Fiscal Year:	FY 2023	**Task Last Updated:**	FY 11/13/2023
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		
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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:**	09/11/2024
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
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Flight Program:	ISS		
Flight Assignment:	NOTE: End date changed to 09/11/2024 per F. Hernandez/ARC (Ed., 6/13/24) 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:	Addition of Seif Tekaya (postdoc) to the team. Seif is an expert in radiation biology and next-generation sequencing.		
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.
Task Progress:	During this reporting period, the major progress has been the resequencing of tardigrade flight and ground control samples to obtain sufficient sequencing depth in a large number of replicates to perform differential RNAseq analysis. Previously, we had extracted and sequenced RNA from our samples. While the sequencing results were of high quality, we felt that greater sequencing depth would allow for a more robust analysis and statistical comparison to be made between flight samples as well as ground controls. Resequencing was performed. This resulted in enough reads to have at least 4 replicates of each sample with sufficient depth for robust comparative analysis. The minimum we had specified was three replicates per sample. In most cases, we now have five. Sequence quality control and trimmer was performed and proceeded as expected, with minimal loss of sequencing reads (an indication of high sequencing quality, which we had observed in our first round of sequencing). Trimmed and quality reads were aligned to our tardigrade reference genome using HISAT2; this included an analysis of read alignment quality. Aligned reads were quantified using DEseq. Principal component analysis (PCA) was performed to assess the degree of similarity (reproducibility) between sample replicates. This analysis reveals stereotypes/responses to each condition, as PCA clustering places replicates from the same exposure/culture conditions together. Exposure to stress conditions was observed in the PCA clustering analysis. Exposure to stress conditions was also observed in a hierarchical clustering analysis.

	Differential gene expression analysis indicated that not only are the changes stereotypical, but robust under certain conditions. Many (tens to hundreds) of genes responded to flight conditions in both a positive and negative fashion. The changes in comparing ground to flight controls show differences, indicating that each environment and time range imposes slightly different stresses on the organism.
Bibliography Type:	Description: (Last Updated: 09/13/2024)