Fiscal Year:	FY 2023	**Task Last Updated:**	FY 09/19/2022
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:	None		
Space Biology Special Category:	None		
PI Email:	Thomas.Boothby@uwyo.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	
Organization Name:	University of Wyoming		
PI Address 1:	1000 E. University Ave., Department #3944		
PI Address 2:			
PI Web Page:			
City:	Laramie	**State:**	WY
Zip Code:	82071	**Congressional District:**	1
Comments:	NOTE: Previously at University of North Carolina until fall 2019.		
Project Type:	FLIGHT	**Solicitation / Funding Source:**	2014 Space Biology Flight NNH14ZTT001N
Start Date:	11/13/2019	**End Date:**	11/12/2023
No. of Post Docs:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:	1	**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA ARC
Contact Monitor:	Griko, Yuri	**Contact Phone:**	650-604-0519
Contact Email:	Yuri.V.Griko@nasa.gov		
Flight Program:	ISS		
Flight Assignment:	NOTE: End date changed to 11/12/2023 per F. Hernandez/ARC (Ed., 2/27/23) NOTE: End date changed to 11/12/2022 per NSSC information. (Ed. 10/29/21)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):			
Grant/Contract No.:	80NSSC20K0283		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	NOTE: Continuation of "Using Water Bears to Identify Biological Countermeasures to Stress During Multigenerational Spaceflight," grant NNX15AB44G, when Principal Investigator was at University of North Carolina. 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 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 (Organismal and Comparative Biology) and CMM-5 (Cell, Microbial, and Molecular Biology) 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 degrees C 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. Chen, X. et al. (2011). Improving the reach of vaccines to low-resource regions, with a needle-free vaccine delivery device and long-term thermostabilization. J. Controlled Release 152, 349–355.
	Aim 1: Aim 1 focuses on distinguishing short and long-term changes in gene expression in tardigrades exposed to the rigors of low-Earth orbit (LEO). Towards this end, we have completed our 61-day flight experiment, culturing tardigrades for 7 and 61 days. These samples have been turned over to us by NASA and we have begun our investigation into the differences between 7 and 61-day flown and ground samples. RNA has been extracted from all 7 and 61-day samples (both flown and ground controls). RNA quantity and quality was assessed using Aligent's TapeStation using a high sensitivity RNA tape kit. Quality RNA was recovered from all specimens. RNA from all samples was sent to University of Colorado (CU) Anschutz's sequencing core facility. RNA was processed and sequencing libraries prepared using Illumina RNAseq technology. Libraries were multiplexed and sequenced using Illumina 150 base pair paired-end reads. Raw reads have been uploaded to University of Wyoming's Teton computer cluster. Quality control and read trimming has been performed on raw reads. Because our samples come from a mixed population containing both tardigrades and their algal food source, it is necessary for us to map our reads to a tardigrade and algal reference genome/transcriptome to parse these reads before

Task Progress:	performing differential gene expression analysis. This will also allow us to ascertain if we need to perform additional sequencing to gain our desired coverage for reads coming from tardigrades. Please note that re-/additional sequencing is easily performed with preexisiting Illmina libraries (CU Anschutz retains leftover sample for sequencing of this type) and no additional flight or ground experiments will need to be performed. In performing this analysis, we found that, indeed, additional sequencing will be necessary to bring our tardigrade fold coverage up to >40X. This resequencing has commenced, and we will perform the above-mentioned processing/quality control on these new reads when they are available. Aim 2: Aim 2 deals with comparing transcriptomes derived from tardigrades exposed to different stress conditions (freezing, drying, simulated microgravity, radiation exposure) to animals exposed to spaceflight conditions (both short- and long-term exposure). We have sequenced and analyzed additional ground-based stress conditions and are waiting for additional sequencing in Aim 1 before we can move forward with comparing ground stresses to flight stresses. Aim 3: Aim 3 deals with testing the functionality of genes identified in Aims 1 and 2 in allowing tardigrades to survive under (simulated) flight conditions. To this end, we have had NASA construct for us a random positioning machine (RPM) capable of simulating different microgravity conditions. We have received this device and have begun optimizing tardigrade culture and monitoring protocols. With these protocols, we are assessing tardigrade health parameters, including: lifespan, number of egg clutches laid, timing of clutch laying, and clutch size.
Bibliography Type:	Description: (Last Updated: 06/28/2023)