Fiscal Year:	FY 2019	**Task Last Updated:**	FY 01/15/2019
PI Name:	Fox, Donald Ph.D.		
Project Title:	Mining Biology's Extremes for New Space Radiation Resistance Strategies		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	TRISH--TRISH		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
PI Email:	don.fox@duke.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	919-613-8756
Organization Name:	Duke University Medical Center		
PI Address 1:	Pharmacology & Cancer Center		
PI Address 2:	DUMC Box 3813, C318 LSRC		
PI Web Page:			
City:	Durham	**State:**	NC
Zip Code:	27710	**Congressional District:**	1
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2017 HERO NNJ16ZSA001N-TRIRT. Appendix C: Translational Research Institute for Space Health (TRISH) Research Topics
Start Date:	10/01/2017	**End Date:**	09/30/2020
No. of Post Docs:		**No. of PhD Degrees:**	
No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:	1	**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:	3	**Monitoring Center:**	TRISH
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Kirsch, David M.D., Ph.D. (Duke University Medical Center)		
Grant/Contract No.:	NNX16AO69A-T0108		
Performance Goal No.:			
Performance Goal Text:			
	Using powerful genetic screening in Drosophila and follow-up work in mice, we will identify unique genes and gene expression that enhance space radiation tolerance in vivo. Our approach will identify new, organism-relevant strategies to provide space radiation resistance. 1-Specific Aims Aim1- A targeted Drosophila screen of candidate factors from Tardigrades (Ramazzottius varieornatus) that enhance radiation resistance. Aim2- An unbiased screen for genes that enhance radiation resistance in the Drosophila hindgut.		

Task Description:	2-Relevance The purpose of this proposal is to uncover new understanding of how a species withstands space-relevant radiation exposure, using validation and safety efficacy studies in model organisms. Drosophila is specifically mentioned, and we have expertise in study of Drosophila radiation resistance mechanisms (Bretscher and Fox 2016, Dev Cell). We will perform genetic manipulation in vivo in flies, targeting potential Tardigrade resilience mechanisms. Finally, we discuss follow-up work in rodents, which we are well-equipped to do, as Duke co-investigator Dr. Kirsch has prior NASA-funded experience in studying space radiation effects in mice at Brookhaven NASA Space Radiation Laboratory (NSRL). 3-Approach Aim1- We will generate novel Drosophila strains expressing candidate Tardigrade genes, and assay their effects on resistance to both high charge and energy (HZE) particles (56Fe), and as a comparison, X-ray irradiation. Tardigrades have recently shown promise for finding factors that enhance radiation tolerance (Hashimoto et al., Nat. Comm. 2016). Genome data for this radiation-resistant organism is now available. From our collaborators Bob Goldstein, we will obtain animals for cDNA generation. We will generate up to 165 unique fly lines, each expressing a Tardigrade gene that, relative to Drosophila or humans, is unique (low homology) and/or induced by radiation. Flies will then be subjected to HZE particles at NSRL or X-irradiation at Duke, and monitored for long-term survival, multi-generational fecundity, and will be sequenced at distinct generations to quantify radiation-induced mutations. Genes with promising enhanced radiation resistance will be pursued further in transgenic mice subjected to similar tests as in flies. Aim2- Relative to candidate screens (Aim1), un-biased fly screens are more applicable to genome-wide study. The Fox laboratory recently identified a Drosophila cell type (hindgut papillar cells) that is highly resistant to X-irradiation, and used a simple in vivo candidate screen to find genes required for the heightened radiation resistance. Expanding on this successful strategy, we will screen 1/5 of the entire genome through ethyl methanesulfonate (EMS) mutagenesis. Mutant strains will be assayed for DNA damage resistance in hindgut papillar cells. Interesting mutants will be sequenced to find causative genes. We will then generate transgenic flies expressing genes that mediate radiation resistance throughout the fly, and perform tests of HZE particle and X-irradiation resistance, including long-term organismal assays and follow-up mouse experiments as in Aim1. 4-Impact and 5-Rationale for mitigating space exploration risks Space radiation poses a significant threat to astronaut health, and novel approaches are needed to limit space radiation damage. Drosophila and mice provide convenient, in vivo-relevant screening platforms, and effects on organism health, such as fecundity, can be scored. Understanding mechanisms that prevent space radiation damage in model organisms may uncover new space radiation resistance strategies to be targeted in humans. Such strategies would accelerate the pace of space discovery while protecting astronaut lives. Bretscher, H. S. & Fox, D. T. Proliferation of double-strand break-resistant polyploid cells requires Drosophila FANCD2. Dev Cell 37, 444–457 (2016). Hashimoto, T. et al. Extremotolerant tardigrade genome and improved radiotolerance of human cultured cells by tardigrade-unique protein. Nat Comms 7, 12808 (2016).
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	In year 1 of our proposal we cloned 96 Tardigrade genes into Fruit Fly expression vectors. These vectors, and the transgenic flies that are deriving from them, represent a new resource for the study of Tardigrade gene products and their potential impact on the biology of extreme environmental stress resistance, including resistance to radiation.
Task Progress:	Original project aims/objectives: (1) AIM1- A targeted Drosophila screen of candidate factors from Tardigrades (Ramazzottius varieornatus) that enhance radiation resistance. AIM2- An unbiased screen for genes that enhance radiation resistance in the Drosophila hindgut. (2) Aim 1- In year 1 we cloned 96 Tardigrade genes into fly expression vectors and began generating transgenic flies from these cloned genes. We also established three independent screening assays to measure radiation resistance in response to both high (56Fe) and low (X-ray) linear energy transfer (LET) radiation. Aim 2- In year 1 we generated approx. 1,500 lethal mutant stocks in the genetic background required for our proposed ethyl methanesulfonate (EMS) screen, and began to optimize our conditions for our un-biased screen. (3) In year 1 we made significant progress in our technology requirement needed for our screens to discover new radiation counter-measures. (4) AIM1- As outlined in our proposal, we aim to identify single Tardigrade genes that, when expressed in another organism, confer increased radiation resistance. We proposed to use Drosophila to rapidly screen through single Tardigrade genes in a whole organism context. In year one, we made progress towards this goal on two major fronts. First, we initiated the creation of 96 transgenic fly lines, each expressing a single Tardigrade gene. Second, we established protocols for screening each transgenic line for increased radiation resistance. We have now cloned 96 distinct Tardigrade genes into fly expression vectors. For 36 of these genes, we have already performed all of the plasmid amplification, sequence verification, and embryo injection to produce stable transgenic lines. In the coming year we see no issues with meeting our proposal's stated goal of establishing approx. 100 transgenic lines, each expressing a Tardigrade-unique gene. Additionally, we established 3 independent assays for radiation resistance. Specifically, we now have repeatable assays to measure an irradiated animal's acute survival, lifespan, and fecundity. For low LET radiation, we have established 15 Gy of X-irradiation (at .16 MeV) at Duke's irradiator as our standard dose, and for high LET radiation we established 10 Gy of 56Fe (at 600 MeV/n) as our standard dose. The latter dose was determined in our first trip to NSRL in June. To summarize, at the end of year 1 we are in an excellent position to begin screening our Tardigrade gene-expressing flies in year 2, while continuing to generate and test additional lines. AIM2- As outlined in our proposal, we plan to conduct an unbiased screen for genes that are required for DNA damage resistance in the Drosophila hindgut. In year 1, we established 6161 fly lines from mutagenized parents. From these, we recovered 1467 independent lines carrying recessive lethal mutations on the X-chromosome. We will maintain these lines as

	heterozygotes, but the lines are in a background that permits site-specific recombination on the X-chromosome to yield homozygous mutant hindgut cells. In year two, we are now well-positioned to carry out the proposed screen of these now established lines for genes required for radiation resistance.
Bibliography Type:	Description: (Last Updated: 09/04/2023)
Awards	Fox D. "Invited speaker at the 64th Annual Meeting of Radiation Research Society, Chicago, IL, September 2018." Sep-2018
Awards	Clay D. (Delisa Clay) "National Science Foundation graduate fellow, September 2018." Sep-2018