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Developing Protocols for the Space Algae-2 Mission



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Space Biosciences
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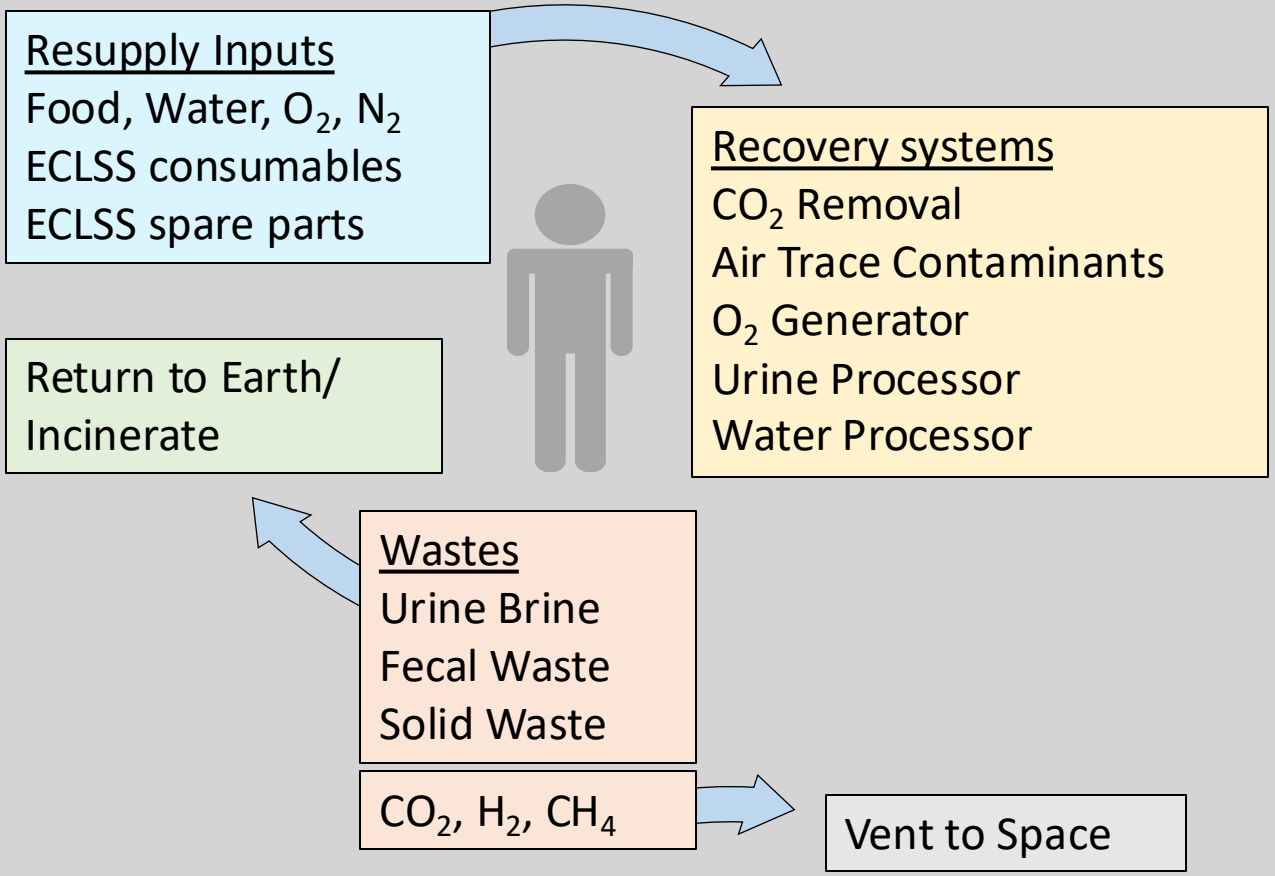
BPS
BIOLOGICAL AND PHYSICAL SCIENCES

Presentation Outline

- The Status of Life Support Aboard the ISS
- What is BLiSS?
- The Role of Algae in Building BLiSS
- Space Flight Risks for Algae
- Summary of Space Algae-1
- Background for Space Algae-2
- Challenges of Developing Methods for Space Algae-2

ISS has regenerative technologies to reduce inputs and waste in the life support system



- Highly efficient recovery of water
  ”Yesterdays coffee is todays coffee”
- CH_4, CO_2 and H_2 are vented into space
  Wasted!!

Monje O, et al (2025) 54th International Conference on Environmental Systems. ICES-2025-460. https://hdl.handle.net/2346/102834

What is BLiSS?

Broadly: A fully or partially closed–loop, self-sustaining biological life-support systems that produces food, clean water, renews air, and processes waste.

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- Biological systems could be more resilient (self-repair) than mechanical systems.
- Would enable to fully recycling of crew waste to crew food ("**Last weeks smoothie is next weeks smoothie**") and thus limit needed supplies for long-duration missions.
- Support crew health

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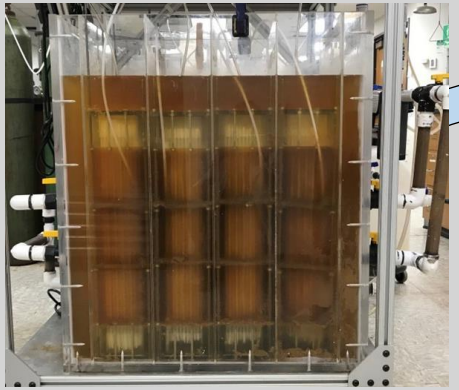
Cons:

- Minimal technological development to date

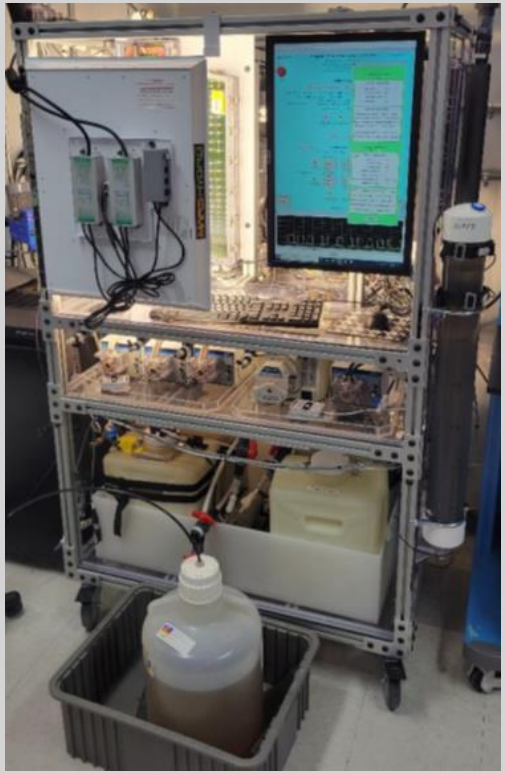
Building BLiSS: Kennedy Space Center is leading development and testing of microbial membrane bioreactor wastewater treatment systems for integration with food production.



Aerobic Digestion



Phototrophic Nitrogen Conversion



Anaerobic Digestion

Food, Water, O_2



Urine
Gray water

Fecal Waste
Solid Waste

Images from: Triana, et al (2025) NASA Technical Reports Server
https://ntrs.nasa.gov/citations/20250002776

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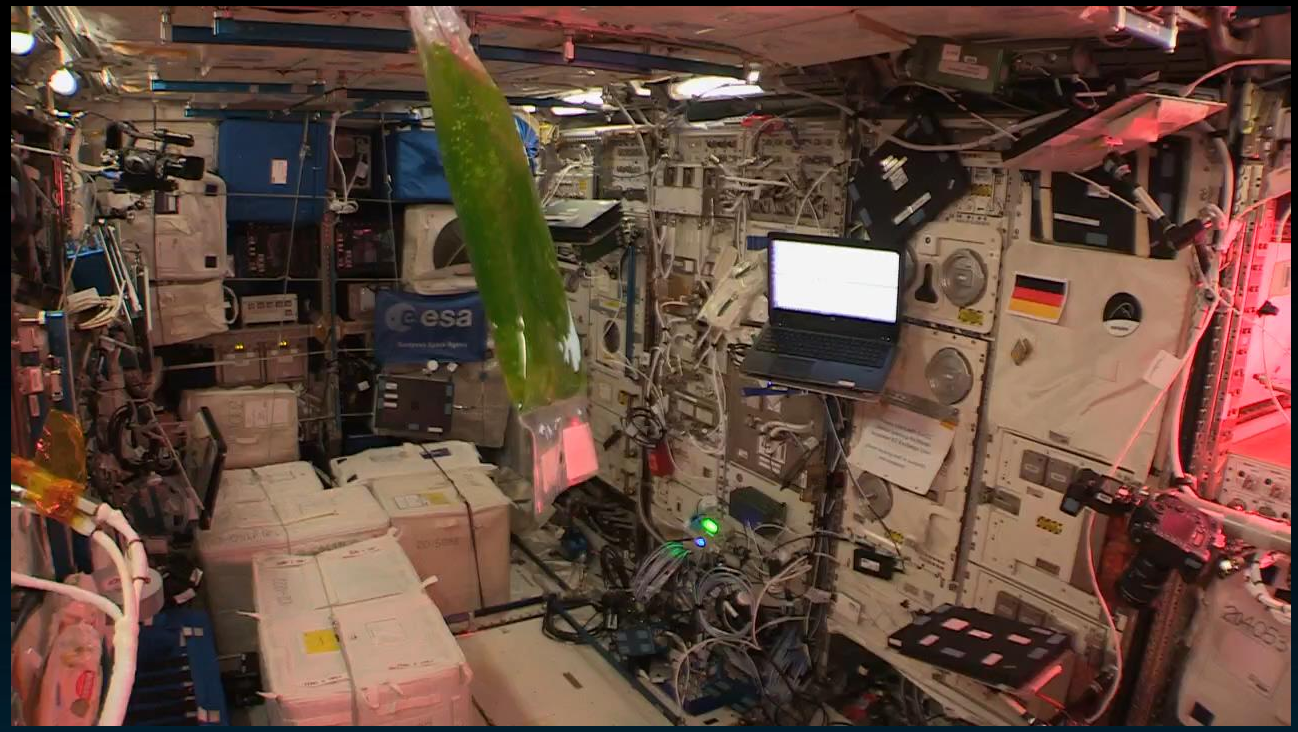
Building BLiSS: Microalgae for photosynthetic production in space

Algae recycle carbon dioxide into oxygen and generate useful biomass

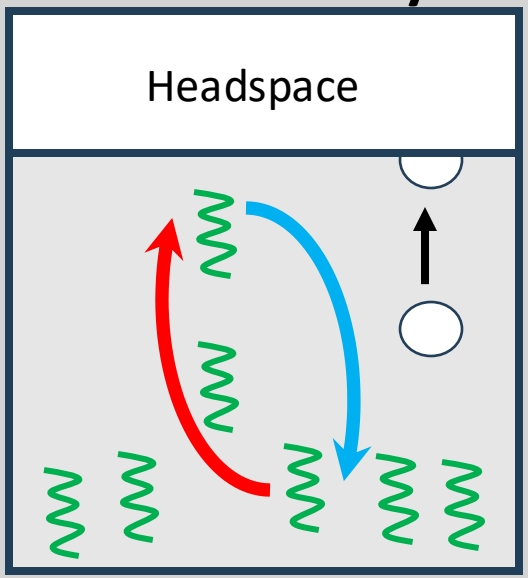
- Food & animal feed
- Biofuels & organic feedstocks
- Vitamins & essential amino acids



Space Algae-1 completed a competitive growth assay of UV-mutagenized *Chlamydomonas reinhardtii* during 35 days of spaceflight.

Space Risk: Microgravity aboard the ISS alters environmental conditions in liquid media

Earth Gravity



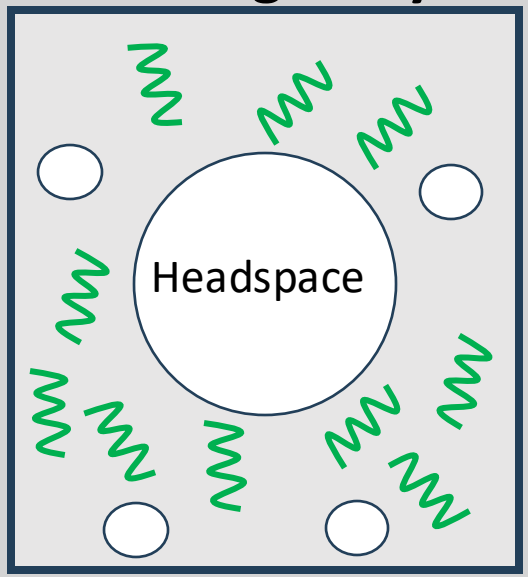
Cultures in microgravity…

- No convection mixing
- No cellular sedimentation
- Headspace is centrally located
- Stable gas bubbles

Modeling of microgravity in low-shear force conditions only prevents sedimentation

Tricones Bubbles

Microgravity



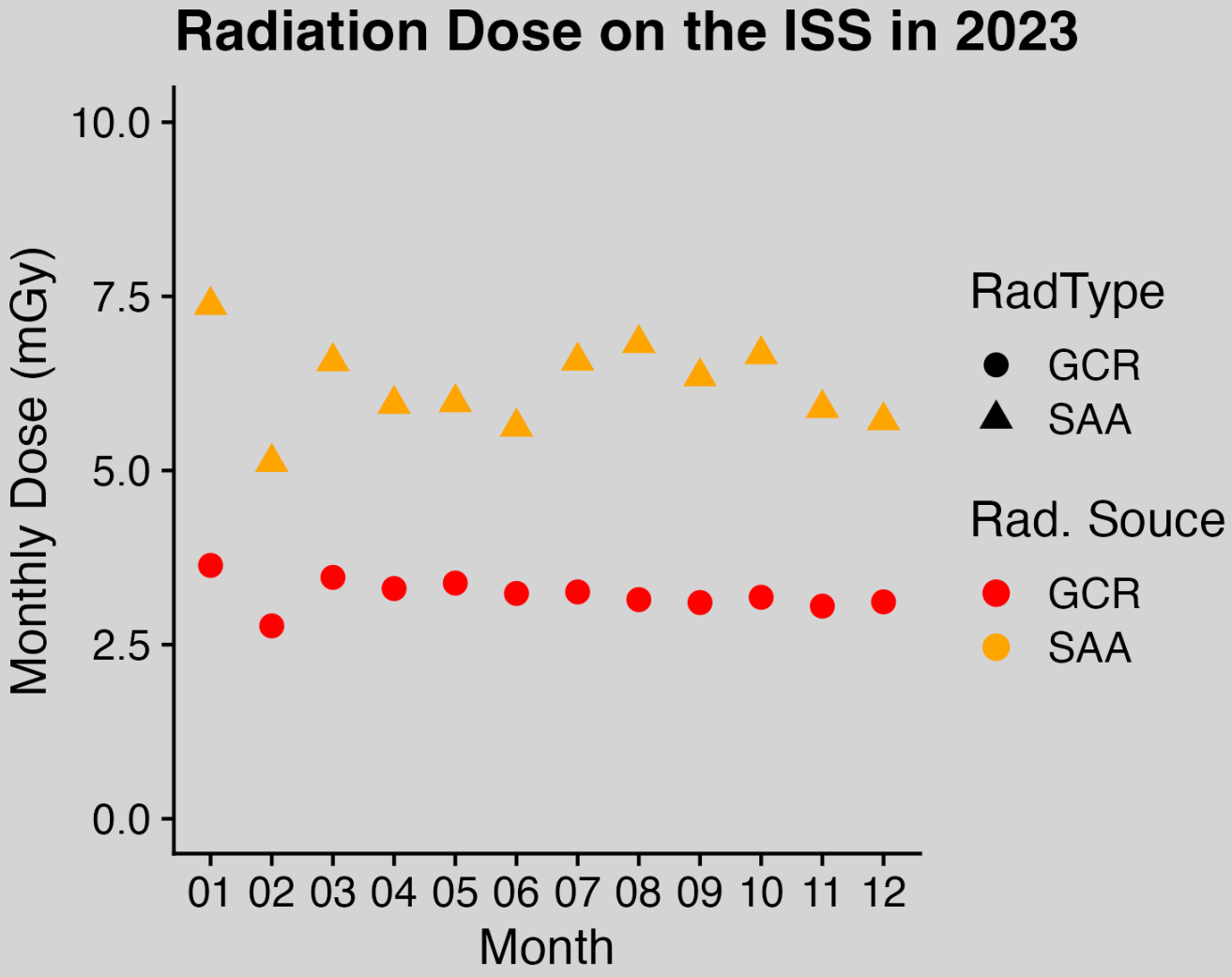
Previous bioproduction experiments have noted diffuse bubbles during growth in microgravity





Space Risks: Elevated radiation environment

- **Galactic Cosmic Ray (GCR):** High energy radiation originating from deep space.
- **South Atlantic Anomaly (SAA):** Dip in the magnetosphere where increased Van Allen belt radiation reach the ISS.
- In 2023, the ISS averaged 3mGy of GCR & 7mGy of SAA radiation
- Over the six months of SA-2 **~60mGy** of radiation is expected
- A crewed mission to Mars is expected to experience no more than **200mGy** of radiation





Summary: To build BLiSS we need to understand the risks posed by space-specific stressors to biological systems

My time de-risking BLiSS

- **PhD at UT Austin studying impacts of radiation on microorganism**
  - DARPA B-SURE: Hardening species used for synthetic biology against space radiation.
- **NPP at NASA ARC with Mark Settles**
  - BioNutrients: Quantifying the mutational burden of long-term storage aboard the ISS in synthetic biology species.
- **Staff Scientist with BMS at NASA ARC**
  - Space Algae-2: Quantifying the mutational burden of long-term cultivation aboard the ISS in a production species of algae

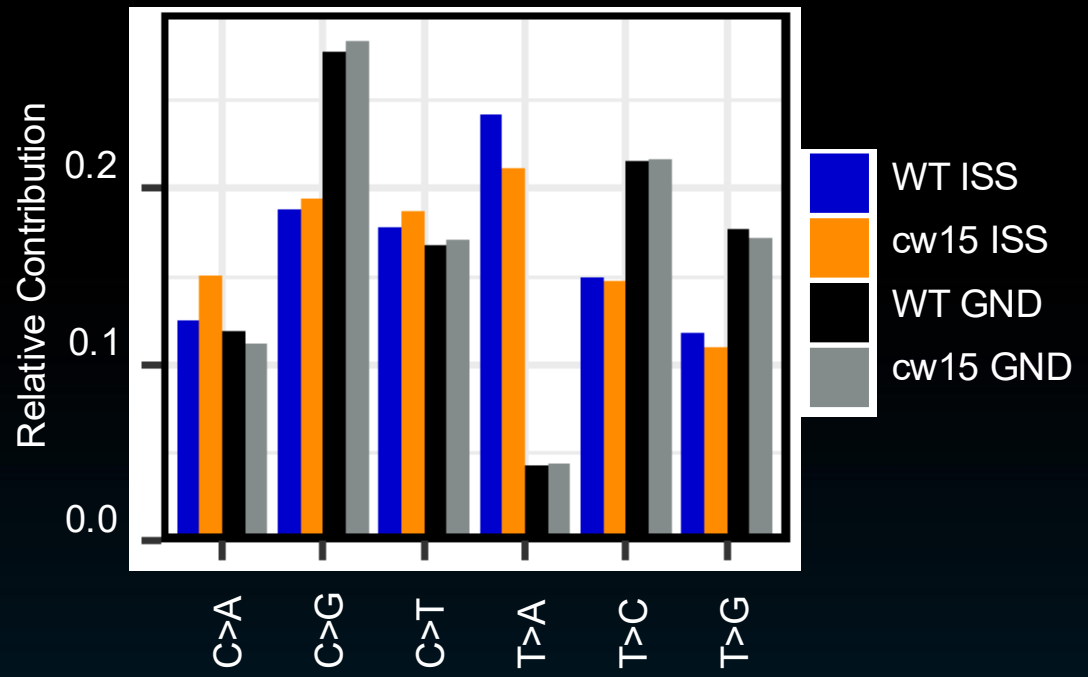


Space Algae-1 was passaged on the ISS for 21 Days



- Whole genome sequencing of 2 strains with 3 biological replicates per strain.
- Four growth cycles with three serial passages to fresh media.

Video credit: NASA



- UV mutagenesis caused C>G, C>T, T>C , and T>G mutations
  - Ground controls had 21-22k point mutations
  - ISS strains had 32-33k point mutations
- The additional 11-12k mutations were enriched for T>A changes.
- Average radiation exposure in space was 285 µGy/d.

Space Algae-2

Motivation

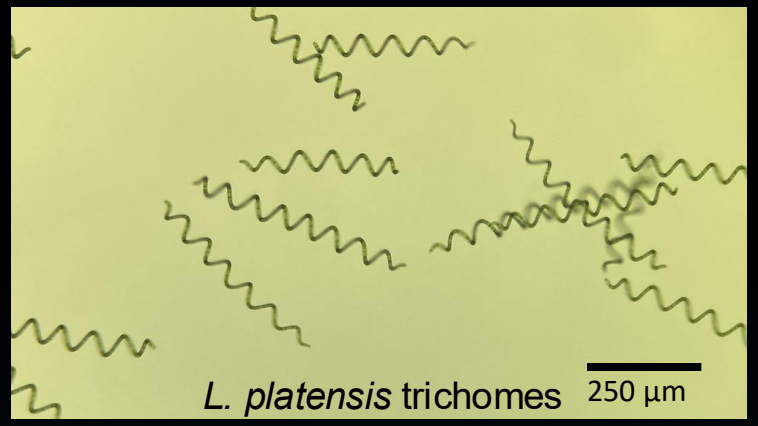
- Are there risks for long-term cultivation of other algae in space?
- *Limnospira platensis* (spirulina) is relatively resistant to acute radiation exposure.

Approach

- Space Algae-2 will grow *L. platensis* for 5-6 months through serial batch cultures.
- Sampling every 2 weeks to test for genome, transcriptome, metabolome, and phenotype effects from spaceflight.

Central Goal.

- Determine feasibility of producing microalgae in space.



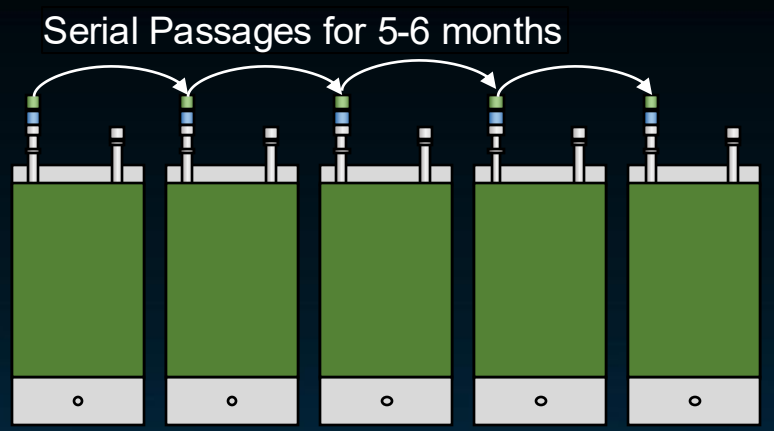
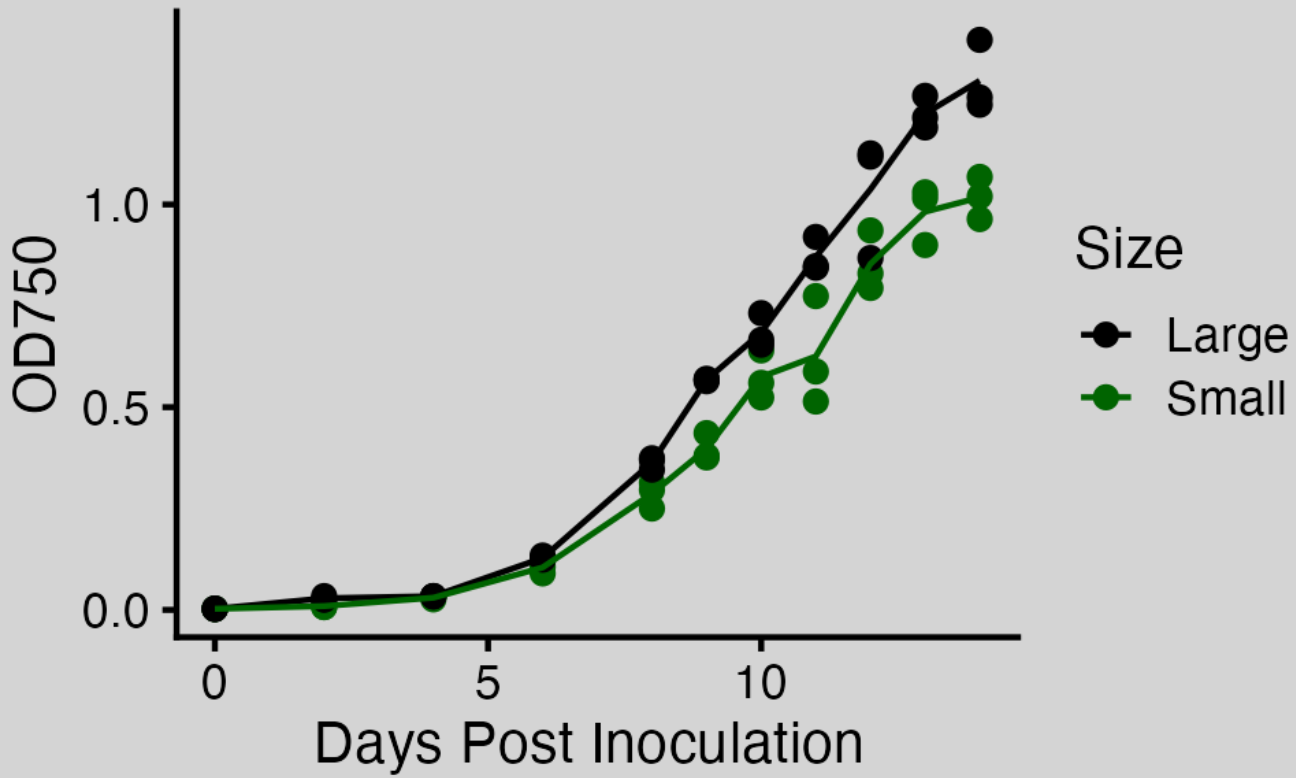


Image credits: K. Fisher & A.M. Settles

Over the 6 months Space Algae-2 at least 100 generation are expected

- *L. platensis* can double every 8h allowing for 540 round of cell division over 6 months, if cells were kept in exponential phase
- Based on growth dynamics we expect a minimum of 9 doubling per passage allowing for 108 total generations
- Few ISS experiments have looked at multi-generational effects of space flight

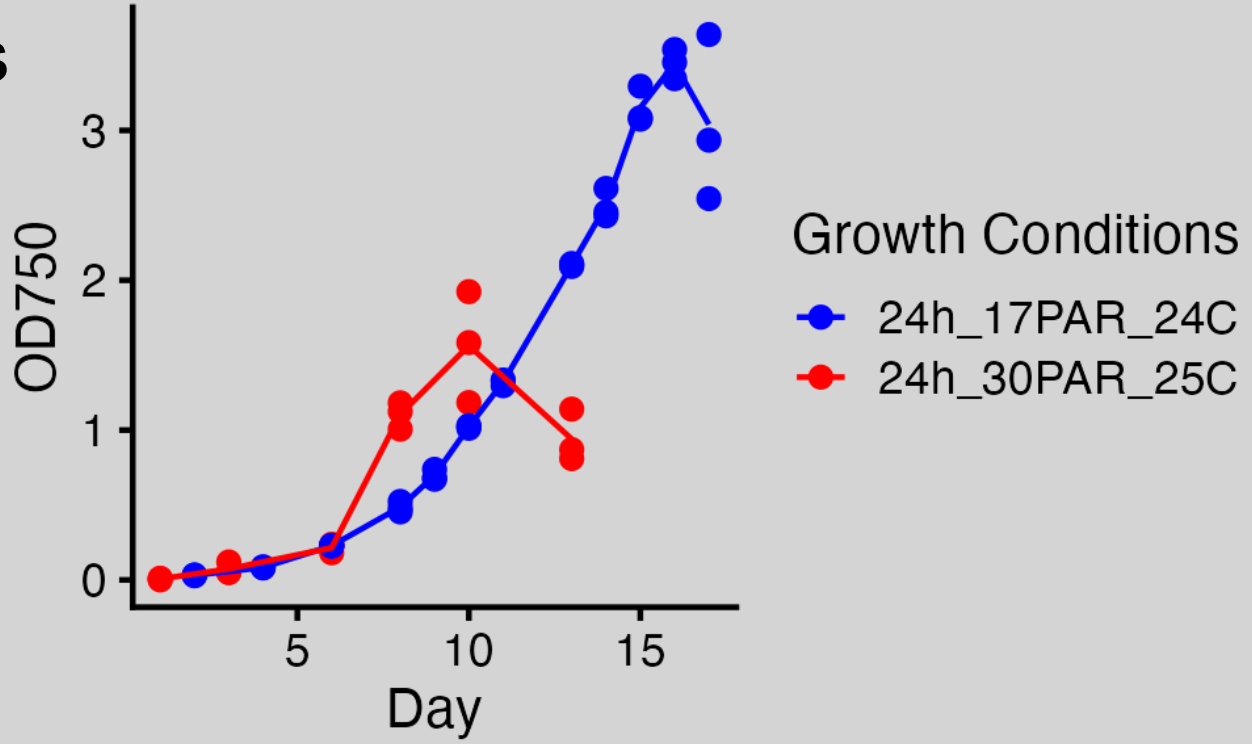


What's required for an experiment aboard the ISS?

- **Simplified experimental procedure**
  - Implemented in microgravity
  - Most astronauts have limited biology experience
- **Robust experimental design**
  - Needs to be adaptable to changes
- **Long term storage**
  - 6 month experiment
  - Multiple months of lead time before launch
  - Limited refrigerated storage

Flight Considerations: Preventing Culture Crash

- A long exponential growth allows for maximum generations
- If *L. platensis* overgrows the culture can crash
- Limited crew time means we can't passage early, even if cultures are looking sick
- Growth conditions need to allow cultures to reliably last 14 days



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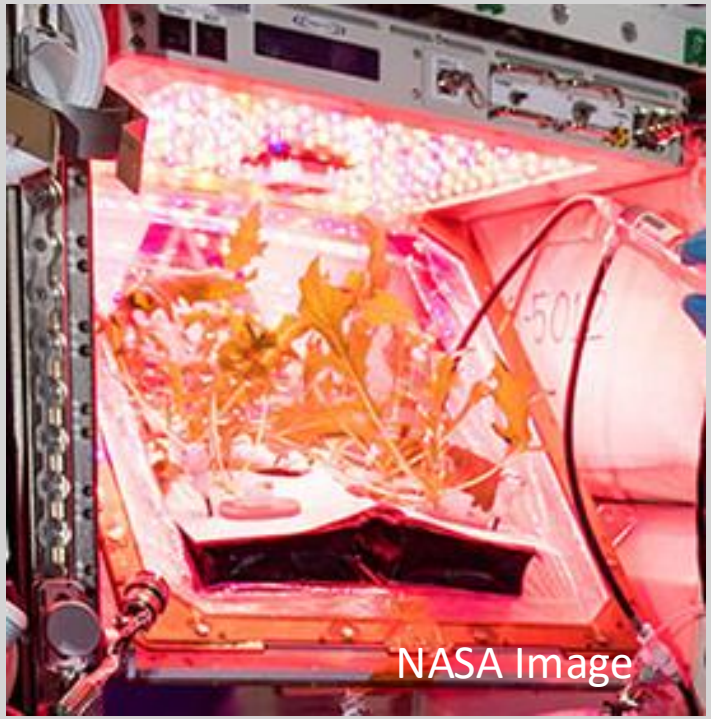
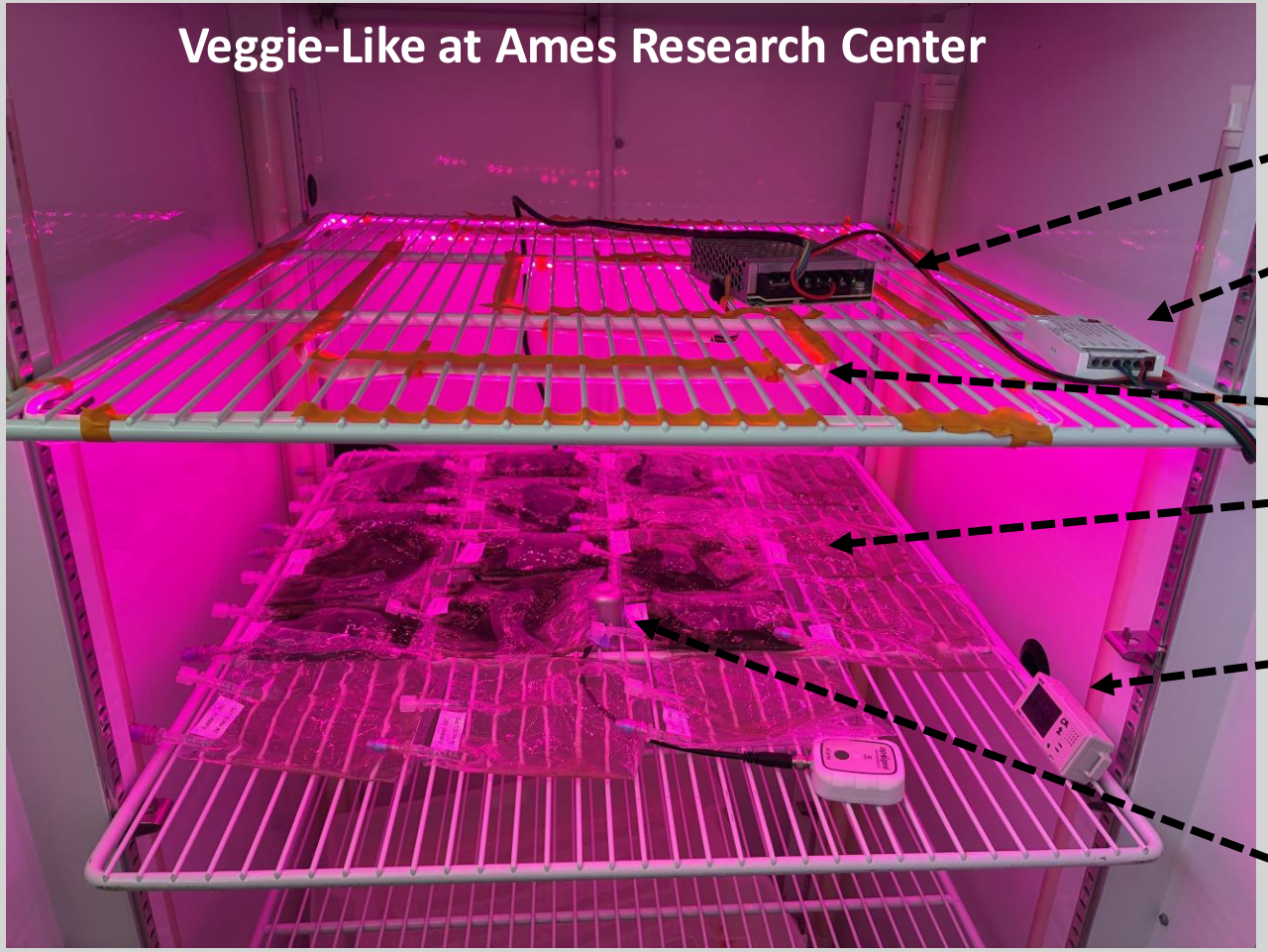
Reproducing the Veggie unit for the development of flight operation

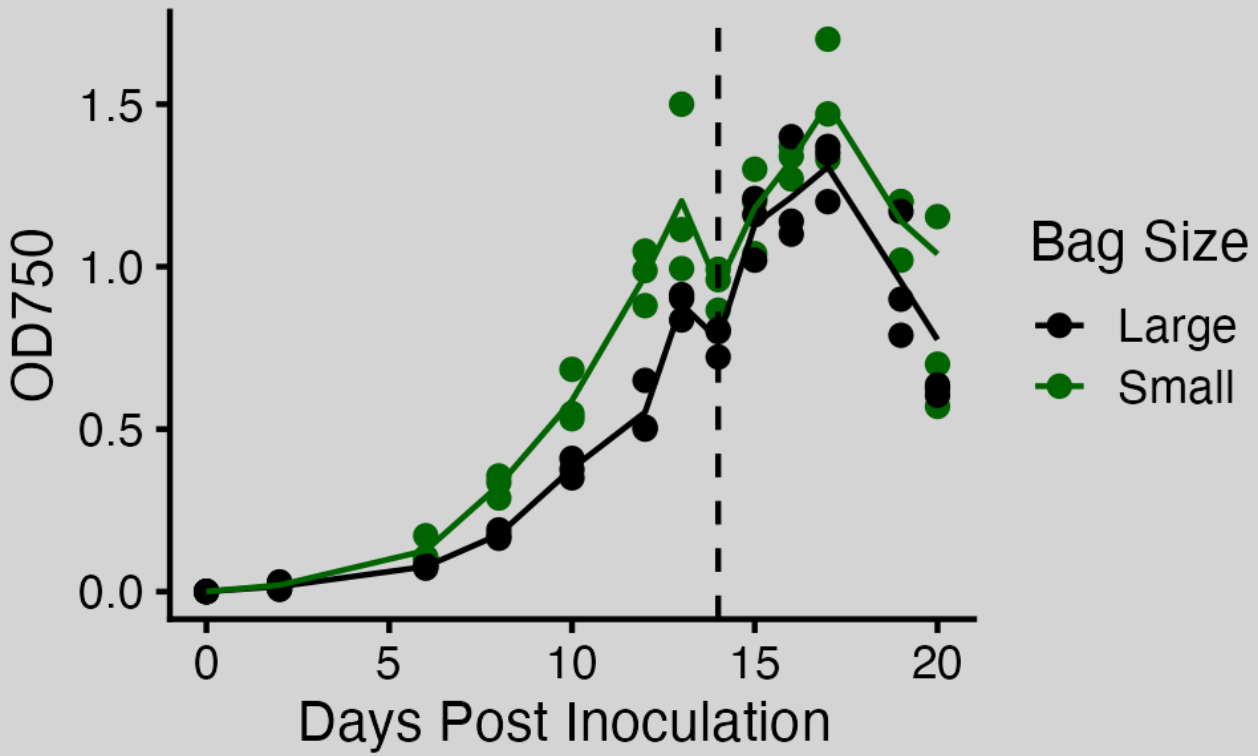


Veg-04B experiment on the ISS



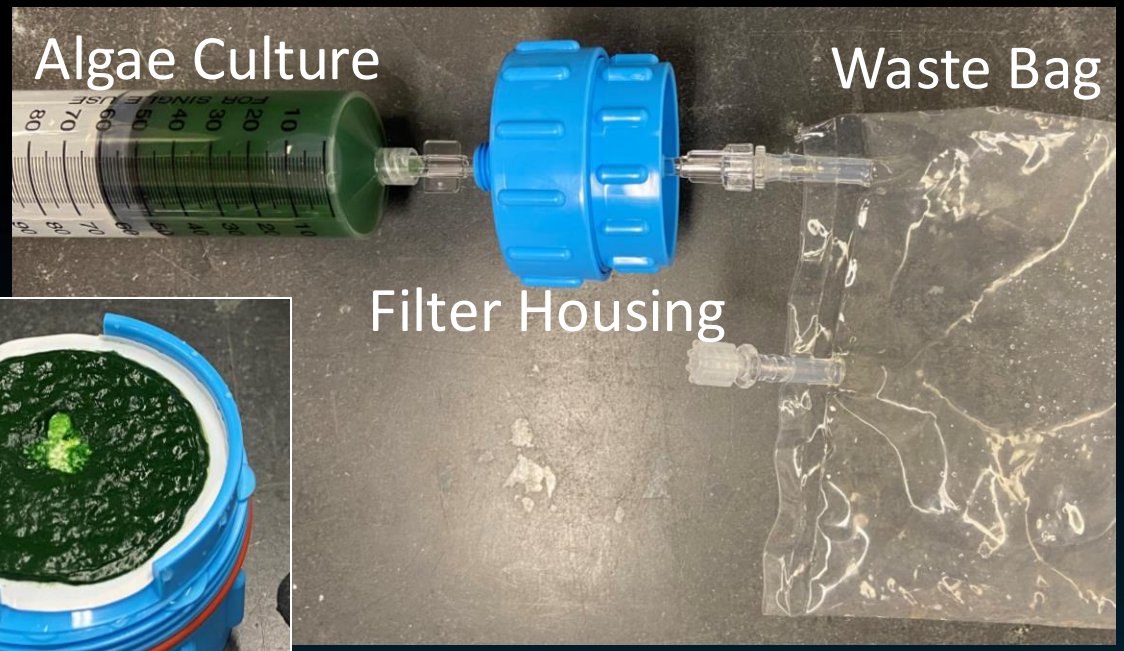
Diurnal cycles support long-term cultures

- Temperature cycle programmed to match previous Veggie experiments
- Lights programmed to remain on for 16h and allow 8h of darkness
- LED's have similar wavelengths as Veggie



Flight Considerations: Minimizing frozen down-mass by dewatering aboard the ISS

- Science-grade freezer space aboard the ISS is limited.
- And even more limited on return capsules.
- Dewatering of Space Algae-2 samples immediately after passaging greatly reduces freezer space needs.

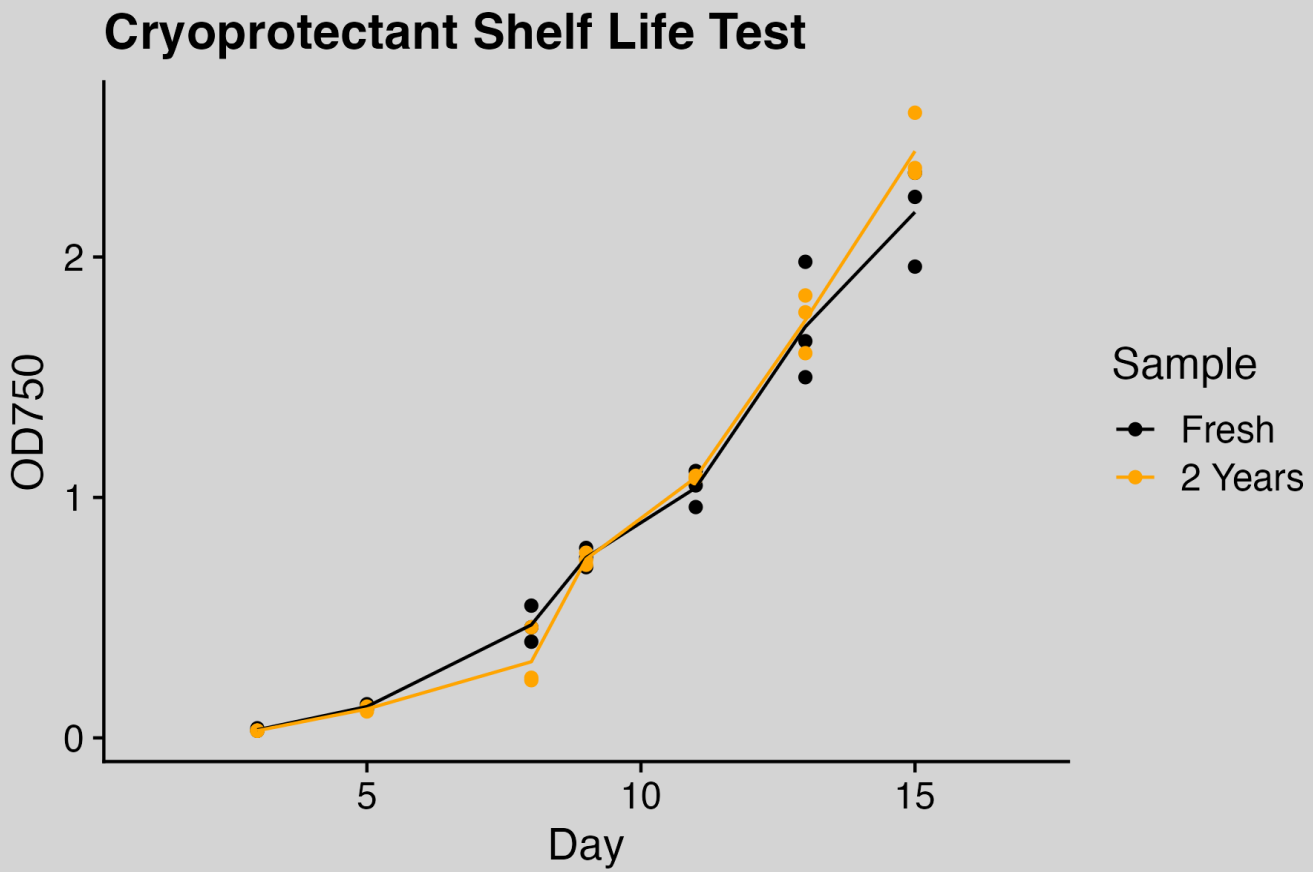




Dewatered Algae

Flight Considerations: Materials must have extended shelf life

- Experimental kits (growth media & cryopreservation mixture) must be prepared in advance of launch
- Stored at room temperature throughout the length of the 6-month experiment
- Similar considerations for long-duration space missions



Acknowledgments

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- Stephen Lantin

Past Members

- Pierce Jestude
- Katie Fisher

