DUCKWEED

A Tiny Aquatic Plant with Enormous Potential for Bioregenerative Life Support Systems

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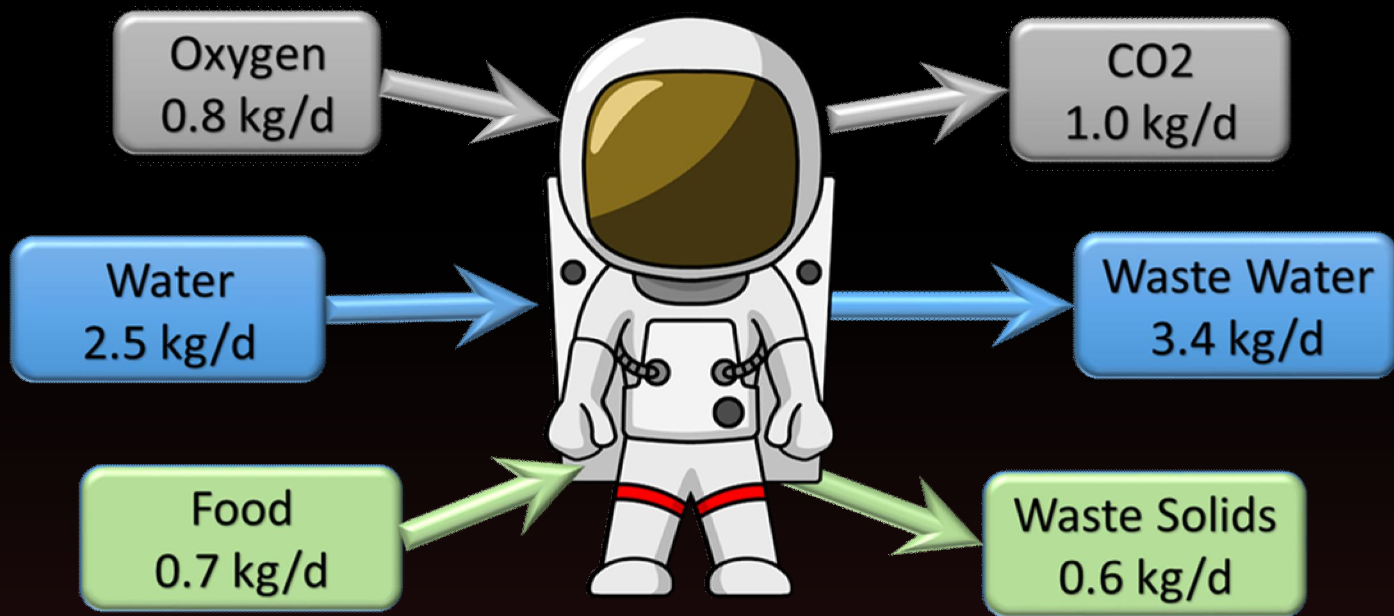
OVERVIEW

I. Motivation: Aquatic Plants for Space Life Support
II. Duckweed 101
III. Water Treatment
IV. Edible Biomass Production
V. Duckweed in Space
VI. Research Needs and Challenges

Environmental Control & Life Support Systems

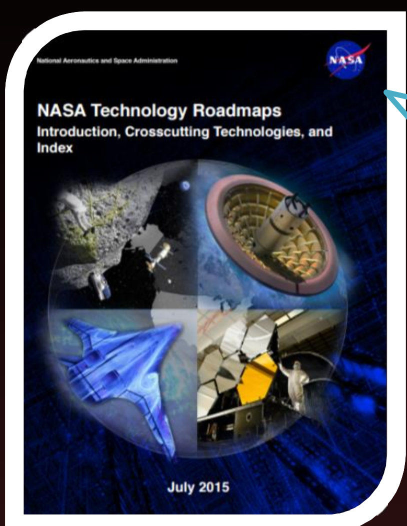
Provide conditions to support human life



Control Temperature | Maintain Pressure | Maintain Relative Humidity

Human Metabolic Inputs & Outputs, Data Source BVAD 2015 (pp 50, 53, 64, & 106)

It's a long way to Mars....



Life Support Self Sufficiency

- Resource Recovery
- System Closure
- High Reliability
- Autonomous Control
- Minimal Expendables

The content of this slide is subject to the propriety statement on the title slide.

Stabilized Packaged Food for Space Missions *(www.nasa.gov)*

Regenerative Food Production for Long Duration Space Habitation

Biregenerative Life Support

Use of living organisms (plants, animals, etc) to renew or recycle the atmosphere, provide clean water, and food.

Multi-Functional

Aquatic Plants for Bioregenerative Life Support

Challenges of Land Plants

Å Gravitropic complications in propagation
Å Nutrient delivery in microgravity
Å Large volumes of inedible biomass

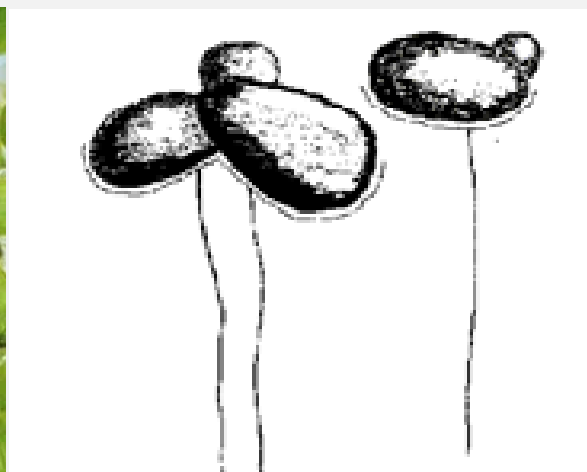
Benefits of Aquatic Plants

Å Consumed around the world
Å Often 100% edible and fast growing
Å Thrive in nutrient rich water
Å Growth less sensitive to gravity

Duckweed 101

What is Duckweed?

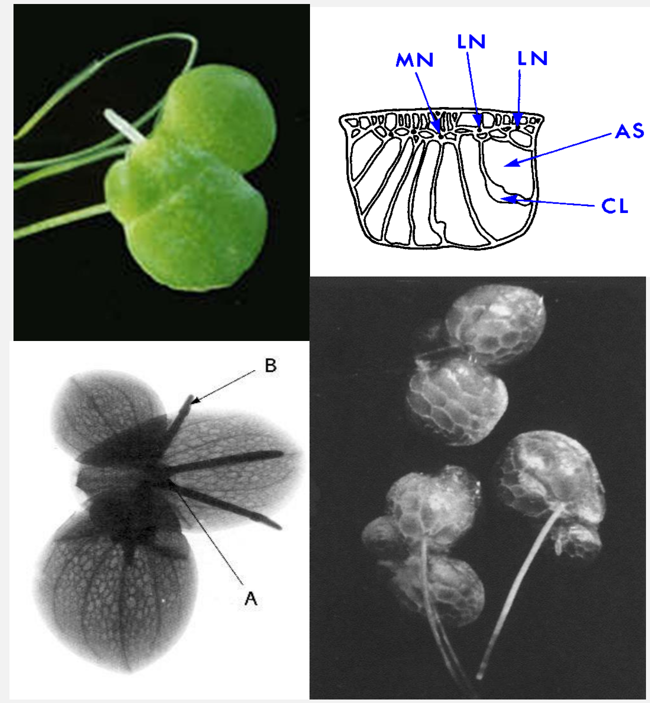
- Smallest flowering plants on Earth (*Journey et al., 1991*)
- Among fastest growing plants in the world (*Ziegler et al., 2015*)
- Family Lemnaceae, 4 genera, and over 40 species
- Free floating or submerged, found in still/slow flowing fresh water
- Common in lakes, ponds, canals, rice fields, ditches, even mud



Cross, 2002

Duckweed Anatomy

- Leaf-like ovoid vegetative body resembles a thallus (neither stem nor leaf), known as 'fronds' that are typically 1-20 mm across, with a variety of shapes
- Fronds grow singly or in small groups, connected by a 'legs' called stipe (narrow part of frond)
- Vascular system 'practically absent'
- Frond composed of chlorenchymatous cells, separated by intracellular air-filled spaces providing buoyancy
- Little structural tissue needed to float ➔ little fiber
- Upper epidermis highly cutinized (unwettable)
- Stomata on upper side of frond
- Turions (dormant fronds) form in low temperatures
- Minerals absorbed through all surfaces of frond
- Hair-like adventitious roots are photosynthetic
- Roots provide stability & absorb nutrients in dilute water



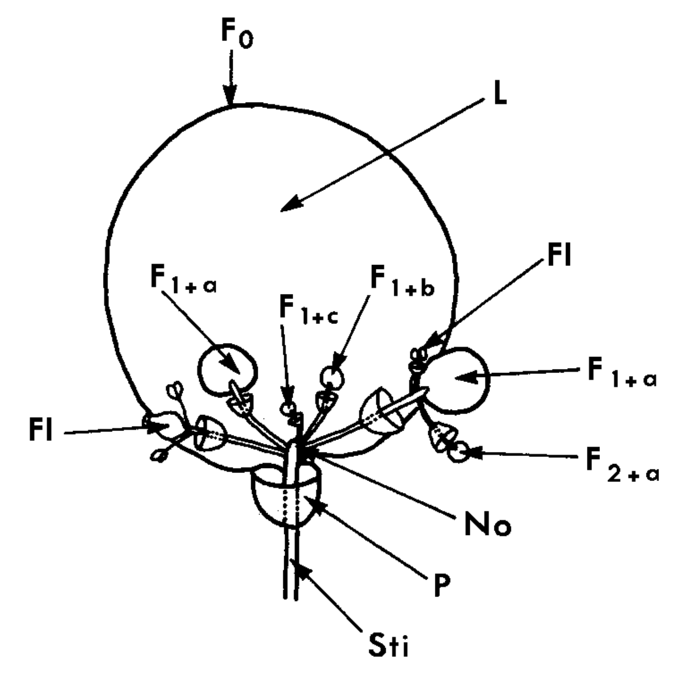
AS = air space, CL = Cell layers surrounding air spaces, LN = lateral nerve, MN = median nerve

http://www.mobot.org/jwcross/duckweed/

Duckweed Reproduction

- Primarily asexual reproduction (unlimited vegetative budding)
- Is a flowering plant, though flowering rarely observed
- One mother frond produces up to 10 daughters in a 10 day period before dying (*Journey et al., 1991)*
- Doubles its biomass in 1-3 days under ideal conditions (*Ziegler et al., 2015)*
- Cyclic senescence & rejuvenation growth patterns occur over ~1 month period

F_0: mother frond	**L**: leaf-like body
F_1: 1^{st} gen. daughter	**No**: node
F_2 : 2^{nd} gen. daughter	**P**: prophyllum
a, b, c: 1^{st}, 2^{nd}, 3^{rd} fronds	**Sti**: stipule or stipe
Fl: flower	



http://www.feedipedia.org/node/15306

Duckweed Environmental Requirements

- **Nutrient composition**: [1]Leng, 1999 [2]Iqbal, 1999 [3]Hasan, 2009
  - Duckweed thrive on water rich in nutrients & dissolved organic compounds
  - Opportunistic growth spurts when flushes of nitrogen occur
  - Utilize a variety of N sources, including urea; prefer NH_4-N over NO_3-N
  - P & K needed in low amounts (concentrates in tissue during rapid growth)
  - Can metabolize sugars if nutrients not present, *without sunlight*
- **Temperature:** 6-33C (growth)[1]; 25-31C (optimal)[2]
- **Sunlight:** Increasing intensity increases growth; optimal range uncertain
- **Salinity:** Fresh to slightly brackish; saline water can be used
- **pH:** 3-10 (growth) & ~7 (optimal)[3]
- **Mat Density**: Full coverage that still accommodates rapid growth (400-800g/m^2)[3]
- **Water Velocity:** Maximum of 0.1[2]-0.3[3] m/sec
- **Water Depth:** Grows on water mm to meters deep – effects sunlight & water temp
- **Water O_2 Concentration:** Grows equally in aerobic & anaerobic water

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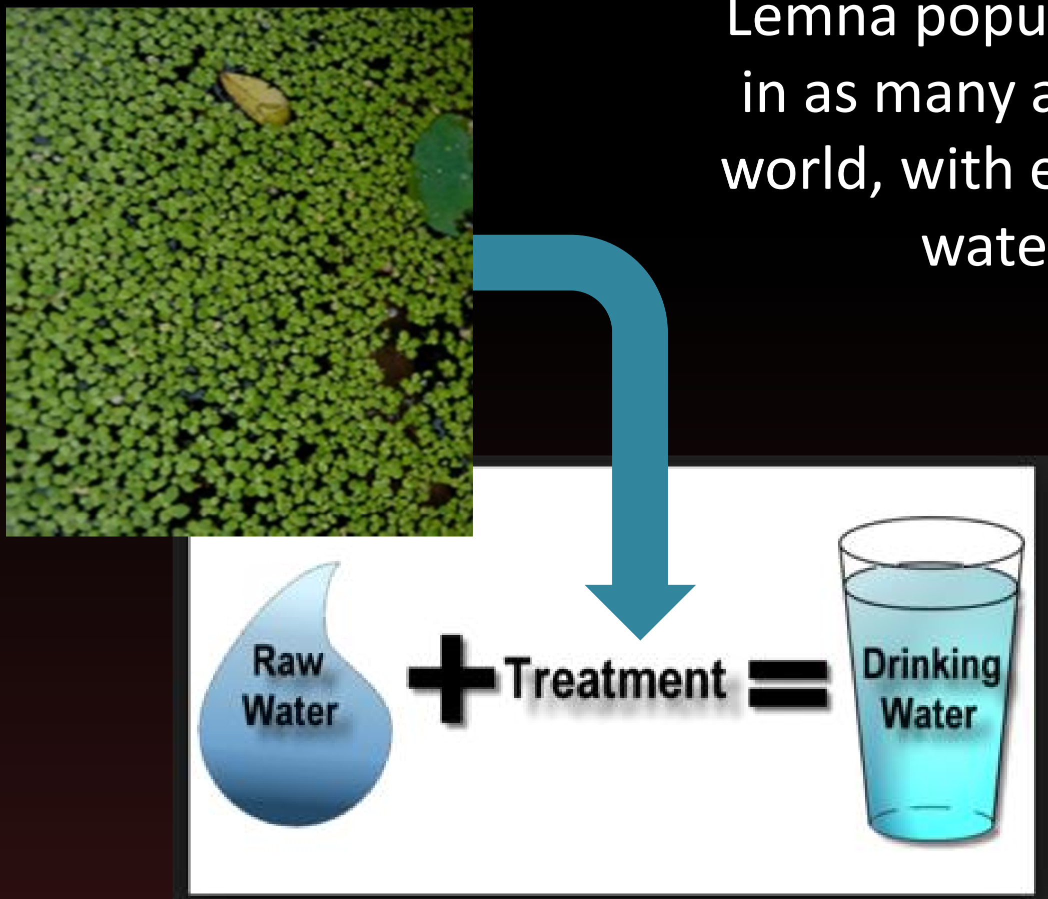
Water Treatment with Duckweed?



Lemna populated lagoons treat sewage in as many as 100 facilities around the world, with effluent often exceeding US water quality standards.

Leng, 1999

Duckweed Wastewater Treatment Facilities

Duckweed-based polishing lagoon in the USA, that receives 500 m^3/d of combined municipal, septic & industrial wastes

(Iqbal, 1999)

Duckweed-covered serpentine plug-flow (continuous flow through) lagoon in the USA for tertiary treatment

(Iqbal, 1999)

Water Treatment with Duckweed

Many laboratory and field studies observe high rates of nutrient removal (nitrogen and phosphorous) as well as TSS, BOD, & COD

- >98 nutrient removal in duckweed ponds *(Mohedano et al., 2012)*
- N/P removal rates of 120-590 & 14-74 mg/m^2/d observed in lab study (Korner et al., 1998)
- In lab experiments with human urine, removal of chemical oxygen demand, phosphorus, and nitrogen exceeded 80%, 90%, and 50% of initial concentrations, respectively (Iatrou et al., 2016).
- Some nutrient uptake may be indirect by attached algae & bacteria
- TSS, BOD, and COD are also removed
- Fecal coliform reduction of 50-90% observed in ponds *(Karpiscak et al, 1996; Van der Steen et al., 1999)*
- Direct contribution of duckweed to water stabilization is unclear (Iqbal, 1999)

Biomass Composition and Yield

➢ **100% Edible**

➢ **Water:** 92-94%[1]

➢ **Protein:** up to 45%[1] (comparable to soybean), high quality

- Rich in lysine & arginine (usually low in plants), leucine, threonine, valine, isoleucine & phenylalanine; low in methionine & tyrosine

➢ **Fiber:** <5%[1]

➢ **Carbohydrates:** 30-35%[2]

➢ **Vitamins:** Good source of vitamin A and pigments (e.g. beta-carotene & xanthophyll)[2]

➢ **Calcium Oxalate:** Duckweed stores calcium as calcium oxalate crystals

➢ **Yield:** Reports vary widely (2-50 tons/hectare/yr[3]; 0.2- kg/m^2); dependent upon species, climate, location, nutrient supply, water depth, and crop management practices (like harvesting rates)

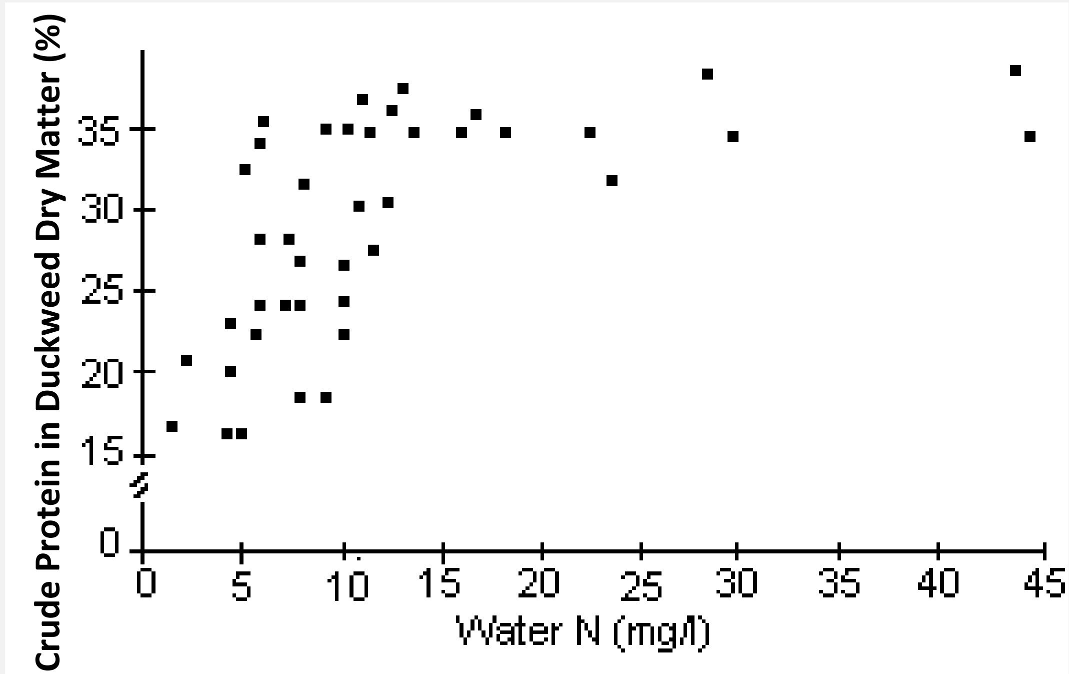
[1] *Journey et al., 1991*
[2] *Ansal et al., 2010*
[3] *Iqbal, 1999*
[4] *Hasan, 2009*

Nitrogen Concentration vs. Protein

Protein content of biomass depends upon nitrogen availability.

- Few experiments on optimum nutrient composition for protein production: 7-30 mg/l N[1] vs. 20-60 mg/L N[2]
- Under nutrient-poor conditions, fiber percentages rise and protein content decreases.[2]

[1]Hasan, 2009; [2]Leng, 1999

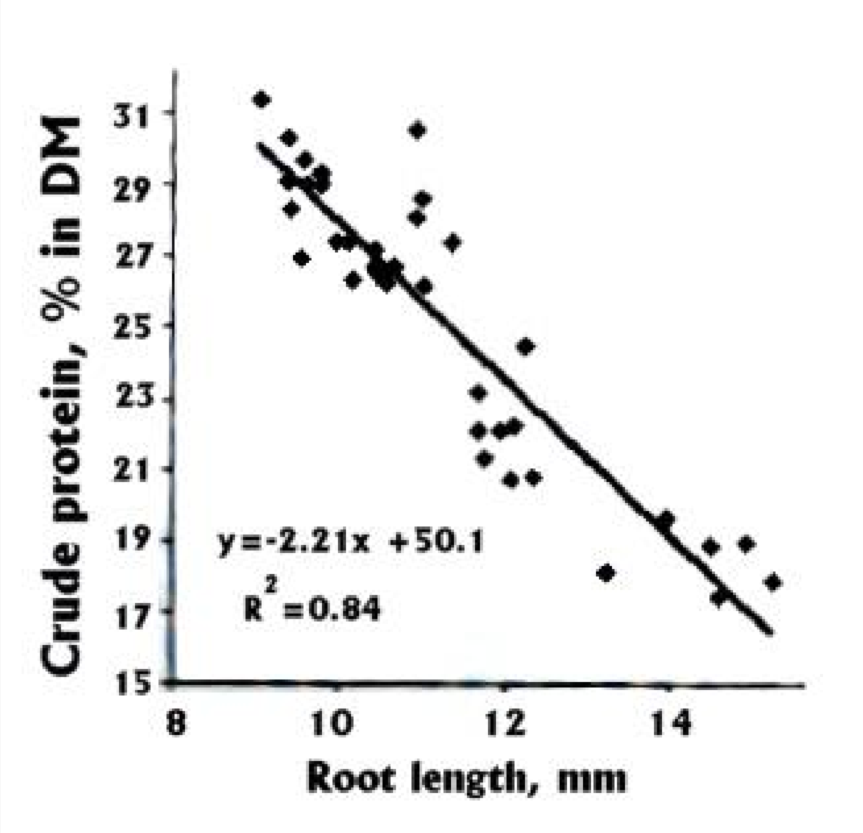


Spirodela spp grown on diluted effluent from a piggery. The P levels in water varied from 1.2-6.1 mg P/litre (Leng et al., 1994).

Root Length vs. Protein

Root growth is negatively correlated with protein content and positively correlated with fiber content

(Leng, 1999)



Relationship between root length and protein content in duckweed (Lemna minor)

Use as Feedstock and Human Food

- Duckweed farms produce feedstock for animals (poultry, swine, and cattle)
- Anecdotal reports of duckweed used throughout South Asia as a human food source as well
- Burmese, Laotians, and Northern Thailand people consume the genus Wolffia as a vegetable.
- Thailand people refer to duckweed as “Khai-nam” or “eggs of the water” and regard it as highly nutritious.
- Also referred to as the “water lentil”

(Leng, 1999)

The Next Superfood?

Amino Acid Composition	Meets/ exceeds World Health Organization recommendations, comparable to legumes
Polyunsaturated Fats	48-71% of fatty acid content
Omega 6 to Omega 3 Ratio	0.1 to 0.75 (FAO recommends <5)
Phytosterol	5-fold higher than most other plant oils (lowers cholesterol absorption)
Antioxidants	High concentrations of lutein and zeaxanthin
Mineral Composition	Presence of trace elements depends on nutrient medium

Appenroth, 2017

The Next Superfood?

Lentein™ Complete from Parabel,
made from “Water Lentils”, i.e. Duckweed

Image Source: www.lentein.com

Duckweed in Space

Close the Loop

- ✓ High Protein Food Supplement
- ✓ Recycle Waste Nutrients
- ✓ Provide Potable Water
- ✓ Remove/reduce CO_2
- ✓ Provide Oxygen

Potential Utilization in a Space Habitat

"One of the most attractive higher plants" for long-duration space life support *(Yuan & Xu, 2016)*

- Preferential **uptake of ammonia** as a nitrogen source (making it attractive for waste processing)
- **Rapid, uniform growth** through vegetative budding (unlimited asexual production)
- **Robust:** Grows under a wide range of conditions and can "survive and recover from extremes of temperature, nutrient loadings, nutrient balance, and pH." (*Journey et al., 1991*)
- **Volume Efficient:** Can grow on thin films of water, allowing more growth area in less volume
- **Indefinite maintenance of stock cultures in light, or in darkness** with sugar supplement, allowing survival and rapid restart of the crop in the event of prolonged darkness from power or lighting failure
- Inactive, **permanently open stomata**, allowing for irregular photoperiods and continuous CO_2 uptake
- **100% harvest index** of highly **nutritious** material (10-45% protein)

Potential Utilization in a Space Habitat

In an environmentally controlled system, yield and biomass composition can be optimized in a small volume

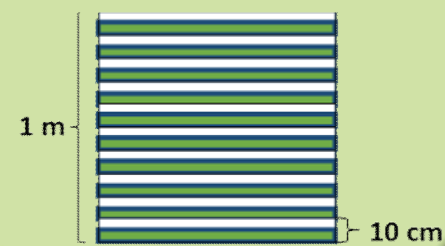
2-3 m^2 of duckweed can treat wastewater of one person *(Iqbal, 1999)*

→

10 m^2 to **treat waste of 4-person crew**

→

Thin (10 cm) stacked 1m^2 trays = 10 m^2 growing area in **1 m^3 volume**



←

Could produce up to 0.15-0.25 kg DM/day in 1 m^3 volume[1] ➔
17-28 g protein/crew member/day!

[1]Gale et al, 1989

Flight Experiments

Numerous orbital experiments w/ aquatic organisms, including floating & submerged duckweed experiments

- STS-67 flew experiment testing influence of microgravity on duckweed anatomy, grown on sugar in the dark (Eichorn & Fritsche, 1996)
- Paragon SDC's Autonomous Biological System (ABS) flew on STS-77, STS-79/81, & SS 86/89 w/ transfer to MIR; almost 18 months of flight (McCallum et al, 1998)
- Closed Equilibrated Biological Aquatic System (C.E.B.A.S.) flew on STS-89, STS-90, & STS107 (Bluem & Paris, 2003)
- In a simulated microgravity study, duckweed growth increased, possible due to changes in cellular structure (Yuan & Xu, 2016)

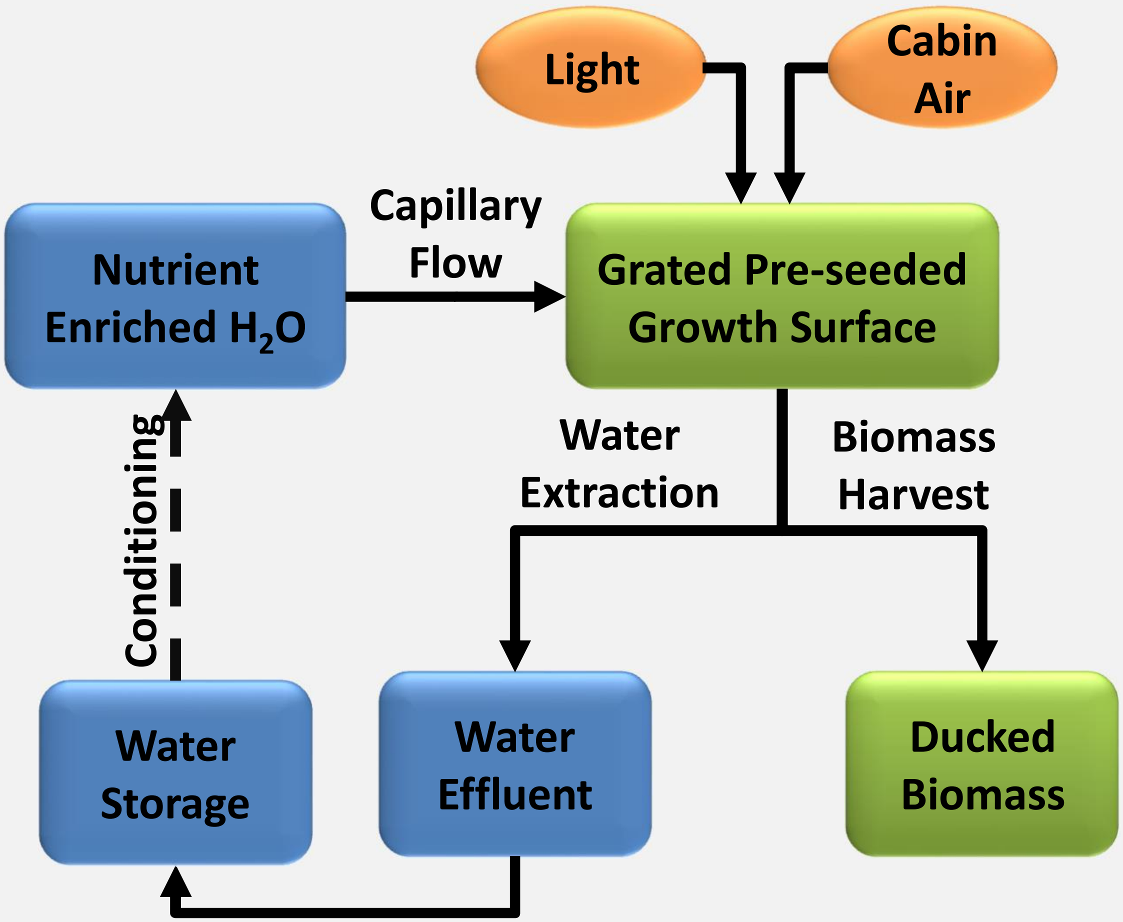
Floating Plant Cultivation in Microgravity

Maximize production of edible biomass with minimal launch and use costs (power, crew time, mass, volume, and consumables such as water)

- Provide needed water, nutrients, light
- Maintain suitable atmosphere (CO_2, O_2, H_2O, pressure)
- Maintain suitable temperature (air & roots)
- Protect plants from environmental hazards
- Support full life-cycle (seeding, budding, and harvesting)
- Operate autonomously (preferably)

Primary challenge of microgravity is nutrient and water delivery

µG-LilyPond: NASA STTR Phase I



Space Lab Technologies & University of Colorado at Boulder

Other System Operations:

- Pre-seeding
- Cleaning biofouling

Operational Challenges, Food Production

CHALLENGE	GOAL	POTENTIAL SOLUTION
Biomass Quality Control	Å Maximize protein Å Reduce oxalic acid	Å Control nutrient composition in water Å Cook/process biomass
Food Preparation	Å Maximize nutritional value Å Improve taste & digestibility	Å Boiling, Pulping, protein extraction, or drying
Daily Harvest Requirements	Å Reduce crew time needed for operation	Å Autonomous harvesting mechanisms

Operational Challenges, Wastewater Tx

CHALLENGE	GOAL	POTENTIAL SOLUTION
Pathogen Control: Water Influent	Å Prevent pathogen growth (biomass & treated water) Å Prevent system biofouling	Å Chemical free pre-stabilization of waste stream (e.g. UV light)
Pathogen Control: Biomass	Å Ensure biomass edibility	Å Baking, UV light exposure, boiling, desiccation
Pathogen Control: Water Effluent	Å Ensure water effluent potability	Å UV light filtration, oxidation
Biofouling	Å Prevent biofouling Å Recover from biofouling	Å Pre-stabilization Å Self-cleaning mechanism

Research Needs

Optimal Conditions & Harvesting Rates?

- Biomass production
- Biomass quality (protein, oxalic acid)
- Nutrient removal (water quality)

Much research exists.

Comparison of published empirical trials is difficult.

Duckweed Research Objectives

- Establish ***Feasibility*** of WWTx in Contained System
- Determine ***Resources*** *Needed* for WWTx (i.e. ESM)
- Determine ***Optimal Controllable Conditions*** (Growth, Quality, & Nutrient Uptake)
- Predict the **Effects of Uncontrollable Conditions** (Wastewater Load or CO_2 Produced by Crew)
- Determine ***Operational Boundaries*** & Failure Modes

Conclusions

❖ Aquatic plants

- enormous potential for biomass production & wastewater treatment
- received little attention as space food crops

❖ Duckweed (family Lemnaceae)

- tiny flowering plant
- one of the fastest growing plants in the world,
- nutritious and high in protein
- 100% edible
- Well suited for metabolic wastewater treatment

By recycling wastewater while rapidly producing edible biomass in a small volume, this organism could provide a fundamental link in a closed space habitat ecosystem.

Conclusions

❖ **µG-Lilypond:**

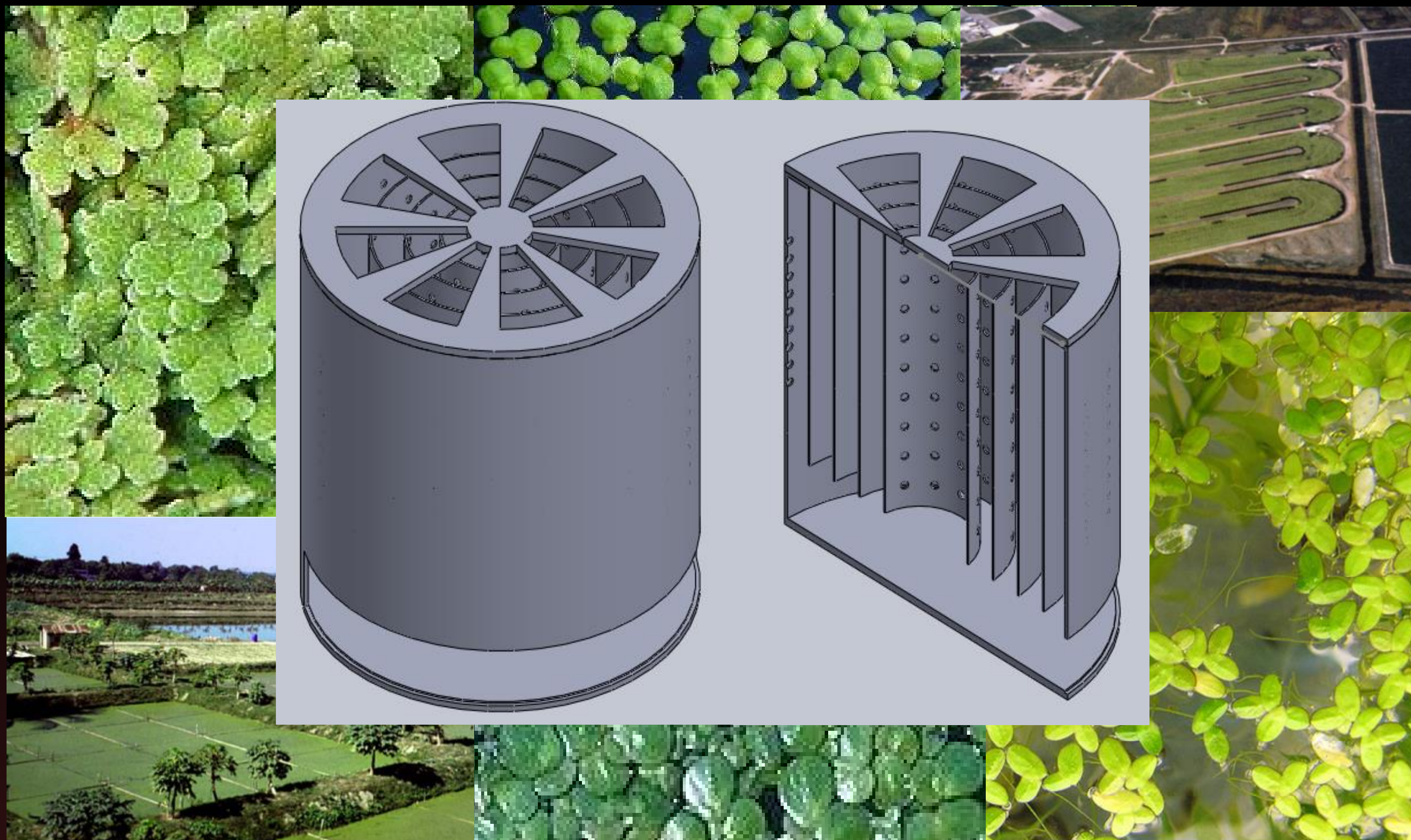
- Collaboration between Space Lab and University of Colorado Boulder
- Phase 1 feasibility study for floating plant production in microgravity

❖ **Research Needed to Define:**

- Optimal conditions for biomass quality
- Food processing techniques
- Daily harvest requirements
- Pathogen control techniques

An understanding of optimal conditions and harvesting requirements for both acceptable water and biomass quality must be determined to exploit this promising plant for spacecraft use.

QUESTIONS?



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