Lighting Protocol to Co-Optimize Duckweed Yield, Nutritional Quality, & Energy-Use Efficiency for Crew Diet Supplementation

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DUCKWEED: NUTRIENT DENSE CROP FOR EXPLORATION

Duckweeds (family Lemnaceae) are tiny flowering plants with enormous potential for bioregenerative space life support. Also known as water lentils or water meal, these small plants are gaining global recognition as a powerful and ecologically friendly means of absorbing nutrients from wastewater. In addition, duckweed has a very high nutritional density and little fibrous material, making it a 100% edible and potentially valuable fresh food supplement to crew diets on long-duration exploration missions.

Spirodela (Large), *Wolffia* (Small), and *Lemna* (Medium) – Landesman (2010)

Space Lab Technologies, LLC and researchers in plant biology at the University of Colorado at Boulder are working to **establish duckweed as a nutrient dense space crop for deep space exploration**.

WHAT IS DUCKWEED?

- Smallest flowering plant on Earth
- Among the fastest growing plants in the world
- Over 40 species
- Can grow free floating or submerged
- Found in still/slow flowing fresh water
- Common in lakes, ponds, canals, rice fields, ditches, even mud

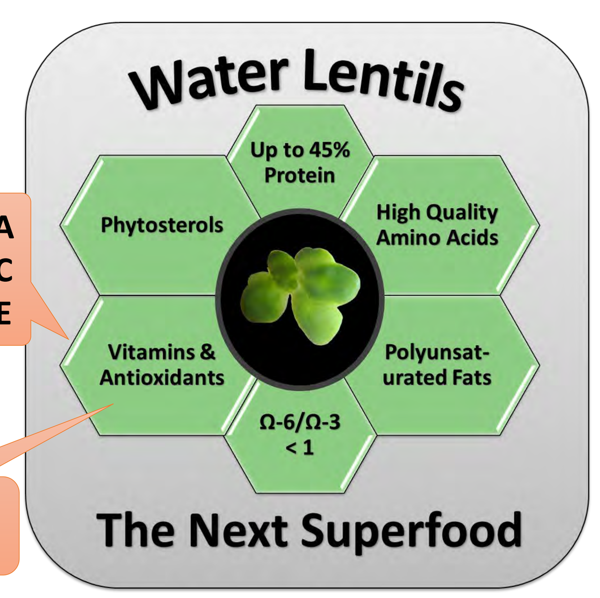
Fronds:

- Oval-shaped leaf-like organs
- 1-20 mm across & grow singly or in small groups
- Take up gases and nutrients
- Permanently open stomata on top
- Cutin (waxy, water repellant coating) on top
- Air sacs provide buoyancy
- Vascular system practically absent
- Little structural tissue needed (floating)

Roots: provide mechanical stability

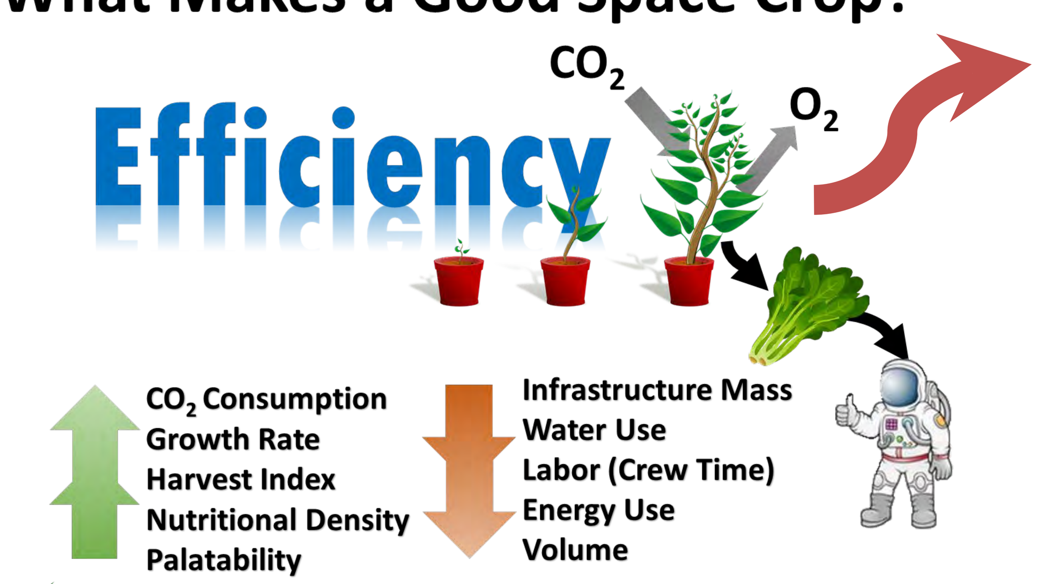
Reproduction:

- Primarily asexual budding
- Flowering rarely observed
- Up to 10 daughter fronds in 10 days before dying
- Doubles biomass in 1-3 days under ideal conditions



AN ATTRACTIVE PLANT FOR SPACE

What Makes a Good Space Crop?



1. 100% Harvest Index
2. Can be eaten raw
3. Highly nutritious
4. High growth rate
5. Vegetative budding
6. Thrives in high CO_2
7. Grows in 24-hr light
8. Grows in shallow water
9. Environmentally robust
10. Palatable
11. Grows in dark on sugar
12. Has a dormant state
13. Prefers ammonium-N
14. Have been grown in µG

THE INNOVATION: CO-OPTIMIZATION OF YIELD & QUALITY

Food Production Mitigates Deep Space Exploration Health Risks

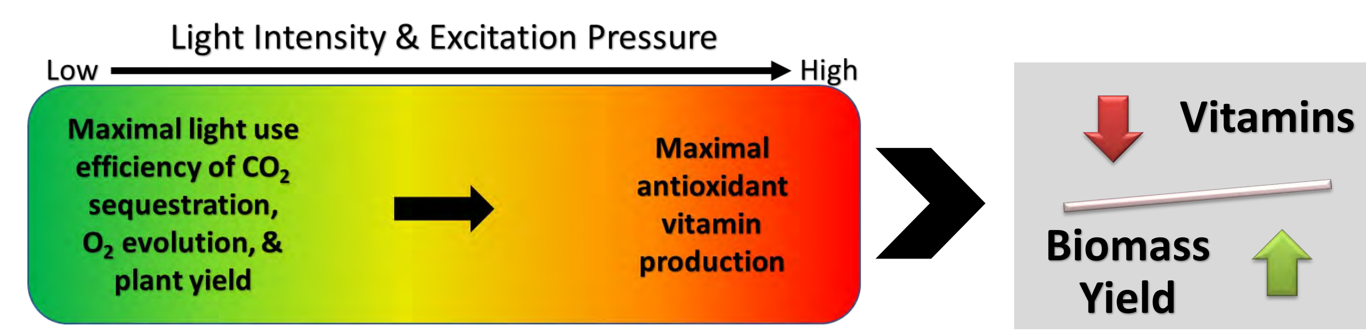
- Packaged food loses palatability & nutrients
- Food rich in antioxidants like zeaxanthin can combat adverse effects of space radiation
- Plants reduce the adverse psychological impacts of isolation, high stress, & distance

A LIGHT RECIPE TO CO-OPTIMIZE:

1. Edible biomass yield
2. Content of human micronutrients
3. Protein content
4. Energy efficiency

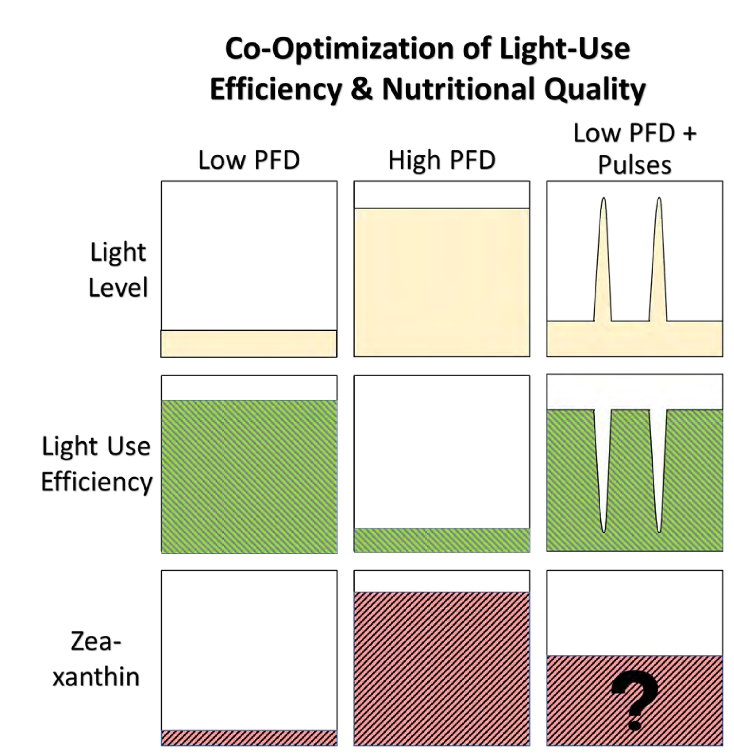
UNDER SPACE RELEVANT CO_2 LEVELS (up to 1%)

Challenge is Co-optimization



Plants growing in the shade of a forest canopy will still make a lot of zeaxanthin if they receive some high-light pulses.

Adams et al. 1999 Plant Cell Environ. 22, 125
Photo: Sunflecks under a *Eucalyptus* canopy, by William Adams, 1994



A combination of low background light and a few high-light pulses will trigger production of human micronutrients by duckweed without loss of yield.

Drs. Demmig-Adams and Adams demonstrated technique with *Arabidopsis thaliana* (Cohu et al. 2014 Acta Astronaut. 94, 799).

TRISH BRASH 1801 Research Objectives:

1. Determine continuous photon flux density (PFD) for maximal plant yield as well as pulse PFD for production of human micronutrients at space relevant CO_2 levels.
2. Demonstrate increase in human micronutrient (vitamin/antioxidant & omega-3 fatty acid) without loss of plant yield through supplemental higher-intensity light pulses.
3. Determine spectral quality effects on plant yield, micronutrients, & energy-use efficiency.

Response Variables:

- Relative growth rate (RGR)
- Photosynthetic capacity (as O_2 evolution)
- Excitation pressure (from chlorophyll fluorescence)
- Pigments (chlorophyll, zeaxanthin, β-carotene, etc.)
- Macronutrients, especially protein

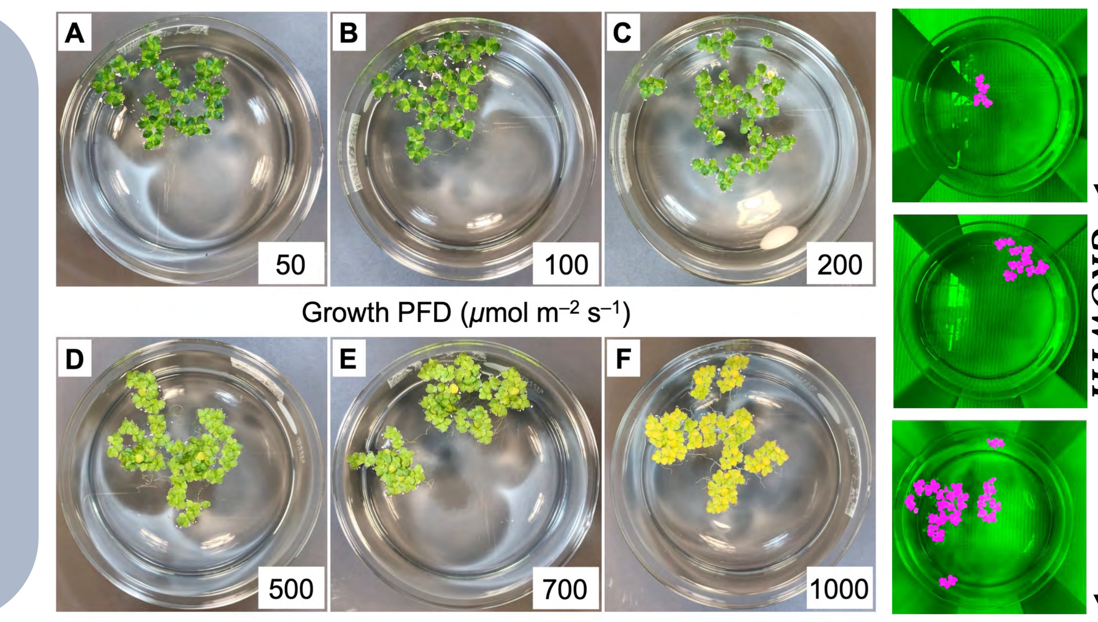
Treatment Variables:

- Continuous & pulsed PFD (50 to 1000 µmol photons $m^{-2}s^{-1}$)
- CO_2 concentration (0.04-1%),
- Spectral quality (white, red, blue, green)
- Species (*Lemna gibba* & *Wolffia arrhiza*)

TRISH BRASH 1801: YEAR 1 GROWTH TEST RESULTS

Growth Conditions

- **Nutrient Medium:** ½ strength Schenk & Hildebrandt
- **pH:** 5.5
- **Temperature:** 25 °C
- **Initial Frond Density:** 20 *L. gibba*
- **Acclimation:** 3 days
- **Growth Period:** 4 days



Lemna gibba grown under ambient CO_2

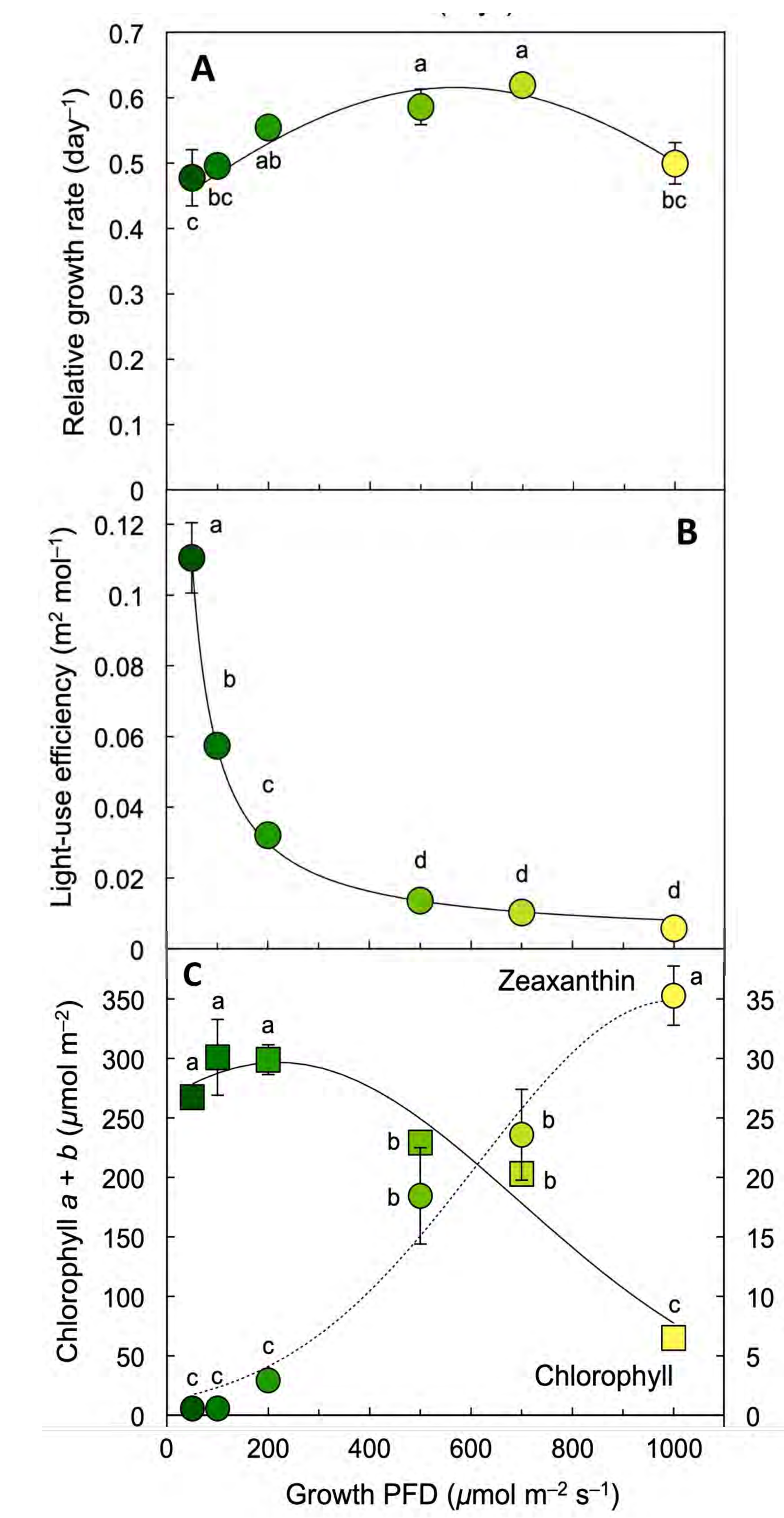


Figure 1 A) Relative growth rate of *L. gibba* (difference in ln-adjusted frond areas per time elapsed), **B)** Light-use efficiency of area production by *L. gibba* (m^2 of frond area produced per mol photons), and **C)** Content (leaf area basis) of light-absorbing chlorophyll (squares) and the carotenoid pigment zeaxanthin that dissipates potentially damaging excitation energy not utilized in photosynthesis as harmless heat (circles). The symbol shades correspond to respective growth PFDs, ranging from deep green (50 µmol m–2 s–1) to yellow (1000 µmol m-2 s-1). Circles show mean values ± standard deviations. Lower-case letters signify statistical differences at $P < 0.05$ via analysis of variance and Tukey-Kramer HSD test. PFD = photon flux density of continuous light.

Year 1 Conclusions:

- *L. gibba* maintains a remarkably similar growth rate over a wide range of growth light intensities, via high levels of light-absorbing chlorophyll (and thin leaves with minimal self-shading) under low light supply and high levels of the antioxidant zeaxanthin for protection against intense light (***Figure 1A,C***).
- Light-use efficiency of frond area production is maximal under low light supply and drops precipitously with increasing growth PFD. (***Figure 1B***).

Challenge is to find a growth protocol that:

- Optimizes zeaxanthin production
- With minimal light input,
- While optimizing protein content,
- Under elevated CO_2.

Next Steps:

- Determine the growth-saturating PFD under elevated CO_2 levels (up to 1%),
- Validate that pulsed lighting boosts anti-oxidant production without lowering growth rates at ambient & elevated CO_2,
- Investigate spectral quality effects on plant growth and antioxidant production.

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