Fiscal Year:	FY 2016	**Task Last Updated:**	FY 08/03/2015
PI Name:	Boothby, Thomas Ph.D.		
Project Title:	Using Water Bears to Identify Biological Countermeasures to Stress During Multigenerational Spaceflight		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Cell & Molecular Biology (2) Animal Biology: Invertebrate		
Space Biology Cross-Element Discipline:	(1) Reproductive Biology (2) Developmental Biology		
Space Biology Special Category:	None		
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Comments:	NOTE: Previously at University of North Carolina until fall 2019.		
Project Type:	Flight	**Solicitation / Funding Source:**	2014 Space Biology Flight NNH14ZTT001N
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No. of Post Docs:	1	**No. of PhD Degrees:**	1
No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA ARC
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Flight Program:	ISS		
Flight Assignment:			
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Goldstein, Bob Ph.D. (University of North Carolina)		
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	For most organisms the stresses associated with spaceflight induce a variety of detrimental effects. To foster a safe and productive long-term human presence in space, therapies and countermeasures to spaceflight-induced stress should be developed. Tardigrades (water bears) are polyextremophiles that have evolved to tolerate multiple extreme environments, which are restrictive to most life. In 2007 tardigrades were shown to survive and reproduce normally during an 11-day low Earth orbit on the Foton-M3 Capsule. We speculate that mechanisms tardigrades have evolved to withstand extreme environments on Earth, may as a side-effect, confer protection against the stresses of spaceflight. This makes tardigrades a uniquely valuable system for studying responses to spaceflight. We have sequenced the genome of the tardigrades Hypsibius dujardini, as well as developed and validated experimental and computational approaches for measuring the effect of different environmental conditions on tardigrade gene expression – allowing us to identify		

Task Description:	mechanisms used by tardigrades to protect themselves from different stresses. We have also developed a reverse genetic approach, RNA interference, for tardigrades that allows us to directly investigate the role of a gene in conferring tolerance to an environment. We will use these approaches to study tardigrades' initial, as well as multigenerational response to spaceflight and use RNA interference to test the functionality of the genes identified in our study. Next-generation transcriptome sequencing will be conducted on tardigrades cultures kept 0 generations (founding generation) and 4 generations onboard the International Space Station (ISS). Differential expression analysis will be conducted to compare ISS spaceflight timepoints, ground controls, and tardigrades exposed to other extreme stresses (e.g., desiccation, freezing). This approach will allow us to identify potential mediators of stress tolerance, which will serve as candidates for functional RNA interference experiments. Understanding how tardigrades tolerate spaceflight will better guide future research into countermeasures and therapies for humans exposed to the stresses of prolonged space travel. This proposal's strengths are: the use of an organism that is suited to studying mechanisms of multigenerational tolerance of extreme environments and that has an established RNA interference method for confirming the function of genes identified in our study, our Preliminary Results that validate our proposed approach and technical capabilities as well as the uniqueness and suitability of tardigrades that will allow us to conduct this study. The participants for this study are comprised of experts in tardigrades' stress response and have considerable experience with next-generation sequencing and analysis of non-model organisms. The proposed experiments directly address recommendation AH16 of the Decadal Survey and are in line with recommendation OCB-5 and CMM-5 of NASA's Multigenerational and Developmental Biology of Invertebrates Research Emphasis as well as NASA's Fundamental Space Biology Plan 2010-2020 goals. Completion of our proposal will identify genes required for tardigrades to survive multigenerational spaceflight and will be a key step towards developing countermeasures and therapies for stresses associated with prolonged human exposure to space environments.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Along with using mechanisms of stress tolerance to counteract detrimental effects of space travel, data from our proposed experiments could be used in the long term toward solving serious problems in the field of human health. Utilizing mechanisms that allow tardigrades to stabilize their cellular proteins and nucleic acids has been proposed as an option for the dry storage and stabilization of vaccines and other biomaterials (Guo et al., 2000; Wolkers et al., 2001; Puhlev et al., 2001). Because current techniques for vaccine production, distribution, and storage nearly always require a constant cold chain (e.g., -80 and 20 0C freezers), these processes are extremely expensive. Some estimates put cold chain costs at around 80% of the total cost of vaccination (Chen et al., 2011). By generating additional stress response datasets, such as response to microgravity, freezing, irradiation, and hypoxia, we will increase our ability and that of other researchers to identify specific mediators of desiccation tolerance, which will then be applied to this and similar problems. Additionally, a better understanding of mechanisms of stress tolerance could lead to the development of drought and/or freeze tolerant crops. Guo, N., Puhlev, I., Brown, D. R., Mansbridge, J., & Levine, F. (2000). Trehalose expression confers desiccation tolerance on human cells. Nature biotechnology, 18(2), 168-171. Wolkers, W. F., Walker, N. J., Tablin, F., & Crowe, J. H. (2001). Human platelets loaded with trehalose survive freeze-drying. Cryobiology, 42(2), 79-87. Puhlev, I., Guo, N., Brown, D. R., & Levine, F. (2001). Desiccation tolerance in human cells. Cryobiology, 42(3), 207-217.
Task Progress:	We have been working to develop and optimize culture and storage conditions for tardigrades onboard the ISS. Below is a summary of our work to date. Long-term inactivation of cultures by freezing To better insure survival, synchronize flight and ground experiments, reduce variability in transport temperatures, and to allow for delays in activation as well as precise temporal activation of tardigrade cultures we tested the potential of using freezing as a long-term method of inactivating our tardigrade cultures. Reproduction of surviving individuals was assessed and in all cases 100% of surviving specimens were found lay viable clutches of eggs. Freezing in 2mL syringes – survival and dispensing Since learning that there have been cracking issues with fibercell cartridges used in the BIOS system during freezing we decided to test if freezing our samples in syringes rather than fibercells would be possible. Additionally we tested the efficiency of inoculating fibercell cartridges using thawed syringe cultures. Dispensing thawed cultures into fibercells was simple and efficient. Thawing temperatures To test if the temperature at which samples frozen in syringes are thawed has an effect on survival we froze subcultures in 2mL syringes for 2 weeks. After 2 weeks syringes were placed at various temperatures and allowed to thaw for 45 minutes. Survival was assessed. Culturing in fibercells We tested if BIOS culture system is a viable option for culturing tardigrades, we assessed the ability of fibercell cartridges to support long-term tardigrade cultures. RNAlater fixation time course at -80 degrees C We have proposed to fix samples using RNAlater to preserve transcriptional profiles until our samples are returned from the International Space Station (ISS). While RNAlater is a well established reagent used for the long-term stabilization of nucleic acids, it has not been tested long-term on tardigrades. To test this we are looking at the integrity of RNA extracted from samples frozen in RNAlater for prescribed periods of time.

Bibliography Type:	Description: (Last Updated: 09/13/2024)