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

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Self-sufficiency of space life support systems is crucial for long-duration exploration missions. NASA's Technology Roadmap states that this will be achieved through resource recovery, system closure, high-reliability, autonomous control, and minimal use of expendables. Regenerative space life support will undoubtedly require food production to recover nutrients and close the carbon loop. Biological plant based systems also provide multiple life support functions such as CO_2 removal, oxygen production, water recovery, and waste recycling. Technologies that both treat waste and produce food with minimal resources (such as energy and water) enable sustainable agriculture on Earth, benefiting populations in water and nutrient scarce regions. Duckweed (Lemna minor) is a tiny flowering plant with enormous potential for bioregenerative space life support. This small angiosperm is gaining global recognition as a powerful and ecologically friendly means of absorbing nutrients and other pollutants from wastewater. In addition, duckweed has a very high protein content and very little fibrous material, making it a 100% edible food supplement for diets lacking in protein. This review explores the capacity of duckweed to provide a food supplement for a spacecraft crew that is high in protein, while recycling nutrients from human metabolic waste, removing and reducing CO_2 from the cabin air, providing oxygen, and purifying metabolic waste water for drinking (ridding of the need to process brine). The review also presents challenges in the safe implementation of a duckweed wastewater treatment and food production system with recommendations for possible solutions and future research.

Nomenclature

ABS	=	Autonomous Biological System
BLSS	=	Bioregenerative Life Support Systems
BOD	=	Biological Oxygen Demand
CEBAS	=	Closed Equilibrated Biological Aquatic System
COD	=	Chemical Oxygen Demand
ECLSS	=	Environmental Control and Life Support System
ISS	=	International Space Station
PAR	=	Photosynthetically Active Radiation
PPF	=	Photosynthetic Photon Flux
TSS	=	Total Suspended Solids
UV	=	ultraviolet
WRS	=	Water Recovery System

I. Introduction

NASA's technology roadmap notes that self-sufficiency of life support systems is crucial for sustaining life on long-duration missions, and that this will necessitate resource recovery, system closure, high-reliability, integrated autonomous control systems, and minimal use of expendables. Regenerative space life support will undoubtedly require food production within a space habitat, to recover nutrients and close the carbon loop. Biological, plant-based systems provide multi-functional life support functions, such as carbon-dioxide removal, oxygen production, water recovery, and waste recycling. Technologies that simultaneously remove waste, produce food, and

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purify water with a high resource use-efficiency will also advance sustainable agriculture on Earth, benefiting populations in regions with low water and nutrient availability. Aquatic plants can recover nutrients from metabolic waste streams, while their edible biomass can provide a protein rich dietary supplement to a spacecraft crew. However, they have received little attention as potential food crops for space applications. Duckweed (family Lemnaceae) is a tiny flowering plant that offers enormous potential for bioregenerative space life support. It is one of the fastest growing plants in the world, growing on slow-flowing or still shallow water bodies. It thrives on wastewater rich in nutrients and dissolved organic compounds (i.e. waste). Duckweed is gaining global recognition as a powerful and ecologically friendly means of removing nutrients and other pollutants from wastewater. Moreover, duckweed has a very high protein content and very little fibrous material, making it not only edible, but also a valuable food supplement to diets lacking in protein. Protein-rich duckweed biomass is best known as feedstock for animals (e.g., fish, poultry, cattle), but is also harvested for human consumption in many countries. For human space exploration, duckweed can provide a food supplement for the crew that is high in protein, while recycling nutrients from human metabolic waste, removing and reducing CO_2 from the cabin air, providing oxygen, and purifying metabolic wastewater for drinking (eliminating the need to process brine). 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.

II. Small Aquatic Plants for Bioregenerative Life Support

Human populations around the world consume many aquatic plants, such as water chestnut, lotus, water spinach, watercress, cattails, and water lilies, as well as numerous species of seaweed (macro algae). In particular, small floating macrophytes like duckweed and Azolla are 100% edible, nutritious, exceptionally fast growing, and able to thrive in nutrient-rich wastewater. According to Ref. 1, higher land plants are the basis for most bioregenerative life support systems (BLSS) concepts developed thus far. Ref. 1 cites several difficulties with land plants for food production, including:

- Gravitropic complications in seed production and reproduction
- Disturbances in nutrient delivery due to changes in gravity (favoring capillarity over convection)
- The need to dispose of large volumes of inedible biomass (roots, stems, etc.)

Ref. 1 therefore suggests plant aquaculture (growing edible plants on water) as a “highly promising contribution” to solving these problems. Investigators have conducted numerous experiments on the growth of aquatic organisms in low earth orbit. In the early days of spaceflight, duckweed studies included both floating and submerged experiments.[2,3] The Closed Equilibrated Biological Aquatic System (C.E.B.A.S.) flew on STS-89, STS-90, and STS107 with the goal of studying the influence of space conditions on aquatic organisms.[1,4] Paragon SDC’s Autonomous Biological System (ABS) was an experimental passively controlled, materially closed aquatic ecosystem.[5] The ABS was a sealed microcosm that included aquatic plants, animals, microbes, and algae. It flew three times (STS-77, STS 79/81, and STS 86/89 with transfer to MIR) and accumulated almost 18 months of flight-testing.[5] On STS-67, Ref. 6 flew an experiment to test the influence of microgravity on the structure of duckweed plants grown on sugar in the dark. In a study of the effects of simulated microgravity on duckweed growth, Ref. 7 found that not only were there no adverse effects, but plant growth rate increased significantly. This increased growth may have been associated with changes in cellular structure or with increased utilization of oxygen or other nutrients in the growth medium when suspended (rather than floating).[7]

III. Duckweed

The duckweed family (Lemnaceae) contains the smallest flowering plants on Earth[8] and is among the fastest growing plants in the world.[9] There are four genera: *Lemna, Spirodela, Wolffia,* and *Wolffiella,* which include about 40 different species in total.[8] Duckweed, shown in Figure 1, is a free-floating aquatic plant that is common in lakes, ponds, canals, rice fields, and ditches.[10] It will occur in most still or slow-flowing water bodies under a wide range of conditions (except in nutrient-poor or highly acidic water).[10] It can even grow in a trickle of water over vertical surfaces, such as seepages in cliffs[10], on mud, or on water that is only millimeters deep.[11] Though it is a flowering plant, duckweed primarily reproduces through vegetative budding. Over a 10-day period, an individual mother frond may produce up to 10 generations of daughter fronds before dying.[8] Only occasionally does it produce tiny flowers and seeds.[8] Duckweed has leaf-like ovoid parts, called fronds, with hair-like roots (thought to provide stability).[8] Fronds range from 1-20 mm across, depending upon the species. Because little structural tissue is required for this buoyant floating plant, fronds contain <5% fiber.[8] Duckweed grows extremely fast, doubling its biomass in 1-3 days,[9] under ideal conditions. In addition to its nutritional value and fast growth, these plants can purify highly concentrated waste streams into safe drinking water.

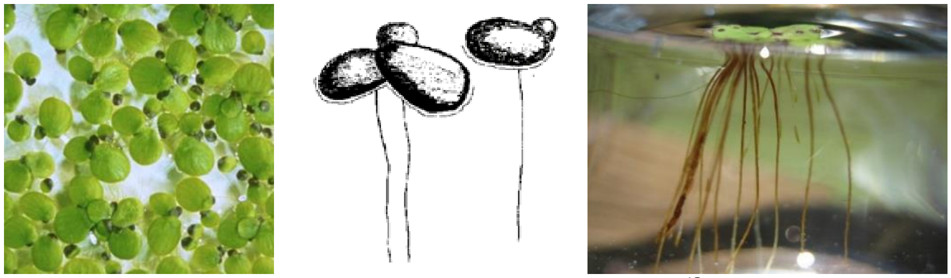

Figure 1. Lemnaceae (Duckweed Family).[12]

A. Water Treatment Capability

Nutrient Uptake: Duckweed grows on water with relatively high levels of nutrients, such as nitrogen, phosphorous, and potassium, while concentrating minerals and synthesizing protein. Therefore, it can grow on thin films of still water containing human urine or other waste solids, while removing nutrients and other pollutants. Ref. 8 explains, "The most favorable circumstance is water with decaying organic material to provide duckweed with a steady supply of growth nutrients and trace elements." Lemna populated lagoons treat sewage in as many as 100 facilities around the world, with effluent often exceeding US water quality standards.[11] Typically, these facilities farm a variety of duckweed species. Many reports have shown high nitrogen and phosphorous removal rates in laboratory tests and field investigations (lagoons and ponds). For example, Ref. 13 observed >98% nutrient removal in the duckweed ponds under study. A laboratory experiment revealed nitrogen removal rates (concentration dependent) of 120-590 mg N/m^2/day and phosphorous removal rates of 14-74 mg P/m^2/day, in a three-day period.[14] Other laboratory and field studies reveal similar nutrient removal rates.[15] About 30-47% of total ammonium-N and 52% of total P uptake is direct, while indirect uptake by algae and bacteria attached in the duckweed mat account for the remainder.[14] In addition to nutrient uptake, duckweed mats also remove total suspended solids (TSS), biological oxygen demand (BOD), and chemical oxygen demand (COD). In fact, some investigations observed 50-90% fecal coliform reduction in waters with duckweed populations.[16,17] The direct contribution of duckweed to coliform removal (versus other environmental parameters or microbial populations) is unclear however.[18]

Duckweed exhibits preferential uptake of ammonium, making it attractive for the treatment of human liquid metabolic waste (urine). In fact, duckweed uses all available ammonium before assimilating nitrate, in contrast to single-cell algae that prefer nitrate.[8] Recently, several laboratory studies have investigated the effects of urine type, dilution, temperature, and nutrient composition on duckweed biomass growth rates.[19] In experiments with human urine and wastewater, Ref. 19 observed that the removal of chemical oxygen demand (COD), total phosphorus, and total nitrogen exceeded 80%, 90%, and 50% of initial concentrations, respectively, while crude protein content reached 31.6% in experiments with human urine and continuous biomass harvesting. Duckweed can even directly remove complex carbohydrates and organic nitrogenous compounds.[8]

Ref. 8 describes the Mirzapur duckweed wastewater treatment plant, operational in 1990. Though not optimized for surface area, this plant treated an average flow of 125 m^3/day of wastewater produced by a population of 2000-3000 people over a 0.6-hectare area.[8] The final treated effluent exceeded the highest water quality standards of the United States.[8] Other treatment plant studies indicate that 2-3 m^2 of duckweed growth area can treat wastewater for one person.[18] If one were to duplicate such a model wastewater treatment system on a small scale for spacecraft life support, a 10 m^2 growing area could treat wastewater for a 4-person crew. Since duckweed can grow on a thin film of water, this would require a relatively small volume, by stacking shallow growing trays. For example, if each tray is 10 cm tall (water depth and air column height), than a 1-m^3 volume could contain a 10 m^2 growing area.

B. Edible Biomass Production Capability

Duckweed farms, like those in Figure 2, have been widely developed terrestrially for wastewater management, as feedstock for animals (poultry, swine, and cattle), and even for human consumption. Ref. 11 describes duckweed based farming systems in Vietnam that rely on manure and excrement collected in eutrophic ponds where thick mats of biomass grow and are harvested daily for duck feed. In Taiwan, pig and poultry farmers use similar methods. Ref. 11 also provides anecdotal reports of duckweed used throughout South Asia as a human food source. For example, 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.[11]

Duckweed fronds contain 92-94% water.[8] The protein content of duckweed is one of highest of all plants (up to 45% of dry mass, depending on nitrogen availability), comparable to that of soybean, and the essential amino acids produced more closely resemble that of animal protein (lysine and methionine).[8] As mentioned previously, the fiber content is low (5%) under ideal conditions.[8] Duckweed dry mass also contains about 30-35% carbohydrates, and are a good source of vitamin A and pigments like beta-carotene and xanthphylls.[15] However, as with any organism, biomass composition changes with diet. The nutrient composition that optimizes growth rate is not necessarily best for protein content and digestibility. Under nutrient-poor conditions, fiber percentages rise and protein content decreases.[8] In a urine treatment study investigators transferred duckweed from diluted urine wastewater to clean water for several days, resulting in increased starch content.[19] Ref. 11 reports >60 mg N/L optimizes protein content.

Figure 2. Left - Duckweed-covered serpentine plug-flow lagoon in the USA for tertiary treatment;[18] Center - Duckweed Mats Fed from Fecal Materials;[11] Right - Duckweed cultivation and wastewater treatment farm at Mirzapur in Bangladesh.[18]

Duckweed reproduces at a very high rate, with doubling times of 1-3 days.[11] Though the plant is renowned for its rapid growth, there is significant variability in growth rates reported in literature due to variability in growing conditions. Productivity reported by authors around the globe varies from values as low as 2 tons of dry mass per hectare per year (t/ha-year DM) to values over 50 t/ha-year. These wide variations are due to differences in species, climate, cultivation area, nutrient supply, water depth, and management.[18] Optimal temperatures are between 25°C and 31°C.[18] Low water flow velocity is preferable (<0.1m/s).[18] Duckweed growth rate is very much dependent upon nutrient concentration and composition, and it responds quickly to nutrient flushes with growth spurts.[11] In addition, growth is dependent upon biomass density, requiring continuous (usually daily) harvesting for optimal growth. Ref. 8 explains that an optimum density is complete cover, but with enough space to accommodate rapid growth. Ref. 18 notes that some of the higher yield values are extrapolations from short-term and small-scale experimental systems operated under controlled conditions. A spacecraft treatment facility would in fact be such an environmentally controlled system. Ref. 13 observed duckweed pond production of over 68 t/ha year of dry biomass, with 35% crude protein content. Assuming a similar yield in spacecraft systems, dry edible biomass production of about 0.17 kg per day could occur within a 10 m^2 growing area. As stated previously, with stacked growing trays, a 1-m^3 volume could contain a 10 m^2 growing area. It is possible that with elevated (injected) carbon dioxide levels and increased light intensity, biomass growth could significantly increase.[2] Up to 0.25 kg per day in a 1-m^3 volume is not an unreasonable projection.[2] Therefore, duckweed could serve as a significant dietary supplement and protein source for the crew, grown only on wastewater alone (not including solid metabolic waste).

IV. Duckweed Implementation in Regenerative Life Support Systems

A. Potential for Utilization in a Space Habitat

Ref. 7 described duckweed as "one of the most attractive higher plants" for long-duration space life support. The enormous potential of this small plant for space habitat utilization is not only the ability to uptake nutrients directly from human urine and rapid growth of edible biomass high in protein. It is also the ability to grow under a wide range of environmental conditions (temperature, nutrient composition, pH, light, and water depth) making it extremely robust for space life support applications. Duckweed can "survive and recover from extremes of temperature, nutrient loadings, nutrient balance, and pH."[8] The high yield of edible biomass and protein for duckweed is not only due to fast growth, but also due to 100% edibility. Duckweed can grow on very thin films of water, requiring less volume, and can even utilize pre-synthesized organic materials (e.g. sugars) to grow without the presence of sunlight.[11] There are several attractive characteristics for its use as a space crop:[2]

- Rapid, uniform growth through vegetative budding (unlimited asexual production)
- 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
- Preferential uptake of ammonia as a nitrogen source (making it attractive for waste processing)
- Inactive, permanently open stomata, allowing for irregular photoperiods and continuous CO_2 uptake
- 100% harvest index of highly nutritious material (10-35% protein with high amino-acid content)

In addition, light from either above or below the plants can drive photosynthesis, allowing for multi-tiered plant-growth systems with shallow, transparent trays and bilateral lighting.[2]

The Water Recovery System (WRS) used on the International Space Station (ISS) includes several processes: 1) chemical urine pretreatment (to reduce ammonia volatility and microbial growth) and storage; 2) gas/liquid separation; 3) solid filtration; 4) catalytic oxidation (removing organic compounds); 5) ion exchange filtration (removing dissolved ions); 6) and finally, iodine addition for pathogen control. In addition to its complexity, disadvantages of this system include the failure to capture and reuse nutrients left behind in brine, brine toxicity created by chemical treatment, lower reliability of complex components like vapor compression distillation (VCD), and non-regenerable consumables (chemicals, filtration & ion exchange beds, and catalyst). Typical terrestrial municipal wastewater treatment systems have similarities to the ISS. Solids are first separated, followed by oxidation (biological or chemical) to remove organic compounds, followed by polishing to remove remaining chemical impurities (such as carbon filtering). Biological oxidation typically occurs with aerobic microbes in reactor tanks. Relative to current ISS water recovery methods and terrestrial municipal systems, duckweed wastewater treatment has several advantages:

Increased Water Recovery: Reclamation of potable water from liquid waste, evapotranspiration condensate, and duckweed biomass will minimize water loss from the system with the potential to achieve close to 100% recovery.

Nutrient Recovery & Dietary Supplementation: Protein-rich edible biomass produced via duckweed photosynthesis, if properly stabilized, can supplement human diets. Most microbial bioreactors produce inedible biomass requiring further decomposition for nutrient recovery. Dried duckweed meal (with <10% moisture) can store without deterioration for at least five years, if protected from light and humidity; and can be pelletized without a binder.[8] Drying also kills pathogens. In fact, in four years of testing, Ref. 20 was unable to culture any viable human pathogens from dried sewage-grown duckweed meal. A waxy coat on the surface of fronds inhibits fungal growth, also contributing to a long shelf life.[15]

Year-Round Operation: Existing duckweed wastewater treatment facilities are typically large outdoor lagoons. By developing a compact environmentally controlled growth chamber, operation can continue with stable, optimal growing conditions, year-round.

Air Revitalization: By using photosynthetic organisms, the system will also take up excess carbon dioxide from the air, while producing oxygen, revitalizing the atmosphere for closed spacecraft habitats. Of course, light incidence and CO_2 concentration drive photosynthetic rates (without nutrient limitation). Ref. 2 observed photosynthetic rates approaching 750 ηmol $CO_2.gDW^{-1}s^{-1}$, with 740 $\mu mol.m^{-2}s^{-1}$ PPF (photosynthetic photon flux) and >1200 $\mu mol.mol^{-1}$ CO_2.

Volume Efficiency: Because duckweed can grow on thin films of water, with lighting from any direction, thin growing trays can be stacked, to enable a large growing area in a small volume.

Power Efficiency: Though lighting consumes power, plant growth does not require power for maintaining higher temperatures (unlike microbial bioreactors). Power efficiency of duckweed wastewater treatment systems may be a significant improvement over bioreactors or distillation processes.

Low Maintenance: Automation of biomass harvest and potable water extraction for continuous culturing would minimize crew time for operation.

B. Conceptual Design for a Microgravity Floating Plant Cultivation System

Under a NASA-funded Phase 1 STTR effort, we are developing a conceptual design for a reliable, flexible, and efficient floating plant production system for use in microgravity. The system must perform reliably in its expected microgravity environment. To be efficient, a crop production system must provide plants with the nutrients, water, light, and atmospheric conditions that maximize the production of edible biomass while minimizing launch and use costs (such as power for lighting, crew time for maintenance, infrastructure mass and volume, and consumables like water). Finally, the flexible system must enable production of a variety of crops from seed to seed, through multiple life cycles.

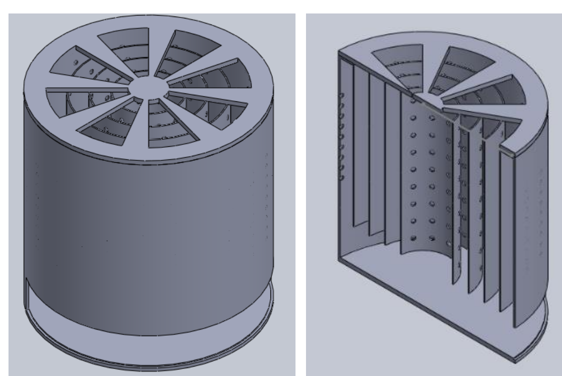

Figure 3. Conceptual diagram of the Duckweed Wastewater Treatment System for

Using existing terrestrial duckweed based wastewater treatment facilities as a baseline, the proposed cultivation system will produce edible biomass, produce oxygen, remove carbon dioxide from the cabin atmosphere, and optionally recover potable water and nutrients from the crew's metabolic waste. This small-scale system will operate in a microgravity environment, on a space station or other spacecraft by using capillary action for water delivery to the plants. The growth chamber shown conceptually in Figure 3 will feature autonomous environmental control and harvesting mechanisms in order to provide suitable growing conditions with reduced crew time needed for operation. The system will feature a compact structure that rapidly produces edible biomass while optionally recycling wastewater.

This collaborative effort between Space Lab Technologies, LLC (Space Lab) and the Bioastronautics research group from the University of Colorado (CU) Boulder Aerospace Engineering Sciences Department will combine modeling, analysis, and engineering design to demonstrate technology feasibility and prepare for detailed prototype design, fabrication, and testing.

Phase 1 Objectives are to:

- Determine feasibility of passive water delivery to floating aquatic plants in microgravity
- Determine feasibility for continuous autonomous biomass harvest and water (effluent) extraction
- Determine feasibility of autonomous floating plant propagation
- Define autonomous environmental monitoring and control methods to support candidate crops
- Estimate cultivation system efficiency, in terms of production capacity versus cost (equivalent system mass of volume, power, infrastructure, consumables, and crew time for operation and maintenance)
- Plan for the future development, verification, and validation of a fully functional flight unit

1. Preliminary Concept of Operations

The chamber will first receive nutrient enriched water (from an external nutrient reservoir or from the spacecraft waste collection system) through capillary tubes. The water source, controlled by electronic valve, will slowly flow from the tubes into grated cylinders pre-seeded with duckweed, spreading a thin film under or around the plants with capillary action. LED strip lights will provide photosynthetically active radiation (PAR) with a controlled intensity, spectrum, and photoperiod. The water film will remain stationary for several days while the duckweed grows or until the water quality has reached desired levels. The cylinders will then be *plunged* allowing water extraction through a filter in the bottom of the unit, where it will then flow to an external holding tank for further processing and/or ultraviolet (UV) light filtration. A tray at the base of the unit captures the duckweed biomass allowing its removal for post-processing. A fraction of the duckweed will remain, captured in the cylinders. The cycle will then begin again.

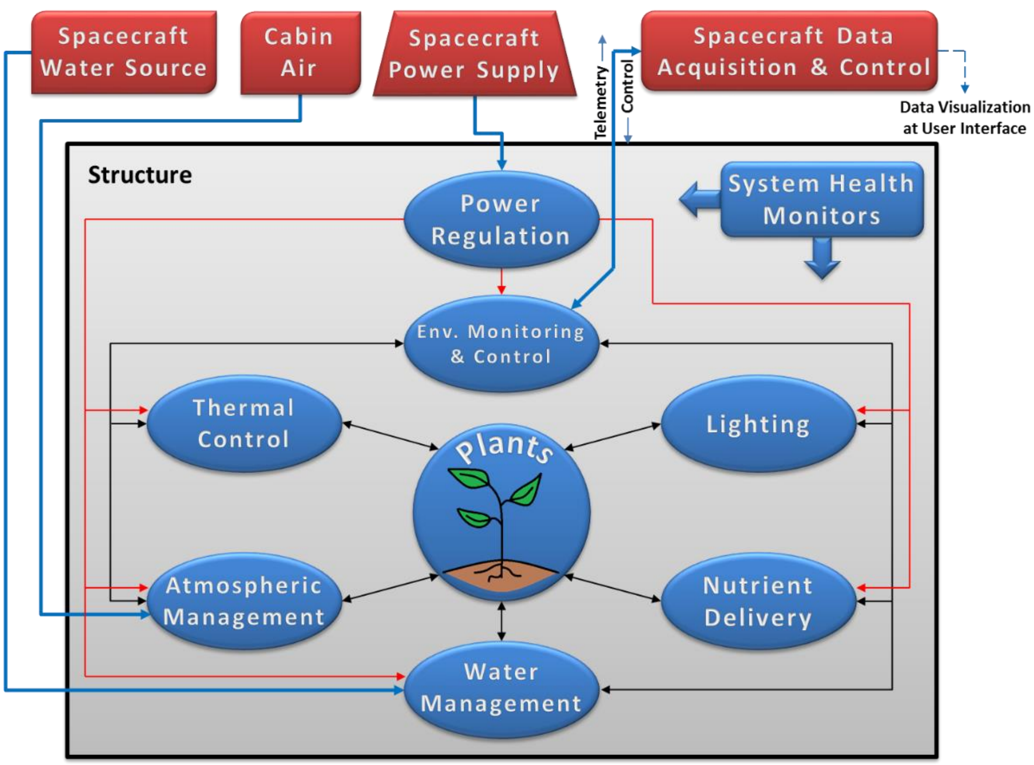

Figure 4. uG-LilyPond Preliminary Functional Architecture

2. Functional Overview

Figure 4 illustrates the main system functionality to be included in conceptual design. The structure will provide low volume, low mass system containment that is robust to the cabin environment; passive water delivery to the plant roots; and mechanisms for biomass harvest, water extraction, and seed propagation. The lighting system will include LED's that allow spectrum, photoperiod, and intensity adjustment. The nutrient delivery system will ensure the proper nutrient conditions (EC/pH) in the water supply, which comes from various sources within the spacecraft. The water management system will ensure proper water flow and temperature, collect condensate and effluent, and recycle/distribute them as needed. Atmospheric management will maintain suitable temperature and air circulation, remove ethylene, and allow for optional CO_2/O_2 control. The need for relative humidity control will be determined in design. The system will allow for gas exchange with the cabin atmosphere, including possible injection of CO_2 from a reservoir and O_2 extraction from exhaust. The spacecraft will provide power externally. Finally, the environmental monitoring and control system includes the sensor suite, data acquisition hardware, and software to collect and process sensor data and to send actuation commands for environmental control.

V. Research Needs and Challenges

There are many challenges still to be addressed in the development of aquatic plants for both food and wastewater treatment in space habitation systems. Focused research efforts can likely resolve these challenges. For instance, NASA considered the use of duckweed for bioregenerative life support early in the space program. Since researchers thought duckweed to be inedible due to high levels of oxalic acid, they gave it little attention for space life support applications[21] relative to higher plants. Although oxalic acid can be toxic in high concentrations and produce a bitter taste (like in spinach), its accumulation in duckweed can be reduced by controlling concentration and balance of minerals in the water supply, water pH, water and air temperature, light incidence, photoperiod, and even biomass density.[11] Studies suggest a positive correlation between high nitrogen availability in the medium and protein content and digestibility.[22] Even growth area geometry may be an important factor. For instance, surface area, rather than depth may influence nitrogen removal rates.[23] 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. Other issues include biomass processing for food (like roasting, or pulping to extract protein), and non-toxic pathogen control methods. Following are some known challenges and possible solutions.

- *Biomass quality control:* Control of the nutrient composition in the waste stream or post-processing (boiling, roasting, or sautéing before eating) may improve protein content, taste, and digestibility. In addition, non-toxic methods for waste-stream pre-stabilization (such as UV light) might eliminate the need for biocides, preventing accumulation of toxic chemicals in the duckweed biomass.
- *Food preparation*: Solutions include pulping, filtration to extract protein, or drying for storage.
- *Daily harvest requirements:* Autonomous harvesting mechanisms will reduce crew time for operation.
- *Biomass pathogen control:* Solutions include baking, UV light exposure, boiling, or desiccation.
- *Pathogen control for treated water:* UV light filtration may provide sufficient stabilization for potability.

Many laboratory and field studies have revealed the effects of environmental conditions on duckweed biomass production and nutrient uptake. However, differences in methodology, scale, and experimental parameters make direct comparisons between published empirical trials difficult.[22] Ref. 22 also points out that optimal and limiting values for growth vary widely between species and between colonies or isolates.

A ground based prototype cultivation system could support a wealth of future studies. Possible *objectives* for focused growth experiments might include:

1) Establish feasibility of producing potable water from urine using duckweed in a controlled environment.
2) Determine the resources required (e.g., power, volume, consumable mass) to treat urine using duckweed.
3) Determine optimal controllable conditions for biomass production and nutrient uptake (e.g. light, urine dilution ratio, or water temperature).
4) Predict the effects of less controllable conditions, such as wastewater load or CO_2 produced by the crew.
5) Determine the system's operational boundaries to predict failure modes and design for failure recovery.

To this end, relevant *research questions* might include:

- What are the maximum and minimum waste loading rates (i.e. dilution ratios) and harvest rates needed for a desired yield and quality of biomass or for a desired level of nutrient uptake?
- Can duckweed absorb ash from solid waste incineration, if mixed into the wastewater stream?
- What is the biomass production, CO_2 consumption, and O_2 production capacity of a system sized to treat wastewater for a four-person crew?

- Does duckweed grown together with algae enhance or inhibit wastewater treatment capacity and/or biomass production?
- What are the limitations for growth? What growth rates occur with a sucrose medium and no light?
- What are the effects of water depth on growth, nutrient uptake, and failure recovery?
- Given optimal urine dilution ratios and water depth, what is the minimum volume required to treat urine generated by a crew of four per day?
- What is required to maintain and restart stock cultures in the event of system failure or shutdown?
- Is duckweed wastewater treatment more or less power efficient than microbial bioreactors?
- What are differences in the wastewater treatment and nutritional capacities for various duckweed varieties and what are the optimal growing conditions for each species?
- What is the nature of symbiotic relationships with root-zone microbes (nitrifying, or other)?
- What environmental parameters (if any) can control biomass composition (especially protein content) and quality (oxalic acid content)?

VI. Conclusion

Regenerative space life support will undoubtedly require food production within a space habitat, to recover nutrients and close the carbon loop. Biological, plant-based systems provide multi-functional life support functions, such as carbon-dioxide removal, oxygen production, water recovery, and waste recycling. Hydrophytes (or aquatic plants) have enormous potential for edible biomass production and metabolic wastewater treatment. However, they have received little attention as potential food crops for space applications.

Duckweed (family Lemnaceae) is a tiny flowering plant that is one of the fastest growing plants in the world, doubling is biomass in 1-3 days.[9] It grows on slow-flowing or still shallow water bodies with high nutrient levels. It exhibits preferential uptake of ammonium making it well suited for metabolic wastewater treatment. There are as many as 100 Lemna populated sewage treatment lagoons in operation around the world.[11] Laboratory and field investigations show high nitrogen and phosphorous removal rates by duckweed. Given reported capacities for terrestrial waste treatment plants, a 10-m^2 duckweed growing area in a 1-m^3 volume could potentially treat wastewater for a 4-person spacecraft crew.

Duckweed farms worldwide provide feedstock for animals and even human food. The 100% edible biomass produced is highly nutritious with low fiber and high protein content that is comparable to that in soybeans. Oxalic acid presence can produce a bitter taste however (like spinach), and may be toxic in high concentrations. Biomass composition and growth rate changes with growing conditions, especially nutrient composition of the water effluent. In an optimal environment, a 1-m^3 growing volume could potentially produce up to 0.25 kg per day of dry edible biomass. For human space exploration, duckweed can provide a protein rich food supplement for the crew, while recycling nutrients from human metabolic waste, removing and reducing CO_2 from the cabin air, providing oxygen, and purifying metabolic wastewater for drinking (eliminating the need to process brine). 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.

Under a NASA-funded Phase 1 STTR effort, the authors, in collaboration with the University of Colorado at Boulder, are developing a conceptual design for a floating plant production system for use in microgravity. In addition, the authors recommend focused research efforts to resolve several operational challenges, which include definition of optimal conditions for biomass quality, food-processing methods, daily harvest requirements, and techniques for pathogen control (in biomass and treated water). 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.

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