Fiscal Year:	FY 2017	**Task Last Updated:**	FY 09/02/2016
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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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:	11/01/2014	**End Date:**	10/31/2017
No. of Post Docs:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	1
No. of Bachelor's Candidates:	2	**Monitoring Center:**	NASA ARC
Contact Monitor:	Sato, Kevin	**Contact Phone:**	650-604-1104
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Flight Program:	ISS		
Flight Assignment:			
Key Personnel Changes/Previous PI:	September 2016 report: Kiera Patanella, an undergraduate at the University of North Carolina at Chapel Hill working on this project, has graduated and obtained her bachelors degree in Biology. Cody Weyhrich, an undergraduate at the University of North Carolina at Chapel Hill, has started working on this project as of 8/1/2016.		
COI Name (Institution):	Goldstein, Bob Ph.D. (University of North Carolina)		
Grant/Contract No.:	NNX15AB44G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	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 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 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.
Task Progress:	While awaiting our flight opportunity, we have continued to carry out our ground-based studies examining the mechanisms and mediators used by tardigrades to survive terrestrial abiotic stresses, such as desiccation, freezing, high temperatures, and irradiation. Using results from transcriptomic sequencing we have identified potential mediators of stress tolerance and are evaluating the function and efficacy of these mediators through a combination of reverse genetic, heterologous expression, and biochemical approaches. Learning more about how tardigrades survive terrestrial abiotic stresses will help build a platform for comparing and contrasting the mediators and mechanisms they use to protect themselves from the harmful effects of spaceflight. A manuscript has been submitted and is under review for publication in a peer-reviewed journal: Boothby TC, Tapia H, Brozena A, Piszkiewics S, Smith AE, Giovannini I, Rebbechi L, Pielak GJ, Koshland D, Goldstein B. "Tardigrades Use Intrinsically Disordered Proteins to Survive Desiccation."
Bibliography Type:	Description: (Last Updated: 06/28/2023)
Articles in Peer-reviewed Journals	Boothby TC, Tenlen JR, Smith FW, Wang JR, Patanella KA, Nishimura EO, Tintori SC, Li Q, Jones CD, Yandell M, Messina DN, Glasscock J, Goldstein B. "Evidence for extensive horizontal gene transfer from the draft genome of a tardigrade." Proc Natl Acad Sci U S A. 2015 Dec 29;112(52):15976-81. Epub 2015 Nov 23. http://dx.doi.org/10.1073/pnas.1510461112 ; PubMed PMID: 26598659; PubMed Central PMCID: PMC4702960 , Dec-2015

Articles in Peer-reviewed Journals	Boothby TC, Goldstein B. "Reply to Bemm et al. and Arakawa: Identifying foreign genes in independent Hypsibius dujardini genome assemblies." Proc Natl Acad Sci U S A. 2016 May 31;113(22):E3058-61. http://dx.doi.org/10.1073/pnas.1601149113 ; PubMed PMID: 27173900; PubMed Central PMCID: PMC4896697 , May-2016