Fiscal Year:	FY 2018	**Task Last Updated:**	FY 09/04/2017
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:**	10/31/2018
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:	NOTE: Extended to 10/31/2018 per F. Hernandez/ARC (Ed., 12/6/17)		
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. 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:	Work/progress on this project can be generally broken down into two categories-- 1.) preparation and hardware testing for our flight mission, and 2.) execution of complimentary ground experiments. 1.) Preparation for flight experiments. Initially we selected the BIOS culture system of our flight experiments and conducted extensive ground testing to verify all parameters of our flight experiment with this hardware. Since the wait time for the use of the BIOS culture system is significant we have been exploring additional hardware options, which at the time we believed might help us accelerate the timing of our flight experiment. We have been in contact with BioServe and Techshot regarding different hardware options and were validating use of BioServe's BioCell. This hardware seems to work well for our purposes, but apparently new information on launch schedules has come out and using this hardware will no longer accelerate our project's expected launch date. Thus, we have settled on using our original hardware choice, the BIOS culture system. 2.) Complimentary ground experiments. Part of our post-flight analysis will be to compare the genes unregulated during exposure to the stresses of spaceflight with ground based stresses. To this end we have been studying the change in gene expression in tardigrades exposed to different Earth-based stresses. Our study of tardigrade responses to drying has resulted in a paper published in Molecular Cell (Boothby et al., 2017). We are currently preparing a manuscript looking at the connection (or lack of connection) between drying and freezing tolerance in tardigrades.
Bibliography Type:	Description: (Last Updated: 06/28/2023)

Abstracts for Journals and Proceedings	Boothby TC, Piszkiewicz S, Mehta A, Brozena A, Tapia H, Koshland D, Holehouse A, Pappu R, Goldstein B, Pielak G. "Tardigrade Disordered Proteins Mediate Desiccation Tolerance." Presented at the 61st Annual Meeting of the Biophysical Society, New Orleans, LA, February 11-15, 2017. Biophysical Journal. 2017 Feb 3;112(3 Suppl):480a. https://doi.org/10.1016/j.bpj.2016.11.2600 , Feb-2017
Abstracts for Journals and Proceedings	Boothby TC, Tapia H, Brozena AH, Piszkiewicz S, Smith AE, Mehta A, Koshland D, Goldstein B, Pielak G. "How Do Tardigades Survive Extremes? Disordered Proteins as Mediators of Tardigrade Stress Tolerance." Society for Integrative and Comparative Biology Annual Meeting 2017, New Orleans, LA, January 4-8, 2017. Integrative and Comparative Biology. 2017 Mar 1;57(Suppl 1):E208. , Mar-2017
Articles in Peer-reviewed Journals	Boothby TC, Tapia H, Brozena AH, Piszkiewicz S, Smith AE, Giovannini I, Rebecchi L, Pielak GJ, Koshland D, Goldstein B. "Tardigrades use intrinsically disordered proteins to survive desiccation." Molecular Cell. 2017 Mar 16;65(6):975-84.e5. https://doi.org/10.1016/j.molcel.2017.02.018 ; PubMed PMID: 28306513 , Mar-2017
Articles in Peer-reviewed Journals	Boothby TC, Pielak GJ. "Intrinsically disordered proteins and desiccation tolerance: Elucidating functional and mechanistic underpinnings of anhydrobiosis." BioEssays. Version of Record online: 13 SEP 2017. https://doi.org/10.1002/bies.201700119 , Sep-2017