biology researchers Rob Ferl and Anna-Lisa Paul recently published the results of a series of spaceflight experiments where Green Fluorescent Protein (GFP) tagged genes were used to identify the activity of growth hormones in plants grown on the ISS. Their findings established the plant hormones sensitive to gravity are located in the root tips, instead of in the primary root.

- The use of fluorescent reporter genes allowed investigators to visually identify the presence and distribution of plant growth hormones in the roots
- They found the distribution of fluorescent-tagged growth hormones in the *primary roots* of plants grown on the ISS was identical to that of the ground controls, but found a more restricted distribution in the root tips of the space plants
- Their findings confirm that the establishment of the auxin-gradient system, the primary guide for gravity signaling in the root, is gravity independent and that root responses to gravity are sensed in the tips

Results of these experiments reveal important information about which cellular parts of a plant respond to gravity. Research into how terrestrial biology responds to space-flight will both refine our understanding of what it takes to explore space and help define how conditions on Earth have shaped biology here.



Above: Fluorescent-tagged genes 'report' their location within the plant root by glowing green. The root at left shows a normal distribution of growth hormones while the two right images of space grown plants show a restricted distribution with the hormones concentrated in the root tips.

Source article: The effect of spaceflight on the gravity-sensing auxin gradient of roots: GFP reporter gene microscopy on orbit. npj Microgravity. Ferl RJ, Paul A-L. 2016 Jan 21;2:15023.

Space Biology Research Accomplishments: Growing Plants in Low Earth Orbit – Started by SB, now SB/HRP collaboration

Decadal Survey Recommendation: P2/3

Gioia Massa

VEGGIE

Astronauts Grow, Harvest, and Consume Fresh Vegetables from ISS Garden

The Kennedy Space Center (KSC) Vegetable Production System (Veggie) "Outredgeous" lettuce experiment (Veg-01b) demonstrated the capability to grow edible vegetables in space - from seed to mature plant. The International Space Station (ISS) crew enthusiastically harvested and consumed the lettuce on orbit. Live media broadcasts captured the vegetable harvest and consumption. The remaining lettuce samples were returned to Earth on SpaceX -8 in May 2016 for analysis by KSC scientists. The Veg-01 experiments revealed acceptable seed viability for long-term seed storage (approximately 1.5 years on ISS). The Veggie flight experiments received high praise from the ISS crew and NASA top management and became a hot-topic in the media.

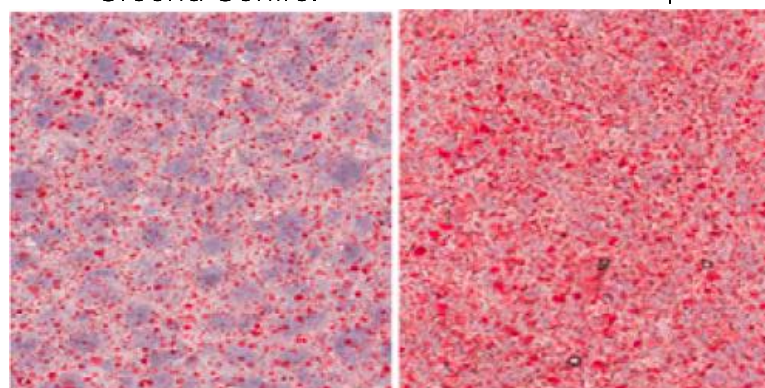
Space Biology Research Accomplishments: New Study Finds Liver Damage in Animals From Two Weeks in Space

Decadal Survey Recommendation: "Further studies are needed that focus on organismal metabolic processes in humans during long-duration spaceflight and/or bed rest to ascertain (1) if there are intrinsic lesions in the mitochondrial system limiting metabolism of fatty acids…" Page 112

Michael Pecaut

A recently published study found troubling signs of fatty liver disease in mice after only two weeks in space aboard the STS-135 shuttle. These findings have contributed important information about potential risks to liver function where little is known and the results could have implications for long-duration exploration missions.

- Mice flown in space lost weight, but redistributed lipids to their livers showing approximately 3.5 times more areas of fat storage (lipid droplets) than ground controls
- Other changes observed in the mouse livers indicate a stress response, which, if left unchecked, could lead to irreversible damage from fibrosis
- Transcriptomic and metabolomic data collected and analyzed in this study have been entered into the GeneLab data system and are publicly available for further analysis by the scientific community



Above: Magnified (40x) photos of liver sections from a ground control mouse (left) and one flown for two weeks aboard the STS-135 shuttle (right). Dark red spots in the images are areas of fat storage.

Source article: Spaceflight Activates Lipotoxic Pathways in Mouse Liver.
Jonscher KR, Alfonso-Garcia A, Suhalim JL, Orlicky DJ, Potma EO, Ferguson VL, Bouxsein ML, Bateman TA, Stodieck LS, Levi M, Friedman JE, Gridley DS, Pecaut MJ. PLoS One. 2016 Apr 20;11(4):e0152877. doi: 10.1371/journal.pone.0152877. eCollection 2016.

Space Biology Research Accomplishments:

The importance of gravity on growth and tissue regeneration

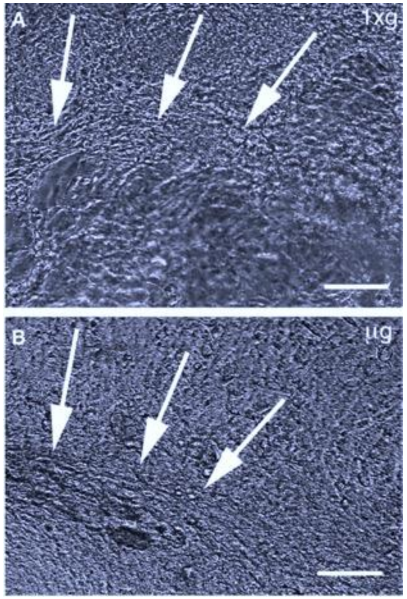
Decadal Survey Recommendation: AH16

Elizabeth Blaber

In a study funded by a NASA Space Biology grant researchers sent cultures of mouse embryonic stem cells into low earth orbit for 15 days on the STS-131 mission to follow the progress of stem cell differentiation, both while in space and then in culture back in normal gravity on earth. What they found in this series of experiments is that mechanical unloading of embryonic stem cells in microgravity inhibits their differentiation and preserves their "stemness"; the essential characteristic of a stem cell that distinguishes it from ordinary cells.

Embryonic stem cells can differentiate into all the specialized cells needed to grow a whole organism, but are also responsible for maintaining the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues. These results may provide important clues to the basis for the inhibition of tissue regeneration in space and explain why bone and muscle cell numbers are reduced after long exposures to microgravity.

Source article: Microgravity Reduces the Differentiation and Regenerative Potential of Embryonic Stem Cells. Blaber EA, Finkelstein H, Dvorochkin N, Sato KY, Yousuf R, Burns BP, Globus RK, Almeida E. Stem Cells Dev. 2015 Sep 28. [Epub ahead of print]. Space biology grant



Top image (A): stem cell controls grown in normal gravity. Bottom image (B): cultured stem cells nine days after spaceflight showing increased numbers of contractile cardiomyocyte colonies. Arrows indicate contractile region of the embryoid body outgrowth.

Space Biology Research Accomplishments:

Research by a NASA astronaut reveals underpinnings of impaired immune system in space

Decadal Survey Recommendation: AH14

Millie Hughes-Fulford

Former astronaut Dr. Millie Hughes-Fulford is a scientist who conducts research on human immunity and has performed several space flight experiments to understand the mechanisms underlying the immune system changes. She has published two papers on the results of her space flight experiments that focus on studying cells that are at the forefront of bodies' defense against disease – the T-cells. Almost every aspect of the adaptive immune response is controlled, in some way, by T-cells. Activation of T-cells is a critical event during which T-cells recognize infections within the body and initiate a defensive response.

In the first set of experiments, whole blood was obtained from 4 healthy human donors. The T-cells were extracted and maintained in cell culture and sent up to space in specialized flight hardware to keep the cells alive. Dr. Hughes-Fulford was specifically interested in tracking how miRNAs were changing in space. miRNAs are a class of small noncoding RNAs that act as posttranscriptional regulators of gene expression and play fundamental roles in regulating immune response and autoimmunity. In the data gathered from that flight experiment, she showed for the first time that the gene transcripts related to immune response were changing as a result of exposure to spaceflight.

In the second set of experiments mice were flown on the STS-131 mission for 15 days. Modified T-cells, an anti-inflammatory, and a substance that enhances the immune response were injected into the mice to see how their surrogate immune systems would respond to a specific antigen challenge while in space. The results from these experiments showed that the surrogate immune cells in the mice over reacted to the antigen creating an exaggerated inflammatory response, indicating that astronauts' immune systems may be hypersensitive to the effects of space. This experiment was the first-of-its-kind study to examine the immune system function inside a live animal host.

Taken together these spaceflight experiments are helping to unravel the mysteries of how T-cells are impaired by exposure to space. T-cells are the sentries on the forefront of protecting us from disease. Understanding how T-cells are affected in microgravity may someday lead to countermeasures to bolster immune systems for astronauts as well as for people on Earth.

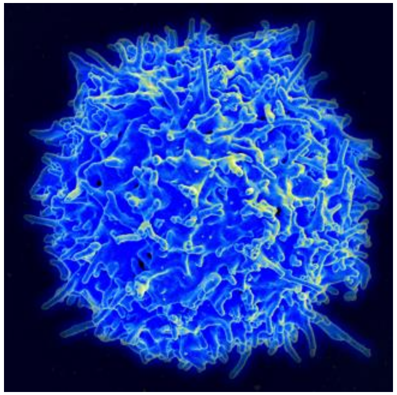
Source articles: Spaceflight impairs antigen-specific tolerance induction in vivo and increases inflammatory cytokines. Chang TT, Spurlock SM, Candelario TL, Grenon SM, Hughes-Fulford M. FASEB J. 2015 Jun 17. pii: fj.15-275073. [Epub ahead of print]

Space Biology Research Accomplishments: Using Microgravity As A Unique Opportunity to Study Immune Function

DS Recommendation: AH14, AH15, CC2

Millie Hughes-Fulford

Decades of past research has revealed that spaceflight not only decreases T-cell activation, but alters the gene and protein expression patterns of these cells as well. Former Astronaut, Millie Hughes-Fulford has dedicated most of her career to characterizing these changes in both human and murine immune system.



Hughes-Fulford and colleagues have recently found that changes in T-cells extend beyond protein-coding genes to those that encode micro RNAs (miRNAs), tiny RNA molecules that attach to messenger RNAs (mRNAs) and prevent these transcripts from being translated into proteins. This novel discovery was made while conducting experiments designed to examine how spaceflight induces changes in gene expression patterns within primary cultures of human T-cells. Her work revealed that the key immunity-regulating miRNA miR-21, which targets many other mRNA transcripts, was also suppressed by microgravity. This surprising finding suggested that gravity was manipulating the immune response through multiple molecular pathways, involving both gene transcription into mRNA, and mRNA translation into proteins.

Another important finding from this study was that exposure of **T-cells to partial gravity (0.5G), resulted in the restoration of miR21 levels to those of cells incubated in 1G**, suggesting that partial artificial gravity can act as a viable countermeasure to the effects of microgravity during spaceflight.

Source articles: Hughes-Fulford, M., Chang, T. T., Martinez, E. M., & Li, C. F. (2015). Spaceflight alters expression of microRNA during T-cell activation. *Faseb j*. doi:10.1096/fj.15-277392

Martinez, E. M., Yoshida, M. C., Candelario, T. T., & Hughes-Fulford, M. (2015). Spaceflight and simulated microgravity cause a significant reduction of key gene expression in early T-cell activation. *Am J Physiol Regul Integr Comp Physiol*, ajpregu.00449.02014. doi:10.1152/ajpregu.00449.2014

Space Biology Research Accomplishments:

Researchers identify genes that predict the loss of bone during weightlessness

Decadal Survey Recommendation: AH2, AH3

Stefan Judex

A principal function of bone is to withstand mechanical loads acting upon it such as the force of gravity on Earth. Mechanical loads are required for bone maintenance and growth. When humans travel into space the absence of mechanical loading during weightlessness causes the erosion of bone and a reduction in strength. There are differences between individuals in the way bones perceive and process similar environmental cues which gives rise to very different outcomes. This investigation sought to identify the specific gene locations which drive bone's responsiveness to unloading.

In this ground-based study adult female mice were subjected to simulated microgravity through the use of hind limb unloading and then subject to a period of reambulation (allowed to move freely in normal gravity for recovery). The femur bones were then analyzed for strength and flexibility using compression tests and the tissues were analyzed to search for the genetic locations (quantitative trait loci) – QTL) related to the changes in bone morphology and strength.

Table 4
Complete list of genes within the 95% confidence intervals of QTLs for changes in peak stress during unloading and reambulation (**in bold**) stratified by molecular signaling pathways important for regulating bone formation and resorption.

Signaling pathway	Associated genes within QTL
WNT	Csnk2a1, Fzd10, Plcb1, Plcb2, Prickle2, Ruvbl1, Wnt7a
Calcium	Adra1d, Atp2b2, Cacna1c, Chrm5, Hrh1, Itpka, Itpr1, Nos1, Orai1, P2rx4, P2rx7, Pde1a, Phkg1, Plcb1, Plcb2, Ryr3, Tacr1, **Adcy2, Agtr1a, Mylk4**
TGF-β	Acvr1, Acvr1c, Acvr2a, Bmp2
Osteoclast differentiation	Il1a, Il1b, Mitf, Pparg, Sfpi1, Sirpa
NF-kappa B	Csnk2a1, Ercl, Il1b
Notch	Cir1, Dll4, Jag1, Ncor2
Hedgehog	Bmp2, Wnt7a, **Gas1, Ptch1**
Actin cytoskeletal regulation	Arpc4, Chrm4, Chrm5, F2, Fgf7, Itga4, Itga6, Pak6, Pak7, Pxn, Raf1, Ssh1, **Mylk4, Pfn3**
ECM-receptor interaction	Cd44, Itga4, Itga6
Focal adhesion	Itga4, Itga6, Pak6, Pak7, Pxn, Raf1, Shc4, **Mylk4, Shc3**
Gap junctional communication	Gjd2, Itpr1, Plcb1, Plcb2, Raf1, **Gas1, Ptch1**

The results of this study indicate that candidate genes have been identified which may be responsible for the mechanical response of trabecular (spongy) bone to gravitational unloading. By revealing novel gene targets that are responsible for responses to gravity it may help to identify and reduce the risk of bone fractures as well as maximizing the recovery of bone strength and flexibility during recovery after space travel.

Source article: Ozcivici, E., Zhang, W., Donahue, L., Judex, S. Quantitative trait loci that modulate trabecular bone's risk of failure during unloading and reloading. Bone. 03/2014; DOI:10.1016/j.bone.2014.03.042.

Space Biology Research Accomplishments: Experimental Drug Treatment Found to Successfully Prevent Bone Loss

Decadal Survey Recommendation: AH2, AH4, AH3*

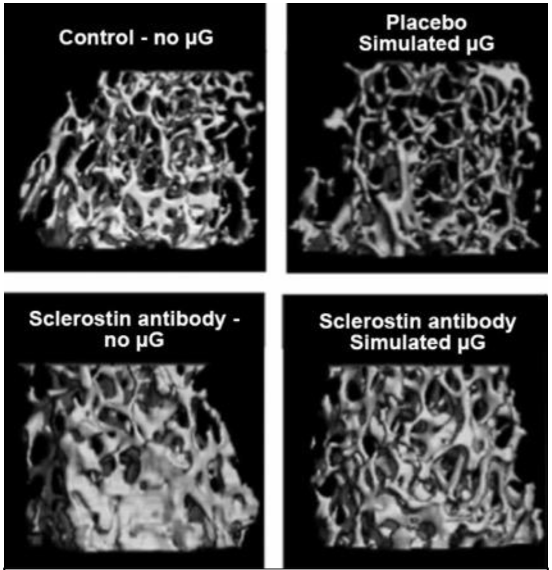
Mary Bouxsein

A study funded by NASA Space Biology examined a novel therapeutic as a means of preventing bone loss induced by exposure to microgravity. In a ground-based study Dr. Mary Bouxsein and her team investigated the use of a sclerostin antibody (SclAbII) on mice subjected to simulated microgravity for 21 days. Sclerostin is believed to be the protein responsible for the inhibition of the cellular pathway which leads to decreased bone formation during unloading conditions. In this study, hind limb unloading was used to simulate microgravity.

The experimental animals that received the sclerostin antibody showed no bone deterioration due to disuse. Moreover, it appeared that the sclerostin antibody was So effective at preventing bone loss that the bone properties of the animals receiving SclAbII were at or above the values of the controls:

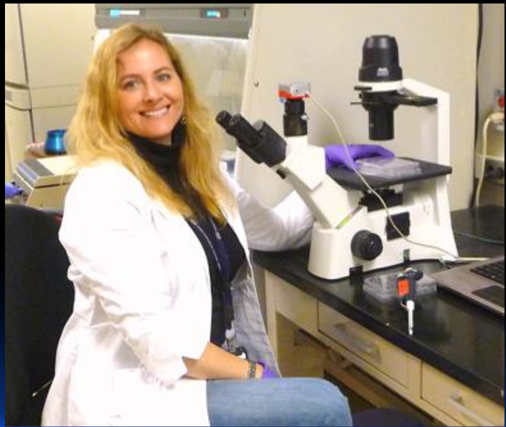
-**Hindlimb bone mineral density decreased 2% in unloaded control animals, whereas it increased 13.1% ± 1.0% in unloaded animals treated with the sclerostin antibody**.

-**Trabecular bone volume was ~2 fold higher in unloaded mice treated with the sclerostin antibody than it was in control unloaded animals.**



Above: micro-CT scans of representative Mice studied in this investigation show visible differences in the volume of trabecular bone between the controls and the drug treatment mice after exposure to simulated microgravity.

Source article: Sclerostin antibody inhibits skeletal deterioration due to reduced mechanical loading. Spatz JM, Ellman R, Cloutier AM, Louis L, van Vliet M, Suva LJ, Dwyer D, Stolina M, Ke HZ, Bouxsein ML. *Journal of Bone Mineral Research. 2012 Oct 29.*



Space Biology Research Accomplishments:

Dried Prunes Surprisingly Effective at Protecting Bones from Radiation-induced Damage

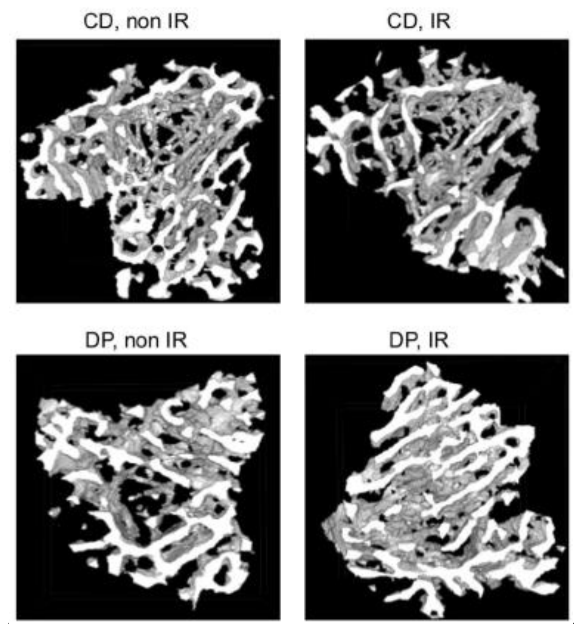
Decadal Survey Recommendation: AH2, CC8, CC9

Ann-Sofie Schreurs

Scientists at NASA's Ames Research Center found a diet of dried prunes given prior to exposing mice to whole-body radiation completely prevented bone loss.

Irradiation of control mice resulted in a 32% reduction in trabecular bone volume, a 25% reduction in trabecular number, and a 13% reduction in trabecular separation. In contrast, irradiated mice fed a diet of dried plums did not exhibit these reductions.

- Radiation-induced bone loss resembles accelerated, age-related structural changes in cancellous or spongy bone and is a risk for astronauts on long-duration, deep space missions
- Cancellous bone experiences significant damage from radiation exposure some of which is irreversible
- Drug countermeasures that have been used to prevent bone loss have limited effectiveness and carry risky side effects
- This is the first study to demonstrate the protective effects of dried prunes against radiation exposure



Representative samples of cancellous bone comparing the effects of a regular diet (CD), no radiation (non IR), to the effects of dried prune diet (DP) and radiation exposure (IR).

Source article: Dried plum diet protects from bone loss caused by ionizing radiation. Schreurs AS, Shirazi-Fard Y, Shahnazari M, Alwood JS, Truong TA, Tahimic CG, Limoli CL, Turner ND, Halloran B, Globus RK. Sci Rep. 2016 Feb 11;6:21343. doi: 10.1038/srep21343.

Space Biology Research Accomplishments: Bion-M1 experiment sheds light on the effects of fluid shifts in space

Decadal Survey Recommendation: AH8

Michael Delp

With some astronauts reporting changes in visual acuity, this has led to speculation that the fluid pressure redistribution in space and increases in intracranial pressure may be some of the factors at work. Mike Delp from the University of Florida was one of a team of space biology investigators who participated In the international collaboration that sent mice into space for 30 days on the Bion-M1 Russian satellite. His research focuses on cardiovascular fluid shifts and pressure changes as a result of exposure to microgravity.

"Without gravity pulling body fluids down toward the feet, fluid will rise toward the brain," Delp said. "When spaceflight alters the function of arteries that precisely regulate blood flow to the brain, it could severely affect many things, including vision."

Dr. Delp's team during post-flight dissection operations in Russia after samples were collected from the BionM1 landing.

Findings from this experiment demonstrated that spaceflight did impair the distribution of brain blood flow during periods of stress. Analyzing cerebral arteries may provide potentially important information about the visual impairment of astronauts induced by spaceflight. Possible solutions may be found in future experiments Mike and his team are planning.

Source article: Sofronova SI, Tarasova OS, Gaynullina D, Borzykh AA, Behnke BJ, Stabley JN, McCullough DJ, Maraj JJ, Hanna ME, Muller-Delp JM, Vinogradova OL, Delp MD. Spaceflight on the Bion-M1 Biosatellite Alters Cerebral Artery Vasomotor and Mechanical Properties in Mice. J Appl Physiol (1985). 2015 Jan 15:jap.00976.2014. doi: 10.1152/japplphysiol.00976.2014.

Space Biology Research Accomplishments: Deep Space Radiation May Cause Heart Problems for Future Travelers to Mars

Decadal Survey Recommendation: CC7, CC8, CC10

Michael Delp

July 28, 2016 – 47 years after humans first landed on the moon space biology researchers have found an increased risk of mortality from cardiovascular disease (CVD) in astronauts who traveled deep into space. Apollo astronauts experienced nearly five times greater risk of death from CVD than astronauts who've traveled to low Earth orbit.

Researchers first compared mortality rates of Apollo astronauts with other astronauts who either never flew in orbital missions or only flew in low Earth orbit. They found a concerning trend in rates of cardiovascular disease that warranted further investigation.

To test the effects of spaceflight on the cardiovascular system directly, investigators subjected mice to simulated conditions of zero-gravity, cosmic radiation, or both combined. After half a year (the equivalent of 20 human years), they found that only the mice that had been exposed to radiation had sustained damage to their blood vessels. In particular, the researchers found damage to the lining of the blood vessels, which is typically the first indication of long-term heart disease leading to a heart attack or stroke. Based on further research in mice, they suggest that the cause of cardiovascular disease in these astronauts may have been deep space radiation.

Principal Investigator for this Space Biology-funded study, Dr. Michael Delp, acknowledges shortcomings of the mortality study of the astronauts, but cautions that their findings indicate the need for more research on radiation effects on cardiovascular function in astronauts. This study was funded in part by the National Space Biomedical Research Institute.

Source article: Apollo Lunar Astronauts Show Higher Cardiovascular Disease Mortality: Possible Deep Space Radiation Effects on the Vascular Endothelium. Delp MD, Charvat JM, Limoli CL, Globus RK, Ghosh P. Sci Rep. 2016 Jul 28;6:29901. doi: 10.1038/srep29901.

Space Biology Research Accomplishments: Mission Success for Wetlab 2

Decadal Survey Recommendation: "[The ISS] is expected to serve as a world-class orbiting national and international laboratory for conducting high-value scientific research and providing access to microgravity resources for major areas of science and technology development." Page 24.

WetLab-2 is a research platform for conducting real-time quantitative gene expression analysis aboard the International Space Station. The WetLab-2 validation experiment has been successfully completed, and, in short – it worked! The team reports that full mission success criteria have been met. April 29, astronaut Jeff Williams tested the system's Sample Prep Module for the first time in space to extract and purify RNA from cells before performing the qPCR analysis. The WetLab-2 team at Ames received data from the space station that clearly shows that Williams isolated RNA in microgravity achieved reverse transcription—conversion of RNA to DNA—and got amplification of that DNA. WetLab 2 enables detailed studies of the quantity of RNA and DNA in identified microbial and other biological samples. See: WetLab-2 validation mission webpage. The experiment was featured in the April 29th edition of the "Weekly Recap From the Expedition Lead Scientist".

Space Biology Research Accomplishments
Top 20 Publications 2010 - 2016

1. Blaber, … Globus, Almeida, et al Microgravity reduces the differentiation and regenerative potential of embryonic stem cells. *Stem Cells Dev*. (2015,24;2605-21. - Mechanical unloading of stem cells in microgravity inhibits differentiation, possibly providing a cellular mechanistic basis for inhibition of tissue regeneration in space and in disuse conditions on earth.
2. Blaber, et al, (2013). Microgravity induces pelvic bone loss through osteoclastic activity, osteocytic osteolysis, and osteoblastic cell cycle inhibition by CDKN1a/p21. *PLoS One, 8*(4), e61372. doi:10.1371/journal.pone.0061372 - Pelvic & femoral regions of mouse skeleton are an active site of rapid bone loss in microgravity, suggesting this loss is not limited to osteoclastic degradation. New evidence for microgravity osteocytic osteolysis, & CDKN1a/p21-mediated osteogenic cell cycle arrest.
3. Chang, . . . Hughes-Fulford et al. (2012). The Rel/NF-kappaB pathway and transcription of immediate early genes in T cell activation are inhibited by microgravity. In *J Leukoc Biol* (Vol. 92, pp. 1133-1145). United States. - Results indicate that microgravity was the causative factor for impaired T-cell activation during spaceflight by inhibiting transactivation of key immune immediate early genes.
4. Choi, Gilroy, et al. (2014). Salt stress-induced Ca2+ waves are associated with rapid, long-distance root-to-shoot signaling in plants. *Proc Natl Acad Sci U S A, 111*(17), 6497-6502. doi:10.1073/pnas.1319955111 - Although plants do not have a nervous system, they do possess a sensory network that uses ion fluxes moving through defined cell types to rapidly transmit information between distant sites within the plant.
5. Crabbe, . . . Nickerson et al. (2011). Transcriptional and proteomic responses of Pseudomonas aeruginosa PAO1 to spaceflight conditions involve Hfq regulation and reveal a role for oxygen. *Appl Environ Microbiol, 77*(4), 1221-1230. doi:10.1128/aem.01582-10 - First study to characterize global spaceflight-induced transcriptional and proteomic responses of Pseudomonas aeruginosa, an opportunistic pathogen present in space. Transcription factor Hfq is first spaceflight-induced regulator acting across bacterial species.
6. Delp, et al. (2016). Apollo Lunar Astronauts Show Higher Cardiovascular Disease Mortality: Possible Deep Space Radiation Effects on the Vascular Endothelium. Sci Rep 6: 29901 - Cardiovascular disease mortality rate of Apollo lunar astronauts, the only humans to have traveled beyond Earth's magnetosphere, is 4-5 times higher than that of non-flight and low earth orbit astronauts.

Space Biology Research Accomplishments
Top 20 Publications 2010 - 2016

7. Hughes-Fulford, et al. (2015). Spaceflight alters expression of microRNA during T-cell activation. *Faseb j*. doi:10.1096/fj.15-277392 – Data suggest that 1) gravity regulates T-cell activation, not only by promotion of transcription, but also by blocking translation via noncoding RNA mechanisms, and 2) that fractional artificial gravity can ameliorate the effects of microgravity.
8. Judex, et al. (2013). Genetic loci that control the loss and regain of trabecular bone during unloading and reambulation. *J Bone Miner Res, 28*(7), 1537-1549. doi:10.1002/jbmr.1883 - Chromosomal regions containing genes that regulate the sensitivity of trabecular bone to weight bearing have been identified in the mouse, which may in turn help to identify individuals most susceptible to unloading-induced bone loss.
9. Kim, . . . Collins et al. (2013). Spaceflight promotes biofilm formation by Pseudomonas aeruginosa. *PLoS One, 8*(4), e62437. doi:10.1371/journal.pone.0062437 - Spaceflight was observed to increase cell numbers, biomass, and thickness of *Pseudomonas* biofilms. Moreover, the biofilms formed during spaceflight exhibited a unique architecture that has not been observed on Earth.
10. Kiss, Millar, & Edelmann. (2012). Phototropism of Arabidopsis thaliana in microgravity and fractional gravity on the International Space Station. *Planta, 236*(2), 635-645. doi:10.1007/s00425-012-1633-y - First to use ISS to examine the behavior of flowering plants in fractional or reduced gravity conditions. At gravity levels ranging from 0.l to 0.3g, red-light-based phototropism was attenuated in both roots and hypocotyls of seedlings. In contrast, blue-light negative phototropism in roots, was enhanced compared with the 1g control, and showed a significant attenuation at 0.3 g.
11. Knox, Venkateswaran et al. (2016). Characterization of Aspergillus fumigatus Isolates from Air and Surfaces of the International Space Station. *mSphere, 1*(5). doi:10.1128/mSphere.00227-16 -Two ISS isolates of *A. fumigatus* were shown to have increased virulence in a neutrophil-deficient larval zebrafish infection model.
12. Kondo, Almeida, Globus et al. (2010). Oxidative stress and gamma radiation-induced cancellous bone loss with musculoskeletal disuse. *J Appl Physiol (1985), 108*(1), 152-161. doi:10.1152/japplphysiol.00294.2009 - Demonstrated that 1) disuse and gamma irradiation, alone or together cause acute cancellous bone loss via increased bone resorption, 2) radiation alone may increase extent of acute bone loss due to reactive oxygen species, and 3) alpha-lipoic acid protects cancellous tissue from detrimental effects of radiation.

Space Biology Research Accomplishments
Top 20 Publications 2010 - 2016

13. Mao, Pecaut, Stodieck, Ferguson, Bateman, Bouxsein, . . . Gridley. (2013). Spaceflight environment induces mitochondrial oxidative damage in ocular tissue. *Radiat Res, 180*(4), 340-350. doi:10.1667/rr3309.1 – First evidence that spaceflight induces oxidative damage that results in mitochondrial apoptosis in the retina, and suggests that astronauts may be at increased risk for late retinal degeneration
14. Marcu, Bhattacharya et al. (2011). Innate immune responses of Drosophila melanogaster altered by spaceflight. *PLoS One, 6*(1), e15361. doi:10.1371/journal.pone.0015361 - Spaceflight alters cellular and humoral immune responses in Drosophila in multiple interacting pathways
15. Millar, Kiss et al. (2010). A novel phototropic response to red light is revealed in microgravity. In *New Phytol* (Vol. 186, pp. 648-656). England - A novel positive phototropic red light response in microgravity was not apparent in seedlings grown at 1g. Blue-light-based phototropism larger in microgravity compared to 1g
16. Schreurs, Globus et al. (2016). Dried plum diet protects from bone loss caused by ionizing radiation. *Sci Rep, 6*, 21343. doi:10.1038/srep21343 - Dietary dried plum supplement may prevent skeletal effects of radiation exposures in space or on Earth
17. Sofronova, S. I., et al. (2015). Spaceflight on the Bion-M1 Biosatellite Alters Cerebral Artery Vasomotor and Mechanical Properties in Mice. J Appl Physiol (1985): jap.00976.02014. - Microgravity attenuates both vasoconstrictor and vasodilator properties of the basilar artery, which could impair the distribution of blood flow in the brain during periods of stress.
18. Spatz, Bouxsein et al. (2013). Sclerostin antibody inhibits skeletal deterioration due to reduced mechanical loading. *J Bone Miner Res, 28*(4), 865-874. doi:10.1002/jbmr.1807 – A murine sclerostin antibody prevented disuse-induced bone loss and increased bone mass by increasing bone formation in unloaded animals.
19. Venkateswaran, et al. (2014). International Space Station environmental microbiome - microbial inventories of ISS filter debris. *Appl Microbiol Biotechnol, 98*(14), 6453-6466. doi:10.1007/s00253-014-5650-6. - First comprehensive effort to assess the viability of microbial cells associated with ISS surfaces, and correlate differential viability with phylogenetic affiliation.
20. Zhang, et al. (2013). Fifteen days of microgravity causes growth in calvaria of mice. Bone 56(2): 290-295. - Microgravity causes changes in calvarial bones that do not bear weight suggesting that fluid shifts alone in microgravity may initiate bone adaptation independent of skeletal unloading .

Education and Outreach Opportunities

NASA Postdoctoral Program (NPP)

- Provides early-career and more senior scientists the opportunity to share in NASA's mission
- Fellows work on 1-3 year assignments at NASA centers and institutes
- Fellows contribute to our national scientific exploration, confirm NASA's leadership in fundamental space research, and complement the efforts of NASA's partners in the national science community.

Space Life Sciences Training Program (SLSTP)

- Trains our next generation of scientists and engineers and enable NASA to meet future research and engineering challenges in the space life sciences
- Students conduct hands-on research, attend technical lectures, develop professional & project management skills, perform a team project and submit an abstract to a professional scientific or engineering organization for presentation

Space Biology - Pioneering Scientific Discovery
Space Flight Data to Knowledge – Genelab Outreach

- New Space Biology project
- Targets high school students
- Pilot program begins Summer 2017

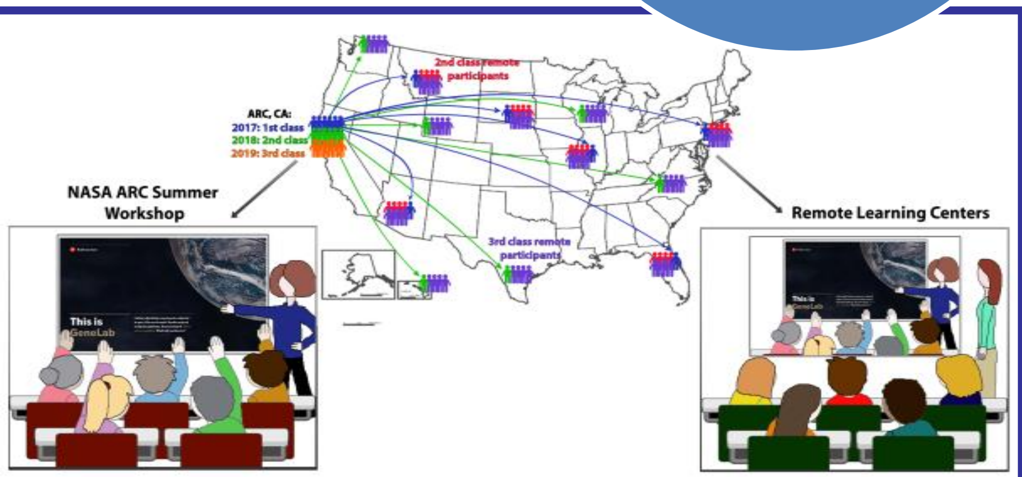
GeneLab for High Schools

Consists of 3 core components:

Core Learning Modules
- Introduction to GeneLab Data Platform and 'omics datasets and analysis tools
- Introduction to model organisms and analysis of model organism datasets

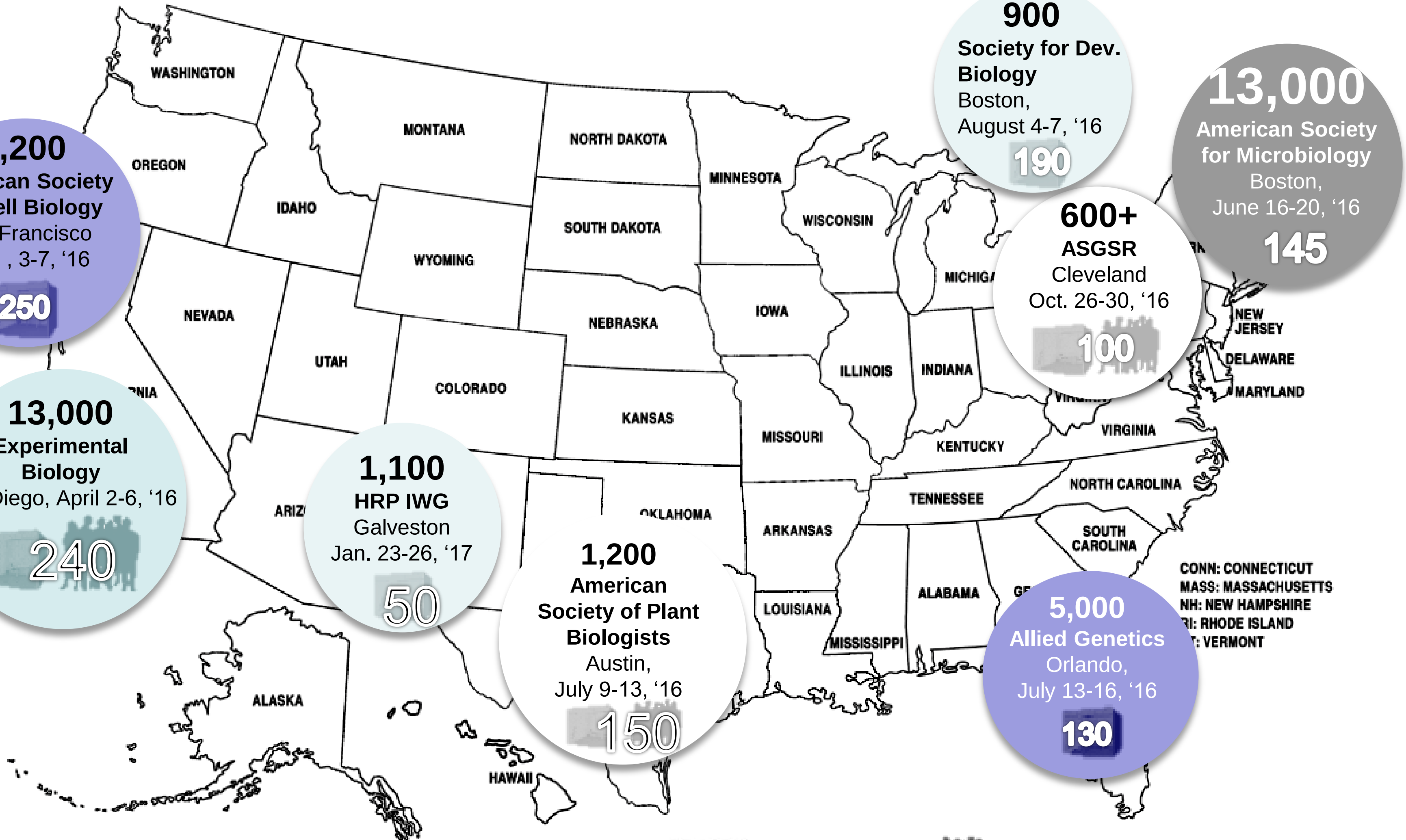
Networking and teamwork component
- Guest lectures by University Professors and Industry experts
- Networking receptions and field trips (University core facilities and Industry)

Independent Learning Project
- Analysis of dataset of choice
- Development of hypothesis and proposal of research project



Elizabeth Blaber, Ph.D.
Development and Organization Lead

Space Biology October 2015 – September 2016
Scientific Exhibits & Workshops



6,200
American Society for Cell Biology
San Francisco
Dec. , 3-7, '16
250

13,000
Experimental Biology
San Diego, April 2-6, '16
240

1,100
HRP IWG
Galveston
Jan. 23-26, '17
50

1,200
American Society of Plant Biologists
Austin,
July 9-13, '16
150

900
Society for Dev. Biology
Boston,
August 4-7, '16
190

13,000
American Society for Microbiology
Boston,
June 16-20, '16
145

600+
ASGSR
Cleveland
Oct. 26-30, '16
100

5,000
Allied Genetics
Orlando,
July 13-16, '16
130

Booth Exhibit Workshop

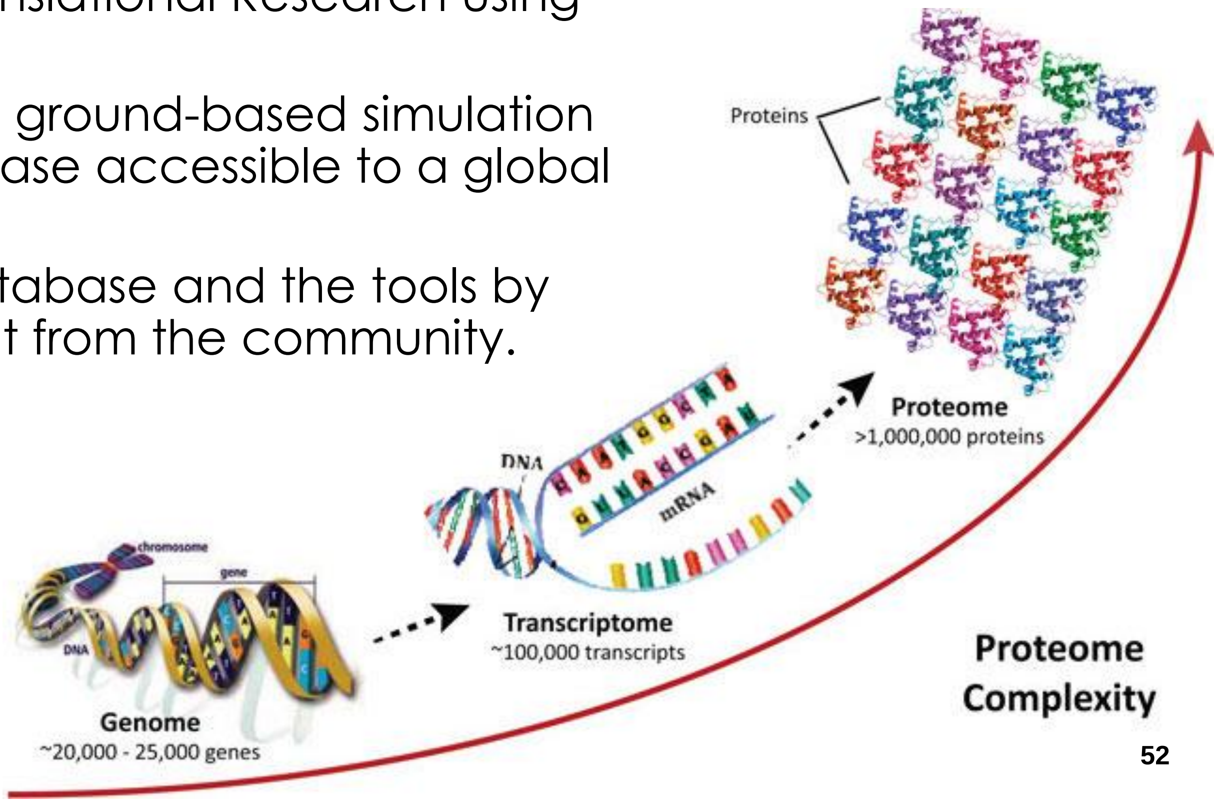
Space Biology: Agenda

Topics to be covered today

- Status
- Planning
- Research findings/Accomplishments
- Genelab Update

Space Biology - Pioneering Scientific Discovery
Space Flight Data to Knowledge – Genelab Goals

- Serve as a resource for researchers to convert spaceflight Life Sciences data to new knowledge to maximize use of ISS Life Science research results .
- Collect and archive spaceflight genomic, transcriptomic, proteomic, and metabolomic data for discovery use.
- Support research community exploration of molecular network responses to spaceflight by maintaining access and tools for performing Translational Research using Genelab data.
- Make all SB spaceflight and ground-based simulation data in the Genelab database accessible to a global researcher network.
- Continually improve the database and the tools by seeking feedback and input from the community.



Space Biology - Pioneering Scientific Discovery
Space Flight Data to Knowledge – Genelab Current Status

- The GeneLab Data System (genelab.nasa.gov) currently houses 88 datasets which are meticulously curated and fully accessible to the public: 49 datasets are from Spaceflight experiments, and 39 from relevant ground experiments.
- Averaging more than 500 file downloads per month, with a data volume of roughly 3 terabytes per month, available worldwide.
- Newsletter updates are currently sent to 4,463 subscribers.
- Collaborated with recent missions (including CASIS) to obtain either tissue samples or data for subsequent omics analyses: Rodent Research-1, Rodent Research–3, Micro-9, Micro-10, Microbial Tracking-1c, BRIC-19, BRIC-20, and the BRIC-23 Process Verification Test.
- Space Biology issued an Innovation Awards Research solicitation (NRA NNH16ZTT001N-GL) in March 2016 - 6 of 34 proposals received were funded after scientific merit and technical feasibility reviews.

Space Biology - Pioneering Scientific Discovery
Space Flight Data to Knowledge – Genelab Planning

Phased Approach:

Phase 1
Searchable Data
FY2014 –2015

Phase 2
Data Exchange
FY2016-2017

Phase 3
System Integration
FY2018– 2019

Phase 4
Full Operations
FY2020 – 2021

Spaceflight Experiments

- ✓ At least two Collaborative Missions (BRIC19 and BRIC20)
- ✓ Establish PI Data Agreements
- ✓ Data analysis validation

Spaceflight Experiments

- ✓ Standards for samples
- ✓ Standards for metadata
- ✓ NRA for Data analysis from initial studies
- ✓ Participate in multiple missions/year
- ✓ Legacy Data
- Minimum Data Set

Spaceflight Experiments/Analysis

- Continue ground controls and process enhancement
- Engage with Scientists external to NASA as part of Outreach Program
- Multiple flight experiments every year

Full Operations

- Full science community engagement
- Analysis and modeling
- Expanding catalog of flight experiments

Data System

- ✓ Public Website
- ✓ Searchable Data Repository
- ✓ Top Level Requirements
- ✓ New Data and Legacy Data

Data System

- Link to Public Databases via Data Federation.
- Integrated Search (e.g., data mashup)

Data System

- Integrated Platform across model organisms
- Build Community via collaborative science analysis & modeling

Full Operations

- Provide access to biocomputational tools for omics analysis
- Provide collaboration framework and tools

Common Threads

- NASA Space Biology objectives and research projects are well aligned with 2010 Decadal Survey priority recommendations
- Every Space Biology research task is required to comply with President Obama's Executive order "Making Open and Machine Readable the New Default for Government Information", enabled through the NASA GeneLab Project
- Potential for more collaboration with other NASA programs, CASIS, or international partners
- In-flight centrifugation is required across all elements and species to determine gravity thresholds, responses to fractional Earth gravity such as will be experienced on Mars or moon, and to provide 1g controls in flight.
- Ability to accomplish goals & recommendations of NRC Decadal Survey is diminished by reduced access to ISS & reduced crew time availability for implementing NASA science
- Need for ground-based research and alternative spaceflight platforms.
- Systems Biology Approach at some level present in 50-70% of all Space Biology tasks.
- Need to actively solicit users of the open science GeneLab database to develop new knowledge from spaceflight omics data.
- Attempt in each element to enlarge NASA's research community by outreach to major scientific professional societies (e.g., Experimental Biology, American Society for Microbiology, American Plant Biology Society, American Society for Cell Biology.)

Supplementary Slides for the Committee

Space Biology Research Accomplishments

Flight Experiments Completed:

- BRIC-21, VEG-01b, VEG-01c
- SpaceX-8 (4/8/16): VEG-03 Tech Demo, MT-1c, Micro-9, Micro-10, WetLab-2 Validation
- SpaceX-9 (7/18/16): BRIC-23 GeneLab Process Verification Test, Rodent Research-3 Biospecimen Sharing

2015-16 Space Biology Publications - Top 20

1. Sofronova, Tarasova, Gaynullina, Borzykh, Behnke, Stabley, McCullough, Maraj, Hanna, Muller-Delp, Vinogradova, Delp. Spaceflight on Bion-M1 biosatellite alters cerebral artery vasomotor & mechanical properties in mice. *J Appl Physiol* (2015,118;830-8).
2. Checinska, Probst, Vaishampayan, White, Kumar, Stepanov, Fox, Nilsson, Pierson, Perry, Venkateswaran. Microbiomes of dust particles collected from ISS & Spacecraft Assembly Facilities. *Microbiome*. (2015,3;50).
3. Delp, Charva, Limoli, Globus, Ghosh. Apollo lunar astronauts show higher cardiovascular disease mortality: Possible deep space radiation effects on vascular endothelium. *Sci Rep*. (2016,28;29901).
4. Jonscher, Alfonso-Garcia, Suhalim, Orlicky, Potma, Ferguson, Bouxsei, Bateman, Stodieck, Levi, Friedman, Gridley, Pecaut. Spaceflight activates lipotoxic pathways in mouse liver. *PLoS One*. (2016,11; e0152877).
5. Vandenbrink, Herranz, Medina, Edelmann, Kiss, A novel blue-light phototropic response is revealed in roots of *Arabidopsis thaliana* in microgravity. *Planta*.(2016,244;1201-1215).
6. Fajardo-Cavazos, Nicholson. Cultivation of *Staph epidermidis* in human spaceflight environment leads to alterations in frequency & spectrum of spontaneous rifampicin-resistance mutations in rpoB gene. *Front Microbiol*. (2016,28; 999)
7. Hughes-Fulford, Chang, Martinez, Li. Spaceflight alters expression of microRNA during T-cell activation. *FASEB J*. (2015,29; 893-900).
8. Blaber, Finkelstein, Dvorochkin, Sato, Yousuf, Burns, Globus, Almeida,Microgravity reduces the differentiation and regenerative potential of embryonic stem cells. *Stem Cells Dev*. (2015,24;2605-21.

Space Biology Research Accomplishments

2015-16 Space Biology Publications - Top 20 (CONTINUED)

9. Ghosh, Stabley, Behnke, Allen, Delp. Effects of spaceflight on the murine mandible: Possible factors mediating skeletal changes in non-weight bearing bones of the head. *Bone*. (2016,83;156-61)
10. Wang, Singh, Benoit, Keyhan, Sylvester, Hsieh, Thathireddy, Hsieh, Matin. Sigma S-dependent antioxidant defense protects stationary-phase Escherichia coli against the bactericidal antibiotic gentamicin. *Antimicrob Agents Chemother*. (2014,58;5964-75).
11. Chowdhury, Seetharam, Wang, Liu, Lossie, Thimmapuram, Irudayaraj. A study of alterations in DNA epigenetic modifications (5mC and 5hmC) & gene expression influenced by simulated microgravity in human lymphoblastoid cells. *PLoS One*. (2016,11;e0147514).
12. Evans, Choi, Gilroy, Morris. A ROS-assisted calcium wave dependent on AtRBOHD and TPC1 propagates the systemic response to salt stress in *Arabidopsis* roots. *Plant Physiol*. (2016,171;1771-84).
13. Chang, Spurlock, Candelario, Grenon, Hughes-Fulford. Spaceflight impairs antigen-specific tolerance induction in vivo and increases inflammatory cytokines. *FASEB J*. (2015,29;4122-32).
14. Ferl, Koh, Denison, Paul. Spaceflight induces specific alterations in proteomes of Arabidopsis. *Astrobiol*. (2015,15;32-56)
15. Gridley, Mao,, Tian, Cao, Perez, Stodieck, Ferguson, Bateman, Pecaut. Genetic and apoptotic changes in lungs of mice flown on the STS-135 mission in space. *In Vivo*. (2015,29;423-33).
16. Kwon, Sparks, Nakashima, Allen, Tang, Blancaflor. Transcriptional response of Arabidopsis seedlings during space-flight reveals peroxidase & cell wall remodeling genes associated with root hair development. *Am J Bot*. (2015,102;21-35).
17. Nislow, Lee, Allen, Giaever, Smith, Gebbia, Stodieck, Hammond, Birdsall, Hammond. Genes required for survival in microgravity revealed by genome-wide yeast deletion collections cultured during flight. *Biomed Res Int* (2015;976458).
18. Singh, Blachowicz, Checinska, Wang, Venkateswaran. Draft Genome Sequences of Two *Aspergillus fumigatus* Strains, Isolated from the International Space Station. *Genome Announc*. (2016,4;e00553-16).
19. Crabbé, Liu, Sarker, Bonenfant, Barrila, Borg, Lee, Weiss, Nickerson. Recellularization of decellularized lung scaffolds is enhanced by dynamic suspension culture. *PLoS One*. (2015,10;e0126846).
20. Andreev-Andrievskiy, Popova, Boyle, Alberts, Shenkman, Vinogradova, Sychev. Mice in Bion-M 1 space mission: training and selection. *PLoS One*. (2014,9;e104830).

Pioneering Scientific Discovery
Space Biology: External Partnership and Collaboration

- International Space Life Sciences Working Group: Forum for developing life science collaborations between NASA, ESA (CSA, CNES, DLR, ASI) and JAXA: ISS experiments, Research Announcements, and GeneLab
- JAXA: OP3 Framework: Rodent Experiments, Rodent Centrifuge and Aquatic Habitat
- U.S./Russian Joint Working Group: Forum for developing life science collaborations with Russia: ISS experiments, Bion, Foton, and GeneLab
- CASIS: collaboration on ISS based experiments with translational relevance

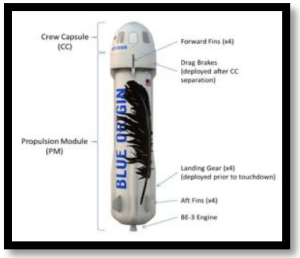
New Platforms

NASA and Other Governmental Opportunities

- Orion: Space Biology Pathfinder Mission
- Flight Opportunities Program
- Advanced Exploration Systems: BioSentinel technology for future LBLEO missions
- Air Force: X37B

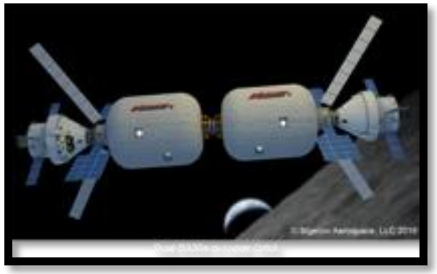
International

- JAXA future Mouse Habitat BSP and Aquatic Habitat for GeneLab
- Bion-M2 Free Flyer Mission



Commercial

- Blue Origin
- SpaceX: Falcon and Red Dragon
- CASIS Rodent Biospecimen Sharing

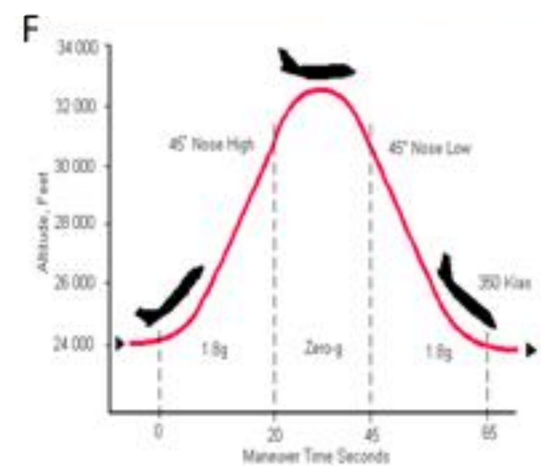


Parabolic Flight Campaigns for Microgravity and Partial Gravity Life Science Experiments

Objectives:

- Establish a **yearly Parabolic Flight Campaign** for Life Scientists that will provide opportunities for short duration **(20-25 sec) micro-g and partial-g** investigations.
- Work towards the expansion of this capability to **90 sec micro-g and partial-g exposures** based upon future modifications to **Commercial F104 jets**.

- A number of conventional (but modified) aircraft are capable of flying a series of parabolas that provide 20-25 seconds of micro-g (or partial-g) at the top of the parabolas.
- Typically, 40 parabolas are flown each flight, and four consecutive flight days can be provided per campaign.



Some Commercial Parabolic Flight Providers. A. Starfighters Aerospace F104. B. Starfighters Aerospace Falcon 20. C. Swiss Space Systems (S3) Airbus A310. D. T-6 Texan WWII Warbird. E. Zero Gravity Corp Boeing 727. F. Parabolic Flight Profile.

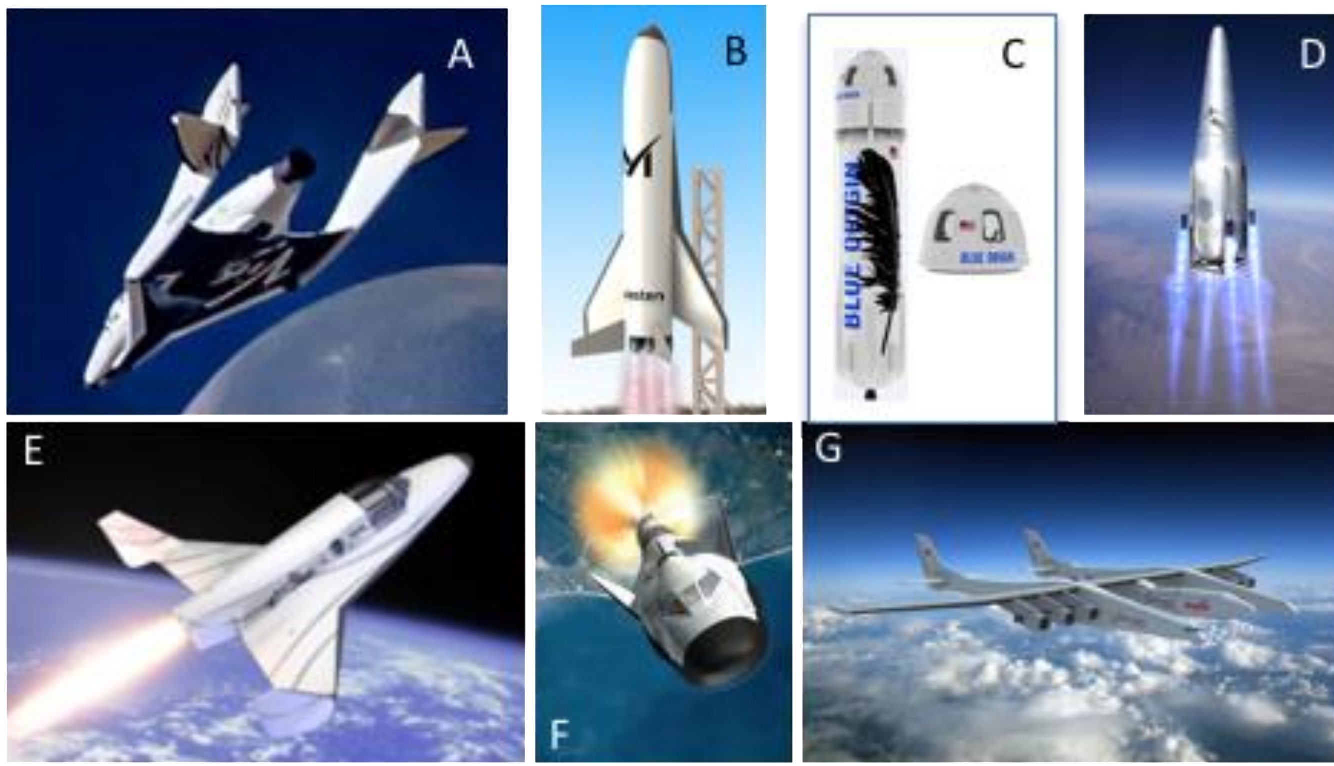
Suborbital Flight Campaigns for Microgravity Life Science Experiments

Objective: Establish yearly Suborbital Flight Campaigns that will provide 200-300 sec micro-g and partial-g exposures for life science investigations.

Suborbital Flight Microgravity Durations

Microgravity ends when the vehicle re-enters the atmosphere. The duration of microgravity is primarily a function of the maximum altitude which is reached during the flight. A few examples:

<u>Altitude</u>	<u>Micro-g Duration</u>
100 km	3:10 min
200 km	5:45 min
400 km	9:10 min



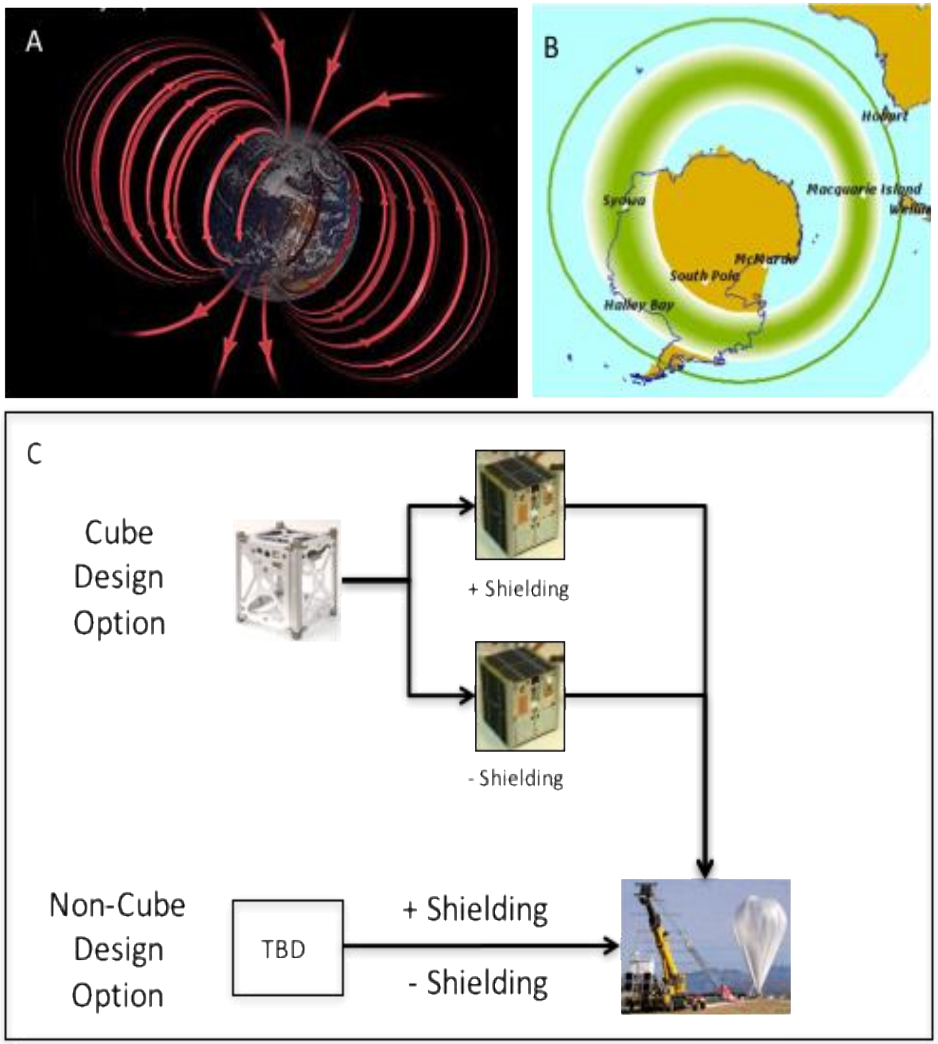
Some Commercial Suborbital Launch Vehicles: A. Virgin Galactic SpaceShip Two. B. Masten Space Systems Experimental Spaceplane XS-1. C. Blue Origin New Shepard. D. Armadillo Aerospace Next Generation Rocket. E. XCOR Aerospace Lynx. F. Sierra Nevada Dream Chaser. G. Stratolaunch Systems Carrier Aircraft.

Antarctic Balloon Campaigns for Long-Term Low Dose Deep Space Life Science Radiation Studies

Objective: Facilitate Deep Space Radiation High Altitude 1-3 month life science investigations.

Earth's Magnetic Field Protects us from Space Radiation:

- Most dangerous particles don't hit the Earth's surface because they are forced by **the Earth's magnetic field** to move around the Earth.
- Particles do enter at the magnetic north and south poles where the magnetic field points directly into the ground, so in those areas **particles from deep space** are free to rain in.
- The result is a **Space Radiation** environment **comparable to what the crew will experience on the way to Mars**.



A. Depiction of the magnetic field lines of the Earth showing polar region areas of deep space radiation penetration. B. View of Auroral Zone's ring shaped region representing the area of deep space radiation penetration with a 2500 km radius around the Earth's magnetic pole (about 2000 km away from the geographic pole). C. Investigators will develop hardware (either Cube-based or TBD) that piggyback on the primary science payload's gondola.

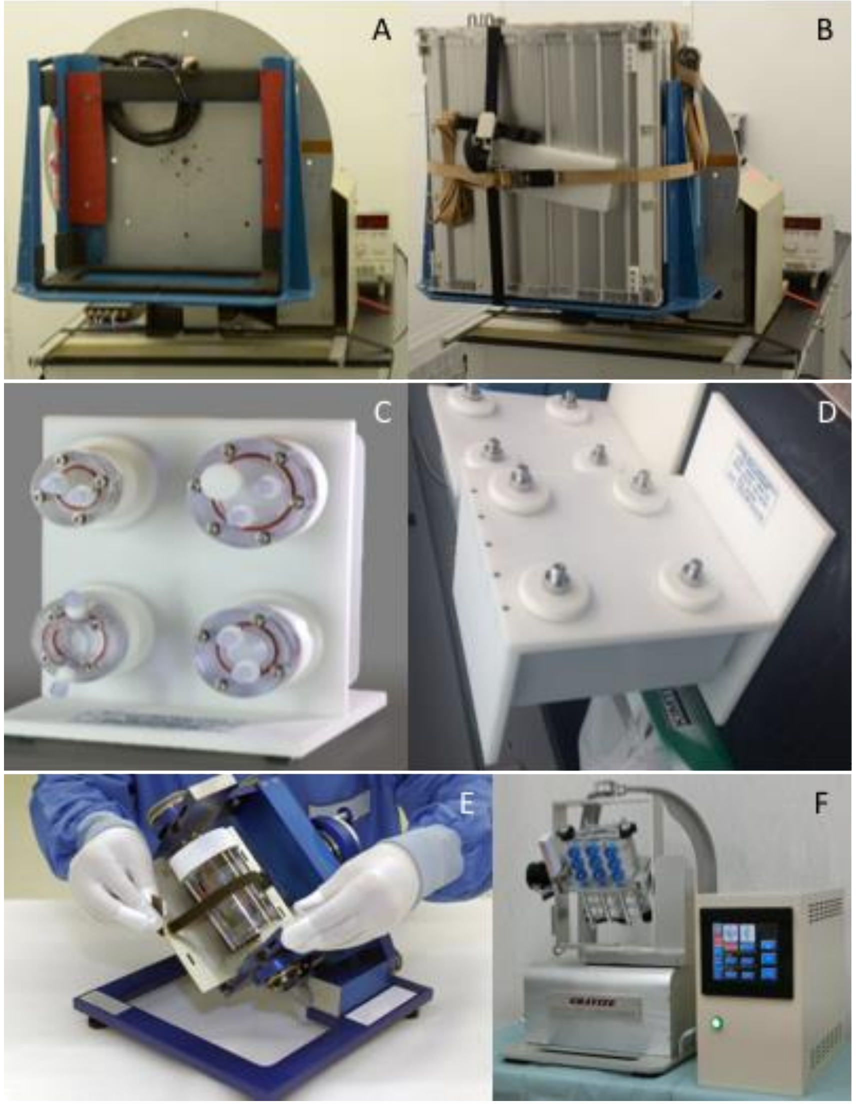
Microgravity Simulators for Ground-Based Gravitational Research

Objective:

Provide the U.S. Space Biology research community a **ground-based micro-g & partial-g simulation capability** composed of devices that negate the directional influence of the "g" vector (e.g. 2D & 3D Clinostats, Random Motion Machines, Rotating Wall Vessels).

Clinostats currently available for use.

A. KSC Slow Rotating Clinostat for large containers (up to 32 kg).
B. KSC Slow Rotating Clinostat in ISS stowage locker configuration.
C. Rotating Wall Vessel (Micro-*g* configured with HARV's attached).
D. Rotating Wall Vessel holder in 1*g* "control" configuration.
E. Airbus RPM 2.0 configured with experimental vessel.
F. Space Bio-Laboratories, Inc. GRAVITE RPM.



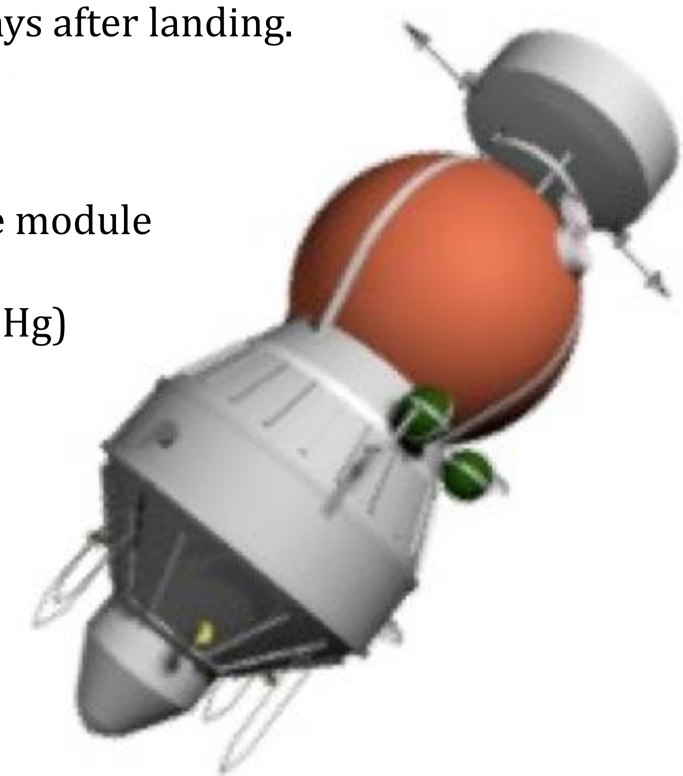
Bion-M2 Free Flyer Mission: Russian/US Collaboration

Goal: To investigate systemic, cellular and molecular mechanisms underlying adaptation of mammals to the combined effects of microgravity, cosmic radiation and other spaceflight factors as well as their readaptation to the terrestrial environment upon 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.

<u>**Bion Characteristics**</u>

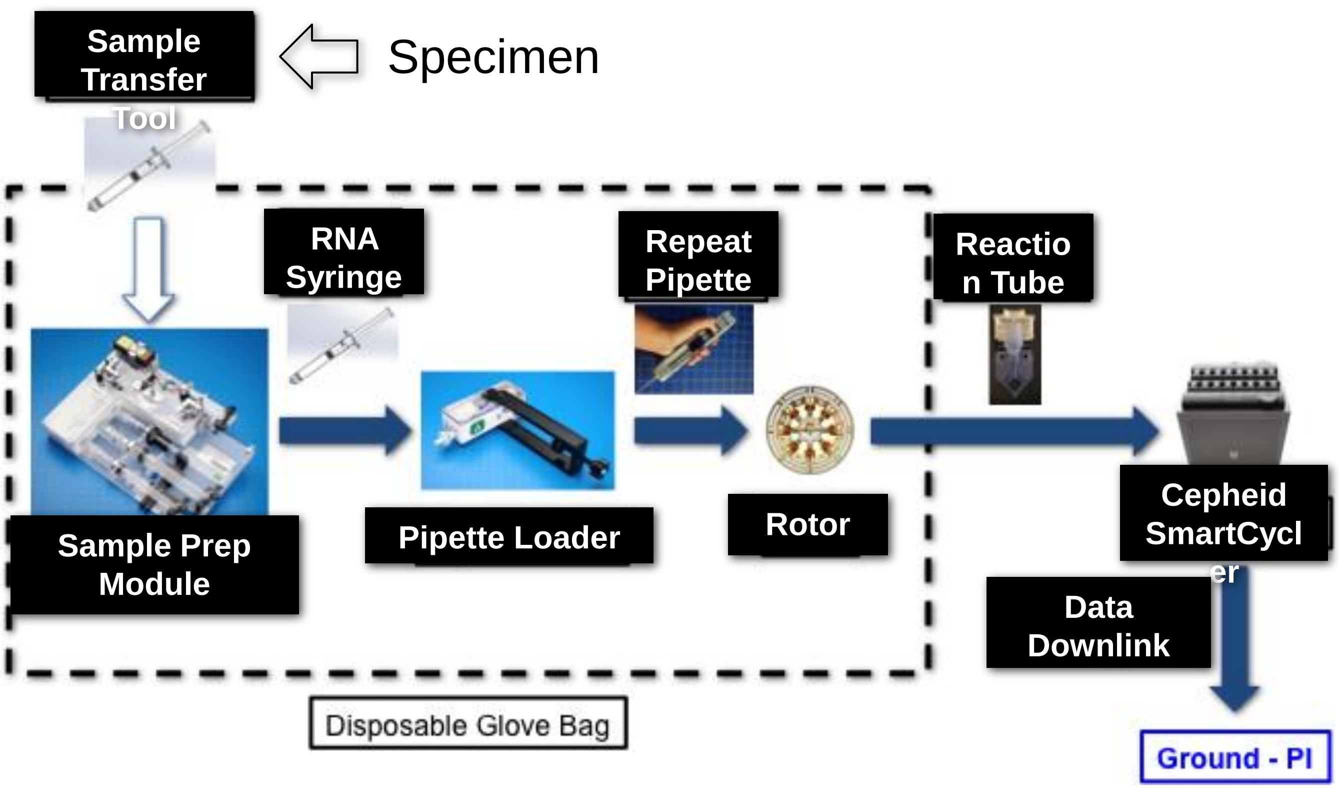
- 2.5 m Sphere used for spaceflight experiments
- 700 kg of payload mass (4 cubic meters) within recoverable module
- 800-1000 km circular orbit for 30 days
- Internal Pressure: 660-960 mm Hg (typically 720-760 mm Hg)
- pO_2: 140-180 mm Hg
- pCO_2: <7 mm Hg (typically <1 mm Hg)
- Relative Humidity: 30-80%
- 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



Wetlab-2
(Validated on ISS during SpX-8)

WetLab-2 System was developed to provide an on-orbit nucleic acid analytical capability. WetLab-2 is a research platform for conducting real-time quantitative gene expression and DNA genetic analyses. The COTS Cepheid SmartCycler and its PCR Reaction Tube were adapted for use on ISS in microgravity. The full system provides the ability to process samples on-orbit, isolate DNA and RNA, reverse transcribe RNA, and perform qPCR. The isolate nucleic acid is amplified and analyzed using the Cepheid SmartCycler. The data is downlinked for analysis within 2 hours of run completion. Purified RNA and DNA may be returned to the PI.

WetLab-2 Suite of Equipment and Workflow



Specifications:

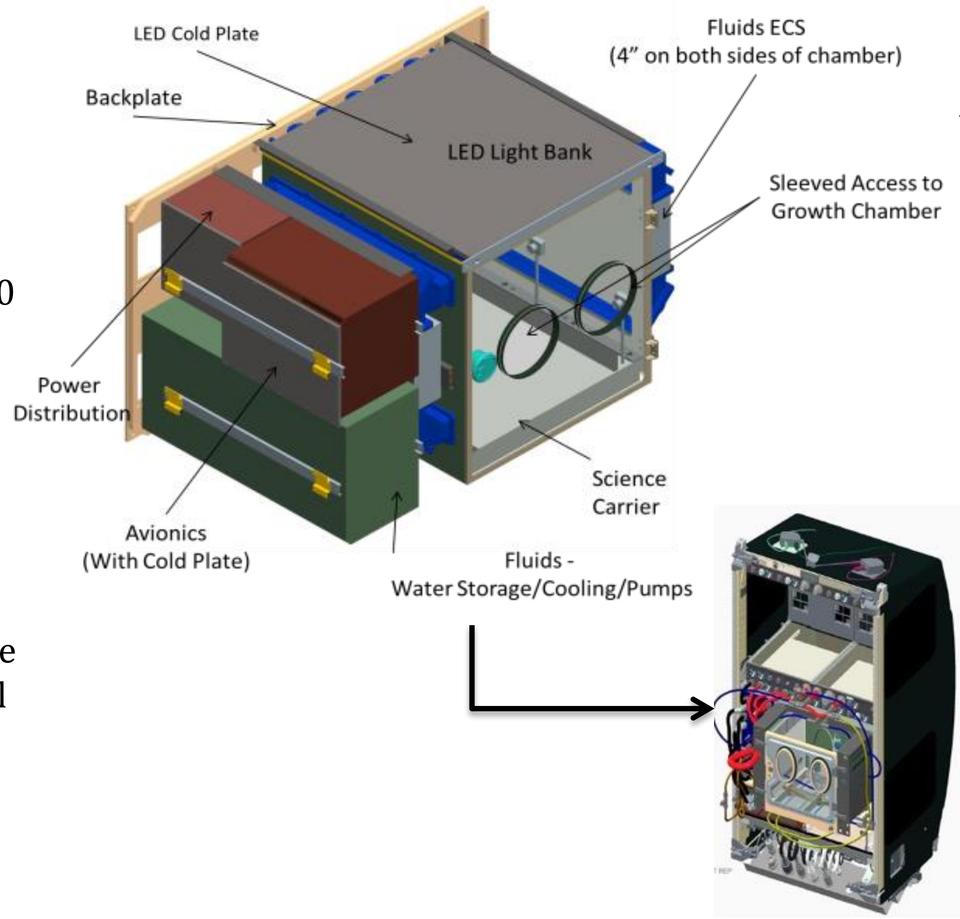
Cepheid SmartCycler
- 16 qPCR in parallel
- 4 optical channels to measure fluorescence

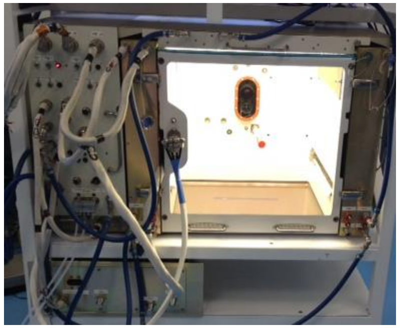
- Reaction Tube
  - Carries reaction solutions and PCR primers
  - Up to quadplex reactions (currently validated for triplex reactions on-orbit)
- Sample Prep Module:
  - Capable of processing microbes, cells, and tissues
  - Sample lysis and extraction and purification of RNA and DNA
- Pipette Loader
  - Provides sample de-bubbler
- Rotor
  - Used with ISS Drill
  - Draws sample down to reaction solutions and primers in the Reaction Tube
- Thermal protocols can be uplinked from ground

Advanced Plant Habitat (OA-7: Feb 2017)

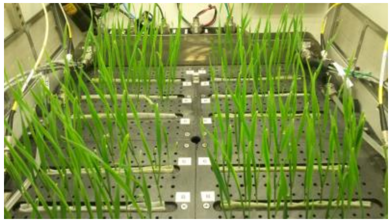
Advanced Plant Habitat (APH) = a large growth volume plant habitat capable of hosting multi-generational studies with environmental variables tracked and controlled in support of whole plant physiological testing (up to 135 days) and Bioregenerative Life Support System investigations.

Specifications:
Max. Shoot Height 45 cm; Root Zone Height 5 cm, Growth Area 1,700 cm^2; Growth Volume 112,500 cm^3; Light Intensity <1000 µmoles [Red 630-660 nm, Blue 450±10 nm, Green 525±10 nm, Far Red 730 nm, White]; Temperature 18-30 C; Relative Humidity 50-90%; Condensate Recycled; CO_2 Controlled (400-5000 ppm); Air Flow @ 0.3-1.5 m/s; Ethylene Scrubbed to ≤ 25 ppb; Air Sampling Ports; Water Sampling Ports; Leaf Temperature Sensor; Root Zone Moisture Level Monitored; O_2 Sensors in both Shoot & Root Zone.





APH Engineering Design Unit (EDU).



Dwarf Wheat Dwarf Wheat Grown within the APH EDU.

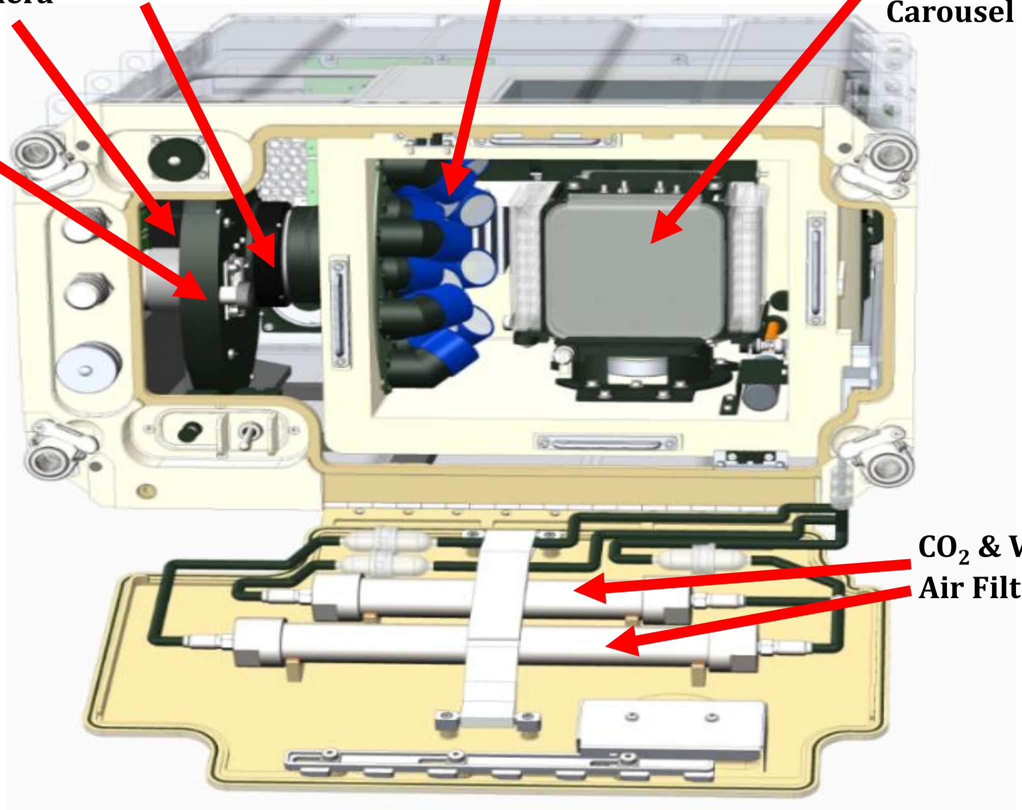
Science Carrier.

Spectrum: Multispectral Fluorescent Imager (SpaceX-15: April 2018)

- 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.

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 µmoles (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)



Schematic displaying front view of Spectrum.

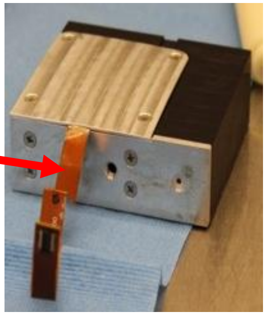
Biological Research In Canisters (BRIC) Light Emitting Diodes (LED) (SpaceX-12: 6/1/17)

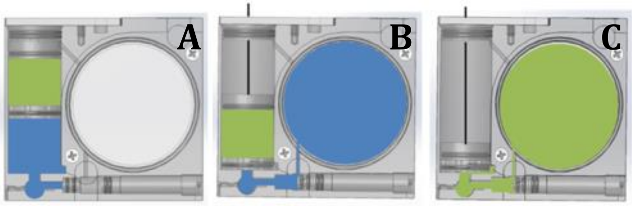
BRIC-LED Hardware Objective: Provide discrete illumination to biological specimens contained in 60mm Petri dishes that are subjected to a microgravity environment.

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



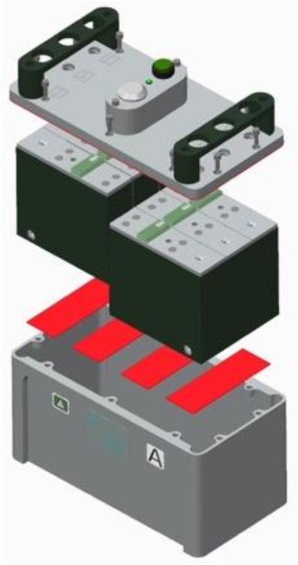


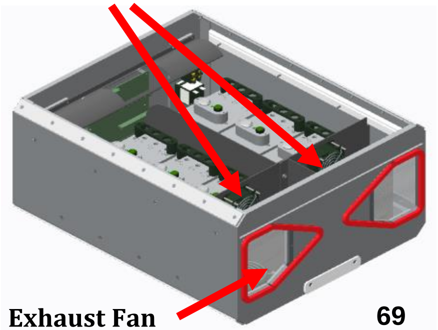
A. Pre-Actuation: Filled with 2 Fluids. B. 1st Actuation: Fluid 1 Dispensed. C. 2nd Actuation: Fluid 2 Dispensed.

BRIC-LED PDFU Lid showing LEDs.

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





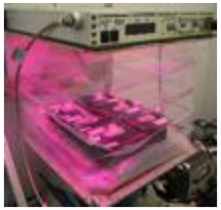
Veggie Unit #2
(SpaceX-12: 6/1/17)

Veggie is an easily stowed, high growth volume, low resource facility capable of producing fresh vegetables and supporting science experiments on ISS. It also provides real-time psychological benefits for the crew, and facilitates outreach activities.

Veggie Configured for Growth of Vegetables

Veggie Light Bank

Veggie Plant Pillow



Veggie + 6 Plant Pillows

Veggie on ISS

Astronaut Steve Swanson harvesting Lettuce.

Veggie Configured for Petri Plate Science Experiments

Petri plate holder with *Arabidopsis* petri plates inserted.

Petri plate holder containing up to 30 *Arabidopsis* plates in Veggie with bellows closed.

→

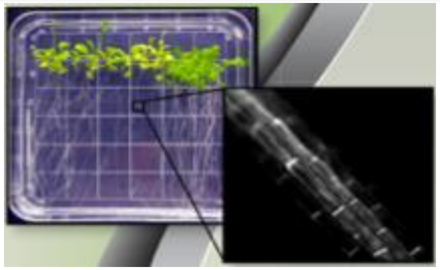


Image of *Arabidopsis* root taken in the Light Microscopy Module (LMM).

→

Astronaut Butch Wilmore fixing plants on the ISS using a Kennedy Space Center Fixation Tube (KFT).

Specifications:

Light:

- 100-500 µmol m^{-2} s^{-1} PPF of Red (630 nm), Blue (455nm) and Green (530 nm)

Cabin Air Fan Settings:

- Low / High / Off

Baseplate Footprint:

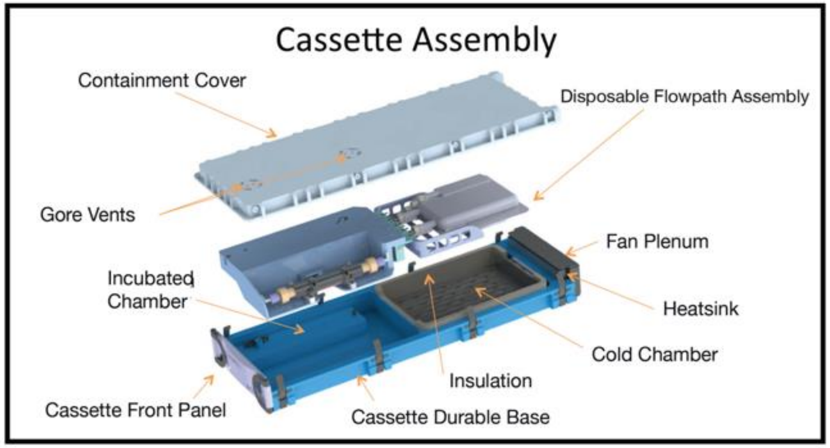
- 29.2 cm x 36.8 cm

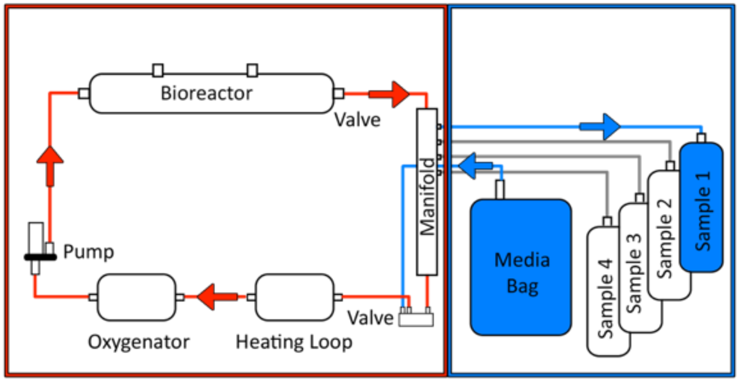
Max. Height:

- 47.0 cm empty
- 41.9 cm with root mat

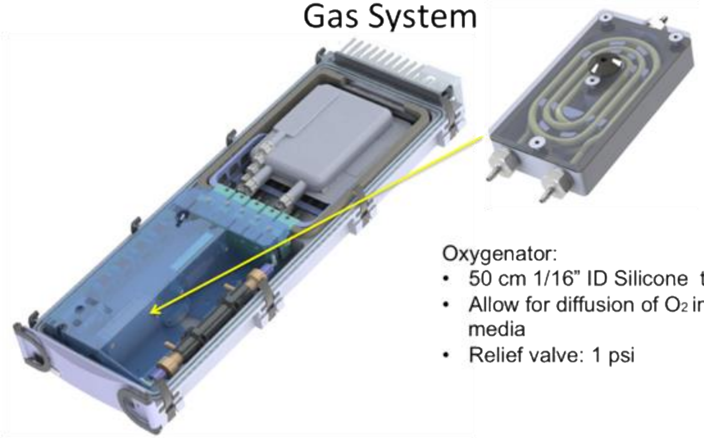
Bioculture System
(SpaceX-13: Sept 2017)

The Bioculture System is a perfusion culture system. The primary component is the hollow fiber bioreactor, designed to efficiently deliver nutrients and remove waste via multiple, tightly packed perfusion fibers. The hollow fiber system is particularly suited for microgravity cell culture where nutrient transfer is limited to diffusion. The increased surface area and low shear perfusion ensures that cells are receiving sufficient nutrients and gas to grow unabated.





Gas System



Oxygenator:
- 50 cm 1/16" ID Silicone tube
- Allow for diffusion of O_2 into media
- Relief valve: 1 psi

Space Biology: Gravity as A Continuum
Example – Gravitational responses of plant roots

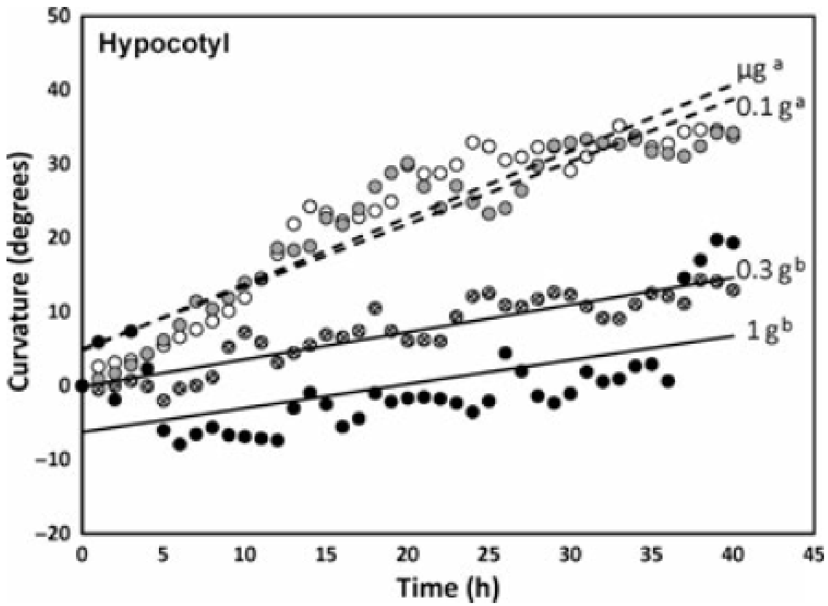


Fig. 3. Time-course studies of positive phototropic curvature in hypocotyls of *Arabidopsis* seedlings in response to red light during the TROPI-2 spaceflight experiment. White circles indicate micro-*g*, grey circles indicate 0.1 *g*, hatched circles indicate 0.3 *g* and black circles indicate 1 *g*. The plots represent the mean, and different letters indicate significant differences among the first-order regression plots. Note the magnitude of the positive response at micro-*g* and 0.1 *g*. The response at 0.3 *g* was not significantly different from the value of the 1 *g* control, and there was attenuation of red light phototropism at 0.3 *g* and 1 *g*.

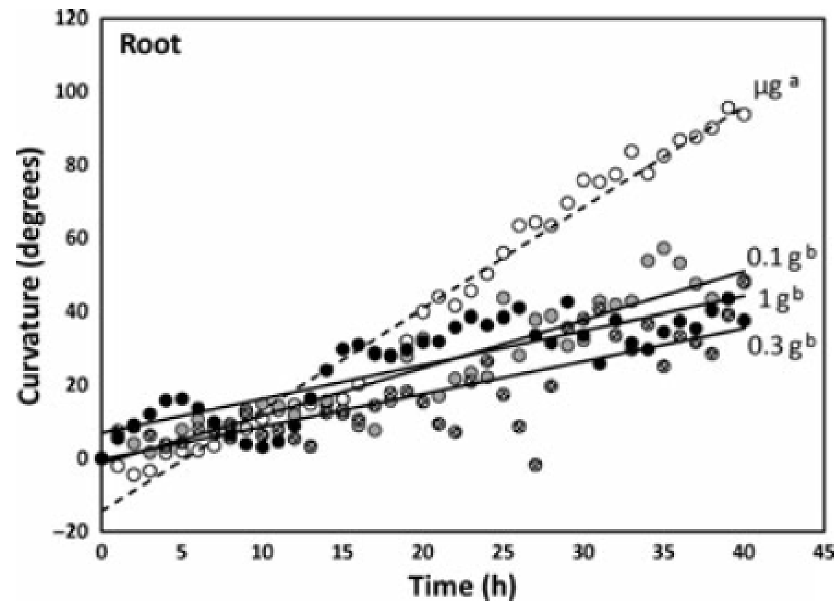


Fig. 4. Time course studies of positive phototropic curvature in roots of *Arabidopsis* seedlings in response to red light during the TROPI-2 spaceflight experiment. White circles indicate micro-*g*, grey circles indicate 0.1 *g*, hatched circles indicate 0.3 *g* and black circles indicate 1 *g*. Note the large magnitude of the positive response at micro-*g*. The responses at 0.1 *g* and 0.3 *g* were not significantly different from the value of the 1 *g* control, and the former values were attenuated compared to the robust response at micro-*g*.

Space Biology - Pioneering Scientific Discovery Partnership and Collaboration with HRP

Integrated Impact of Diet on Human Immune Response, Gut Microbiota, & Nutritional Status During Adaptation to Spaceflight. Lead - Grace Douglas (NASA JSC) Team members - Crucian, B (NASA JSC), Lorenzi, H (J Craig Venter Inst), Ploutz-Snyder, R (USRA), Smith, S (NASA JSC), Zwart, S (USRA) Compare current spaceflight diet to "enhanced" diet, with complete dietary tracking, on immune function, gut microbiota composition, & nutritional status of crew in ground-control, & on ISS. Hypothesis: Increasing consumption of fruits, vegetables & bioactive compounds (omega-3 fatty acids, lycopene, flavonoids) & enhancing overall nutritional intake will improve human immunological profiles, taxonomic profile of the gut microbiota, & nutritional status biomarkers. Will determine diet effects on immune dysregulation, including leukocyte distribution, inflammatory cytokine profiles, T cell function, & other relevant immunological markers, the taxonomic & metatranscriptomic profile of the gut microbiome, & nutritional biomarkers & metabolites at selected intervals, in spaceflight & ground-control data.

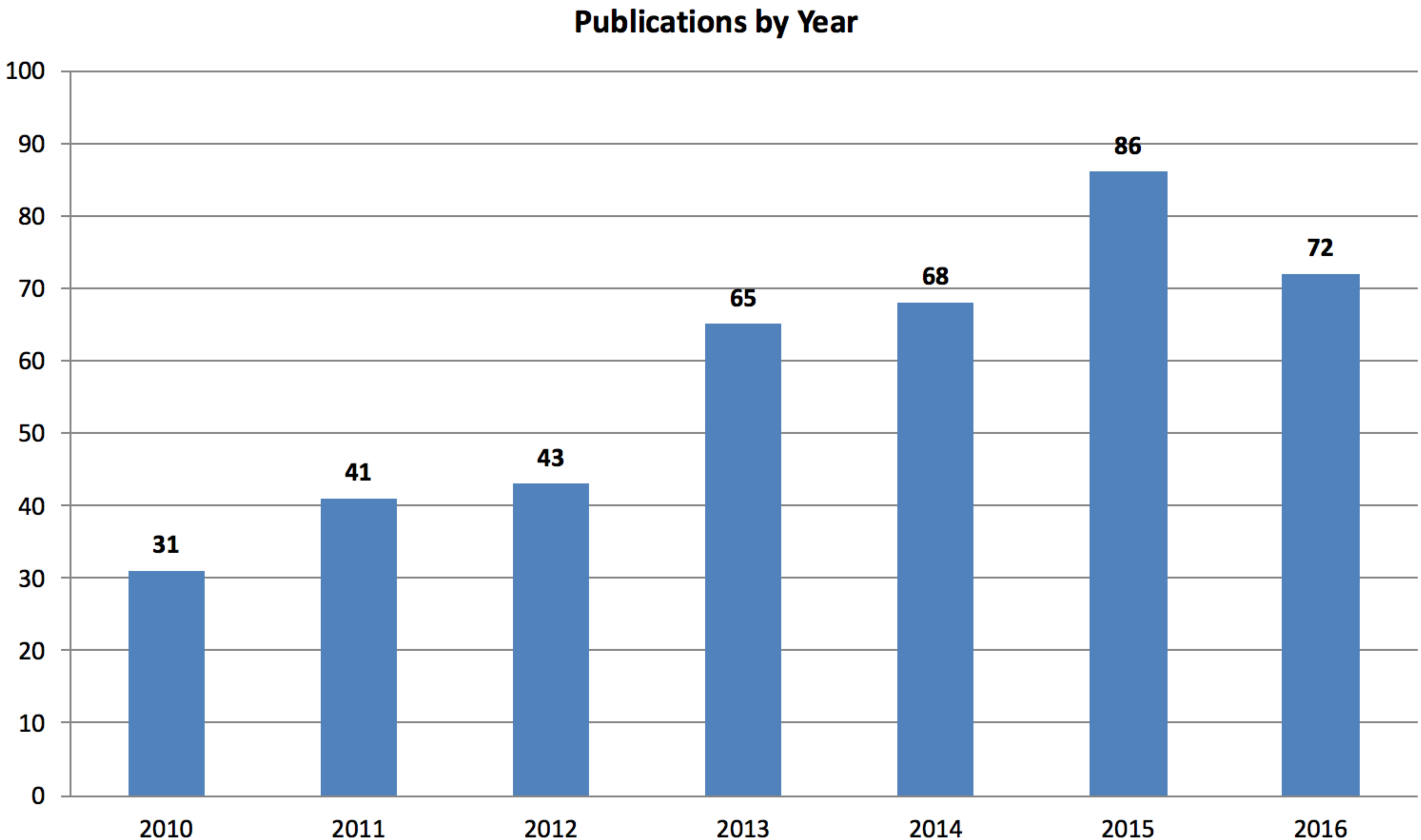
Pick-&-Eat Salad-Crop Productivity, Nutritional Value, & Acceptability to Supplement ISS Food System. Lead - Gioia Massa (NASA KSC) Team members - Douglas, G (NASA JSC) Hummerick, M (Qinetiq NA) Mitchell, C (Purdue) Morrow, R (Orbital Technologies) Wheeler, R (NASA KSC) Young, M (NASA JSC) Williams, T (Wyle) Growing nutritious, palatable food for crew consumption during spaceflight can provide health promoting, bioavailable nutrients, enhance dietary experience, & reduce launch mass for longer-duration missions. The “Veggie” vegetable-production system on ISS offers an opportunity to develop a “pick-&-eat” fresh vegetable component as a step to bioregenerative food production. Growing salad plants in Veggie during spaceflight will determine effects of light quality & fertilizer formulation on crop morphology, edible biomass yield, microbial food safety, organoleptic acceptability, nutritional value & behavioral health benefits of fresh produce. Phase A flight tests use leafy greens and Phase B dwarf tomato. Deliverable will be the development of growth protocols for these crops in a spaceflight vegetable production system.

Pioneering Scientific Discovery - Gravity as a Continuum Partnership between SB and HRP

15-15 NASA AG-0013 "Partial-Gravity Dose Response: Roles of vestibular input & sex in response to AG" - Charles Fuller (University Of California, Davis) Team Alwood, Hoban-Higgins, Ronca - Study male, female & labyrinthectomized rats, who no longer have macular vestibular input. Studies will adapt rats to 2G using chronic centrifugation. Our previous research has shown that animals exposed to chronic centrifugation establish a new steady-state in physiology & behavior. We will then return the animals to 1G Earth gravity, subjecting them to a 1G step down in ambient force. All animals will be studied for 60 days post-centrifugation. Tissues will be collected for endocrine, histological & proteomic analysis. Body temperature, activity & sleep-wake parameters. Body mass, & food & water consumption will be monitored. Body composition & bone structural analysis will be measured & video recordings will be used for behavioral phenotyping.

15-15 NASA AG-0005 "Musculoskeletal response to partial-gravity analog in rats: structural, functional & molecular alterations" - Seward Rutkove (BETH ISRAEL DEACONESS) Co-I Bouxsein One countermeasure to negative consequences of space travel is artificial gravity (AG) via centrifugation. Lower levels of force may be effective for preventing muscle & bone atrophy. This study will evaluate AG for prevention of muscle & bone atrophy & weakness by studying rats using partial unloading developed previously for mice. Musculoskeletal responses of adult male rats will be studied following exposure to 0.2, 0.4, & 0.7G as well as 1 G for 1, 2 & 4 weeks. Measures will include histological analyses, force of muscle contraction, & bone mass, microarchitecture, & strength. Serological & tissue molecular analyses will be used to understand mechanisms and time course of changes. At the conclusion, we will have defined the potential impact of partial artificial gravity for prevention of musculoskeletal dysfunction & deterioration to inform future application of AG in human research & space exploration.

Space Biology Research Accomplishments



Space Biology - Pioneering Scientific Discovery
Space Flight Data to Knowledge – Genelab Results

GeneLab
Open Science
for Exploration

81 Studies and Growing

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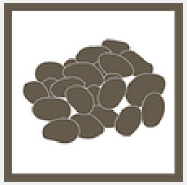
https://genelab-data.ndc.nasa.gov/genelab/projects/

Page 1 of 1 (Total Studies: 80)
Studies Per Page: 100

GLDS-83

Comparative Transcriptomic Analysis of Adult Medaka Tissues Sampled after Adaptation to a Space Environment

Organisms	Factors	Assay Types	Release Date	Description
Oryzias latipes	Spaceflight	transcription profiling	15-Oct-2015	To understand how humans adapt to space environments, many experiments can be conducted on astronauts while they work aboard the Space Shuttle or the International Space Station (ISS). We also need animal experiments that can apply to human...

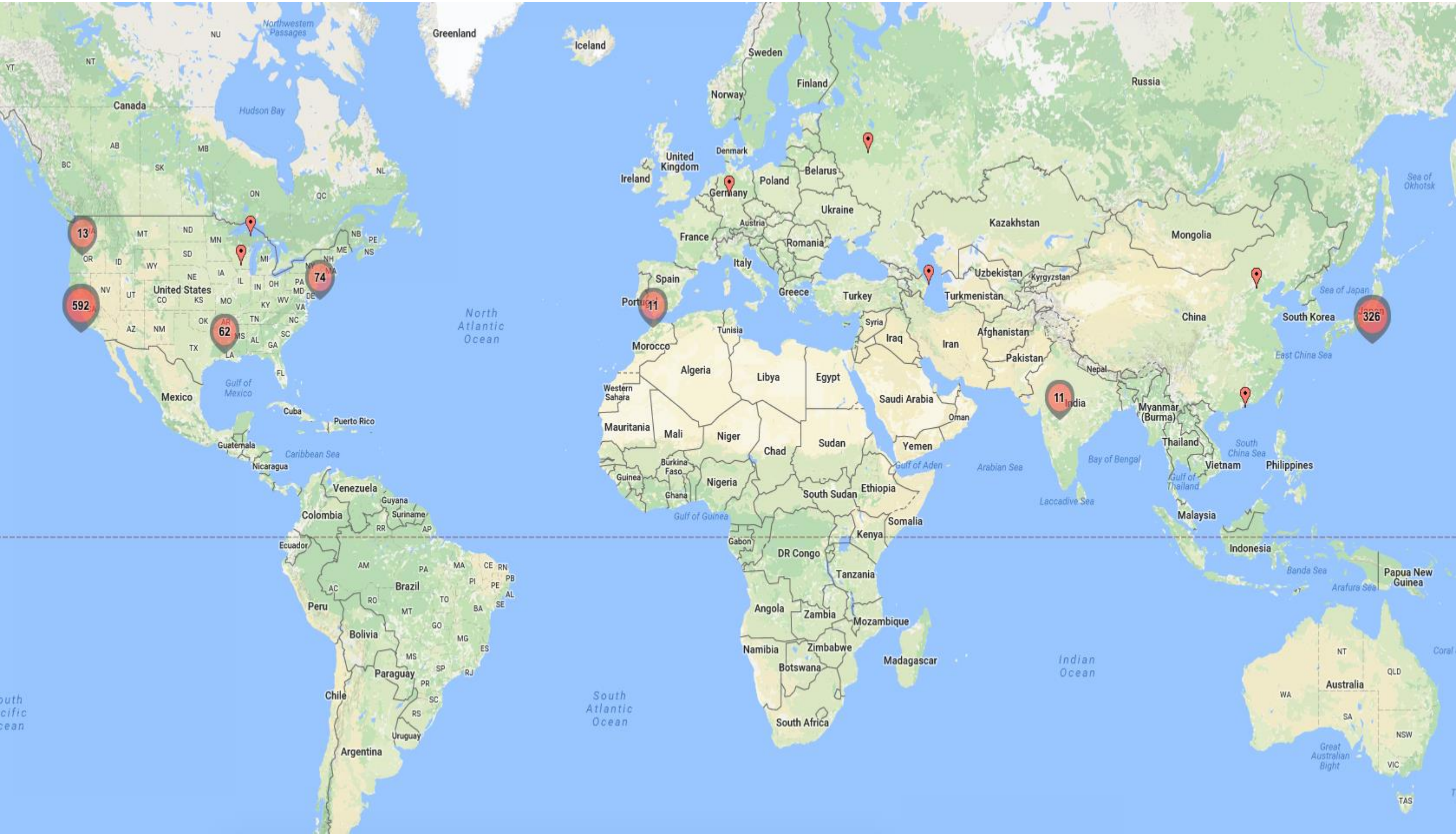


GLDS-81

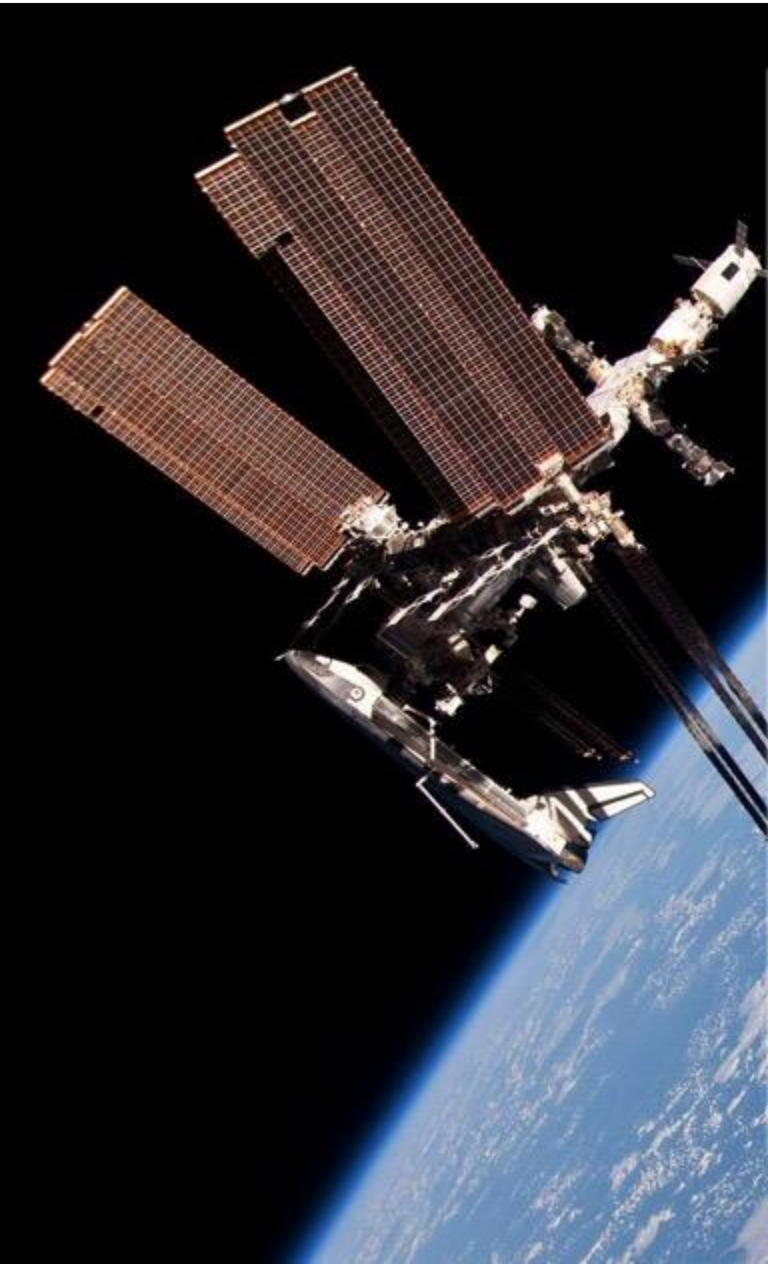
Bacillus subtilis strains at low-pressure: 5 kPa versus 101 kPa growth

Organisms	Factors	Assay Types	Release Date	Description
Bacillus subtilis	strain pressure	transcription profiling	14-Mar-2014	Comparing the transcriptional responses of Bacillus subtilis strains WN624 and WN1106 at 5 kPa and 101 kPa. WN1106 is a 5 kPa-evolved strain with increased fitness compared to ancestor-WN624 strain at 5 kPa. This experiment probed the diffe...

Space Biology - Pioneering Scientific Discovery
Space Flight Data to Knowledge – Genelab Access



Space Biology Research Challenges



Crew Time:

- ISS crew time is oversubscribed; little is available for Space Biology and Physical Science

Rodent Research:

- Severely limited ability to execute rodent research on orbit

Post-ISS Exploration:

- NASA will not be the “anchor tenant” for research in low Earth orbit

New Platforms:

- Continuing research activities on ISS while developing opportunities to move beyond LEO and utilize alternative platforms

Addressing Space Biology Research Challenges

Crew Time:

- Focus near-term on ISS payloads that can fly using reserve crew time
- Focus on automating ISS flight experiments to reduce/eliminate crew time
- Partner with CASIS, Roscosmos, HRP, STMD and other Programs to co-sponsor payloads of joint interest in exchange for prime crew time from those Programs
- Target alternative vehicles to increase flight opportunities and go beyond LEO

Rodent Research:

- Implement a BSP, in collaboration with CASIS, for every Rodent Research Mission to maximize utilization of tissues returned from space and obtain tissues for Space Biology PI research
- Collaborate with JAXA and Russia for BSP and Joint Rodent Research Missions to obtain tissues for Space Biology PI research
- Seek new opportunities for Rodent Research in Space (*e.g*. Bion)

Post-ISS Exploration and New Platforms:

- Working to develop plans to enable increased Life Beyond Low Earth Orbit Activities
- Working to fly investigations on all NASA Exploration missions and increase utilization of new space flight carriers