Fiscal Year:	FY 2019	**Task Last Updated:**	FY 09/10/2018
PI Name:	Mancinelli, Rocco Ph.D.		
Project Title:	Elucidating The Nitrogen Cycle of Eu:CROPIS (Euglena: Combined Regenerative Organic-food Production In Space)		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	SPACE BIOLOGY--Cellular and molecular biology		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Cell & Molecular Biology (2) Microbiology		
Space Biology Cross-Element Discipline:	(1) Reproductive Biology		
Space Biology Special Category:	(1) Cell Culture (2) Translational (Countermeasure) Potential (3) Bioregenerative Life Support		
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Zip Code:	94035	**Congressional District:**	18
Comments:			
Project Type:	FLIGHT	**Solicitation / Funding Source:**	Space Biology Unsolicited
Start Date:	10/01/2013	**End Date:**	10/31/2020
No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA ARC
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Flight Program:	Small Satellites		
Flight Assignment:	NOTE: End date changed to 10/31/2020 per F. Hernandez/ARC (Ed., 8/13/18) NOTE: End date changed to 9/30/2018 per F. Hernandez/ARC (Ed., 3/23/17)		
Key Personnel Changes/Previous PI:			
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Grant/Contract No.:	Coop Agreement via NNX12AD05A		
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Performance Goal Text:			

Task Description:

Editor's Note (12/2/2013): Funding is for Dr. Mancinelli's participation as Co-Investigator on the German Aerospace Center (DLR)'s Eu:CROPIS (Euglena with Combined Regenerative Organic-food Production In Space) mission and the Principal Investigator of the nitrogen cycling portion of the mission, entitled "Elucidating The Nitrogen cycle of Eu:CROPIS (Euglena: Combined Regenerative Organic-food Production In Space)."
The objective of the proposed study is to determine the effect of different gravity levels on the nitrogen cycle leveraging experiments to be flown on DLR's Eu:CROPIS mission. This is of importance to NASA because The National Research Council's Plant and Microbial Biology Decadal Survey (2011) states that there is a need for understanding the role of gravity on microbe-microbe interactions and microbe-plant interactions. The research proposed here will do just that. Nitrogen is an essential element for life. It is present in all living systems, occurring in several important molecules including proteins and nucleic acids. Without nitrogen life as we know it could not exits. Thus, the nitrogen cycle is important to supporting life whether it is on Earth, in space, or on other planets or moons. Because only Earth has a 1 x g environment understanding how the nitrogen cycle operates as a function of gravity is key to sustaining life off of Earth. To change the gravity levels the spacecraft will be maneuvered (by spinning) to produce three different gravity regimes during the courser of the mission. The three gravity regimes will be 0.01 x g - 0.1-x g (essentially microgravity); 0.16 x g (Moon gravity); and 0.38 x g (Mars gravity). Each gravity regime will last for six months. Eu:CROPIS will be used in reducing organic waste and in the development of efficient life support systems. Its core element is a microbiological trickling filter of lava rock – the habitat of a multitude of microorganisms that purify and decontaminate water. The development aims at a wet composting system that may be used in closed life support systems such as waste water recovery. A key component of the system is the nitrogen cycle. So, modeling the nitrogen cycle of the system is essential to understanding how the system functions. It will be the first time nitrogen-transformation reactions will be measured as a function of gravity. NASA has an excellent opportunity to participate in the DLR's Eu:CROPIS mission that allows us to obtain data by leveraging their laboratory work and hardware at a fraction of what it would cost if funded/supported solely by NASA.

Rationale for HRP Directed Research:

Research Impact/Earth Benefits:

The need for fundamental research to understand the role of gravity on microbe-microbe interactions microbe-plant interactions and microbe human interactions in space is recognized in the National Research Council's Plant and Microbial Biology Decadal Survey (2011) on Biological and Physical Sciences in Space (chapter 4). Through the proposed work, data from the Eu:CROPIS mission will address microbe-microbe and microbe plant interactions through cycling of key nutrients, specifically nitrogen, oxygen, and carbon.
Eventually, space travel will require the ability for self-sufficiency. Once mission profiles extend beyond short trips to the lunar surface, the duration of each mission will mean it will no longer remain cost-effective - or indeed feasible - to dispose of all waste and resupply oxygen, water, and food to crew members from Earth. NASA has acknowledged this reality for more than two decades with programs exploring the development of both physicochemical and bioregenerative life support systems. The program on bioregenerative capabilities arose from observations that the only truly long-term, self-sustaining life support system that has a demonstrated stability and efficacy relies upon biological systems for its function; that system is the life support afforded by Earth. Since bioregenerative life support systems are not high on the NASA priority list at this time it was stated in the report: Because international collaborations will be essential to make rapid progress with these aims, NASA should support collaborations, where appropriate, with partners that are already pursuing these goals, such as European scientists….

Eu:CROPIS is a clear example that fits in with this statement. It allows NASA to obtain this data at little cost by using the laboratories, the hardware, and the spacecraft paid for by the DLR. The Eu:CROPIS (Euglena: Combined Regenerative Organic-food Production In Space) experiment will test the feasibility and technology in the areas of life support systems and gravitational biological research. The mission offers for the first time the opportunity of analyzing coupled biological life support systems under different levels of gravity (space, moon, Mars) utilizing state-of-the-art methods for image and molecular analysis. It combines the C.R.O.P. system plant growth water purification system developed at the DLR in Cologne, Germany with the well studied Euglena gracilis space flight system.

Euglena gracilis is a motile, photosynthetic, unicellular flagellate living in ponds and lakes. It uses gravity and light as hints to reach and stay in regions of the water column optimal for photosynthesis and growth. At low light irradiances, Euglena swims toward and at higher irradiances cells swim away from a light source (positive and negative phototaxis). In addition, Euglena typically orients away from the center of acceleration (negative gravitaxis). Euglena is considered a 'professional gravi-sensing organism,' a term that was coined by ESA (European Space Agency). In the past 15 years, Euglena has been established as a model organism for studying gravity perception of single cells. A model for gravitaxis was created by the combination of physiological, biochemical, and molecular biological methods. In this context substantial contributions came from microgravity experiments in space.

The major work this year focused on developing a computer simulation model as well as testing the ground simulation facility. Following is what we accomplished regarding the Computer simulation.
Eu:CROPIS (Euglena: Combined Regenerative Organic-food Production In Space) is a closed ecosystem housed in a satellite that functions as water purifying and plant growth system. At its core is a lava rock trickling filter inhabited with microorganisms that breakdown urea in wastewater and produce nitrate for tomato growth. The Euglena produce oxygen and consume excess ammonium. An integral part of this system is the nitrogen cycle. Nitrogen is an essential element for life. Thus, the nitrogen cycle is important to supporting life whether it is on or off Earth. The objective of the study is to determine if the gravity levels of the Moon and Mars affect the nitrogen cycle of the system. The concentration of oxygen, urea, ammonium, nitrate, and nitrite in the system is monitored as a function of time.

Computer simulation of the filter column and the Eu:CROPIS-system

The simulation was performed with the GameMaker Software 8.1. (YoYo Games, Dundee, Scotland) . Cell types as well as substances were programmed as instances of objects with defined properties. The filter column as well as the subsequent compartments were programmed as 2 D-objects (representing a section of a "real column"). Substances were "flushed" into the system at the top of the column, where they migrated through the column, through the algae/plant compartment, and back to the column. The system enables performance of a "batch-run," where an initial amount of substances is flushed into the system and subsequently degraded, "pulsed runs," where preset amounts of substances are flushed into the system in regular time intervals and a combination of the two methods. In addition,

Task Progress:	substances can be added manually during an actual run. All instance numbers were constantly recorded, displayed on the screen, and stored. Only the cycles of carbon and nitrogen were simulated; other elements (phosphorous, potassium, trace elements) were not. Experimental setup for determination of the filter column (full system without plants) The Eu:CROPIS-test module was constructed mainly with same hardware components as the flight module. In order to allow detailed analysis of the filter-bacteria/algae-interaction no tomatoes were integrated in the system. It turned out in preliminary experiments that a head space of air is important for development of the filter column and Euglena gracilis. For this reason, an air reservoir with the size of the algae greenhouse was attached. A container with dormant states of Euglena gracilis cells was attached to the alga circuit. Activation of the pump of the algae section flushed the cells into the algae tank. The filter column was filled with small lava rocks which were primed among others with nitrifying bacteria (the rocks were part of a CROP-column before and provided from the DLR). The filter material was dry so that the experiments start with desiccated states of the bacteria. Temperature of the system was maintained at 20°C. Algae were constantly illuminated with a photon flux of 83 µmol photons/m2 provided from a mixed array of blue (peak wavelength 470 nm) and red (peak wavelength 660 nm)-high power LEDs. In order to allow initial growth of Euglena gracilis the algae section was started 10 days before the filter circuit was engaged. Summary The computer model seems to reflect what is occurring in the ground module of the system. We will continue to gather more data from the ground module to refine the model in anticipation of the flight experiment.
Bibliography Type:	Description: (Last Updated: 02/22/2023)