On-Land Experimental Cultivation of a Nutritionally Dense Seaweed for Potential Human Consumption in Space

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Why *Ulva*?

***Ulva* as a food source can help to reduce the health risks astronauts face associated with long-term missions.**

- A number of publications have demonstrated the presence of functional properties important for human health (Pirian et al., 2018).

- Regarding carbohydrates, the Ulvan type is found to have up to 30% and is considered to have antiviral, antitumor, and antidepressant functional properties (Domínguez and Loret, 2019).

- *Ulva* species contain phenolic, chlorophyll, and carotenoids, which can act as anticarcinogens (Domínguez and Loret, 2019).

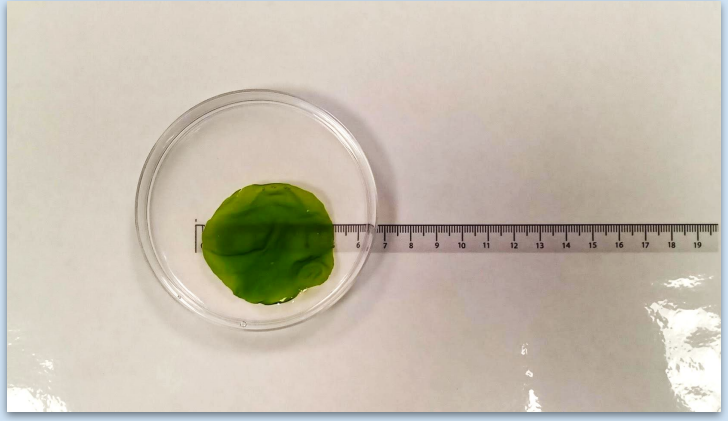
- *Ulva* species are sources of essential amino acids such as histidine, containing levels comparable to those found in legumes and eggs (Domínguez and Loret, 2019).

- *Ulva* has a high protein content (up to 30%), Fe, good unsaturated lipid acid, and vitamins C and E (Domínguez, 2013; Domínguez and Loret, 2019; Lordan et al., 2011; Pirian et al., 2018; Ruangchuay et al., 2012).

Why *Ulva?*

***Ulva* Grows Rapidly.**

- Based on laboratory experiments, a maximum growth rate of up to 10% gr/day or 22% cm/day has been recorded in the presence of high nutrient concentration (Young and Gobler, 2016), and salinities of 20 or 35 ppt (Angell et al., 2015; Ruangchuay et al. 2012).



Ulva being prepared for measurement.

Why *Ulva*?

Researching *Ulva* can directly support NASA's interest in Space Biology.

- Culture technology can positively support NASA's Strategic Goal 2 to "Extend human presence deeper into space and to the moon for sustainable long-term exploration and utilization" (Anonymous, 2018, p. 3).

- Researching *Ulva* can support NASA to "Develop the scientific and technological foundations for a safe, productive human presence in space for extended periods and in preparation for exploration" (Anonymous, 2018, p. 3).

- Large-scale *Ulva* cultures can be used to benefit NASA and America by providing a sustainable food source that can be grown in space while providing the same nutritious benefits to those on Earth. As a result, it will be important to determine *Ulva's* ideal growing conditions in a lab on Earth before researching *Ulva* in space.

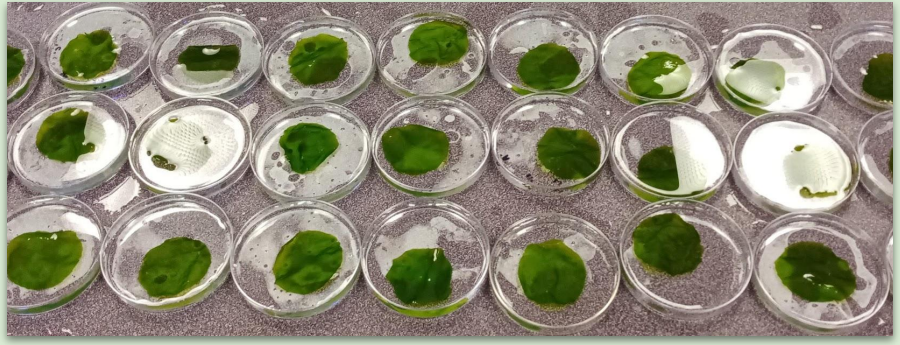
- *Ulva* cultures represent a sustainable source of food production because solar energy and CO_2 are the main carbon and energy sources, not freshwater and soil, which are resources that are limited on Earth but absent in Space.

- We are hopeful that even with our limited resources as a community college and the limited research time available to us, this is a research project that can meaningfully contribute to current and future NASA's strategic plans, directives, and objectives.

Goal of Project

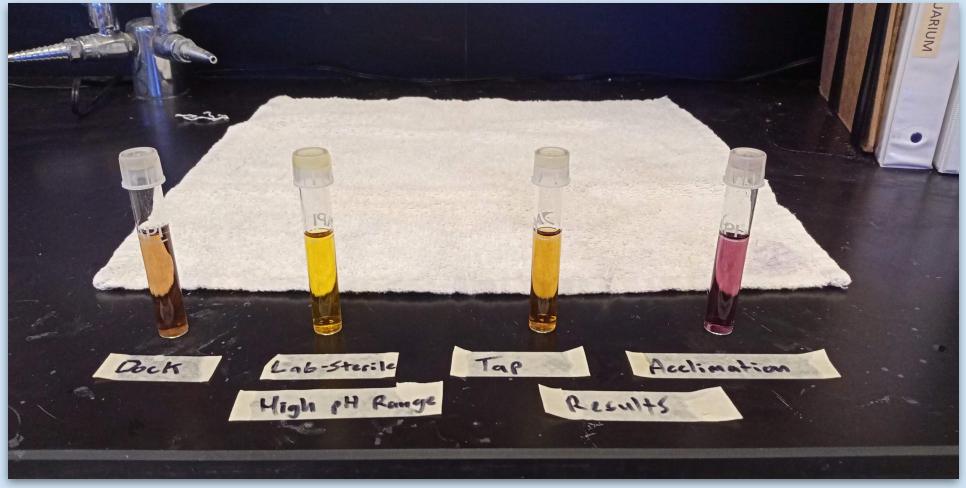
- Determine the ideal combination of nutrient concentrations (1x, 2x, and 4x) and salinity (25, 30, and 35 ppt) that result in the highest growth rate (% increase of length/day) of *Ulva* spp. over the span of two months.

- We also wanted to determine the difference in protein and carbohydrate content present in the *Ulva* tissue of our experimental units by performing a spectrophotometric analysis. Unfortunately, we could not perform the spectrophotometric analysis because of a prohibition of face-to-face activities at the college associated with the outbreak of the COVID pandemic.



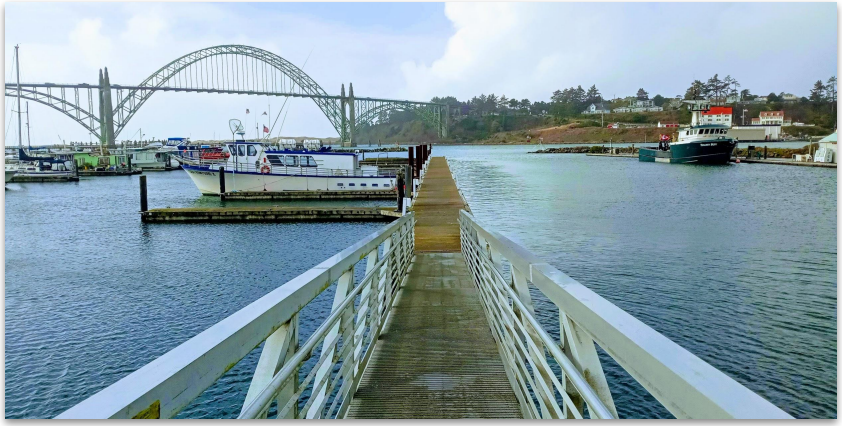
Ulva discs in petri dishes.

Project Execution



Where and How *Ulva* was Harvested

- *Ulva* specimens from the South Beach Fuel Dock in Newport, Oregon on January 26, 2020.
- We used a handheld salinity refractometer to determine the salinity of the water at the site (31.0 ppt).
- A handheld conductivity, salinity, and temperature meter was also used to measure the water temperature at the site (10.60℃).
- Extra seawater was gathered so that its nutrient content could be tested back at the lab.



South Beach Fuel Dock in Newport, Oregon.

A bucket filled with *Ulva*.

Ulva Size

- Our intention was to start with discs 5 cm in diameter.

- This was accomplished by creating a cutting device out of an aluminum sheet placed inside of a 5 cm diameter pipe. The *Ulva* was placed on styrofoam and then punched with the custom cutter.

- The discs were cut on January 26, 2020, but the experiment could not be started until February 8, 2020 due to a delay in the arrival of experiment components. This caused the average diameter of our *Ulva* to be 6.02 cm when we started our experiment.

Ulva being cut into 5 cm diameter discs.

Preparation of Experimental Units

- The *Ulva* was placed into clear plastic petri dishes 10 cm in diameter.

- The petri dishes were first cleaned in a 1:30 bleach/water solution, rinsed, and allowed to air dry.

- As the *Ulva* grew in size, we replaced the 10 cm diameter petri dishes with 15 cm diameter petri dishes that were also cleaned in a 1:30 bleach/water solution.

- We had 27 total experimental units of *Ulva*; three for each nutrient/salinity combination:
  1x nutrient concentration at 25, 30, and 35 ppt salinity,
  2x nutrient concentration at 25, 30, and 35 ppt salinity,
  and 4x nutrient concentration at 25, 30, and 35 ppt salinity.

Modification of Refrigerator

Modified refrigerator containing *Ulva* experimental units.

- A New Air AB-1200X beverage refrigerator was used to maintain a constant temperature of 10.6℃.
- The AB-1200X has 3.4 ft^3 interior space, 5 wire racks, as well as a glass front panel.
- Temperatures were verified with two separate thermometers to ensure accuracy.
- A styrofoam sheet 1” thick was taped over the glass panel, and a 20” Aqueon light fixture was then mounted in the center of the styrofoam sheet.

Lighting

- The Aqueon light fixture was fitted with one 20” LED Day White bulb and one 20” LED Beauty Max bulb.
- LED bulbs were chosen because they do not create a lot of heat and wouldn’t melt the styrofoam.
- We used one Day White bulb and one Beauty Max bulb because they offered the widest spectrum of usable light when used in conjunction (Figs 1 and 2).
- The lights were then put on a light/dark cycle of 10:14 hr to replicate our light cycle in February.

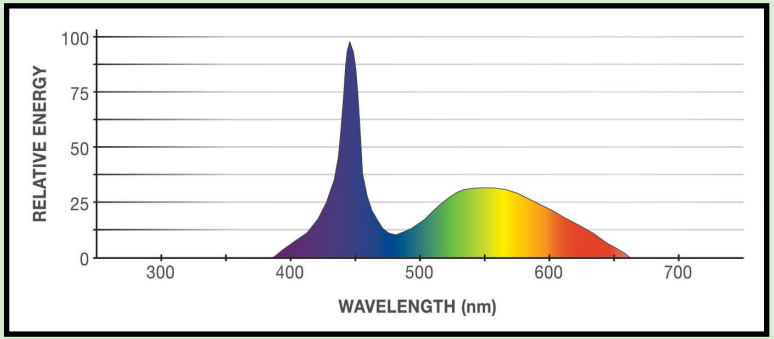


Figure 1: Beauty Max bulb light output.Original images and data from Anonymous (2017).

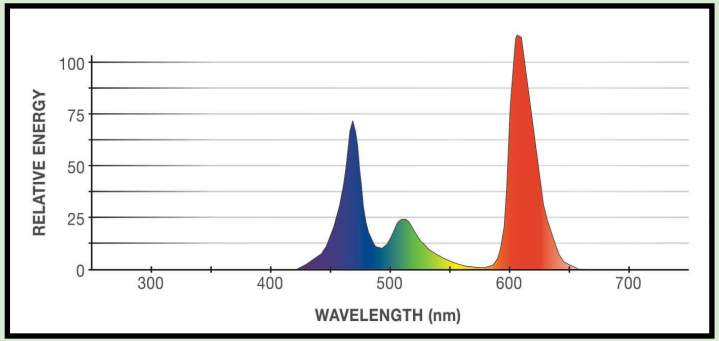


Figure 2 Day White bulb light output. Original images and data from Anonymous (2017).

Nutrients

- Grow More brand Bromeliad Fertilizer was the closest match to the nutrient concentrations (N : P : K, 21 : 8 : 18) cited by Mendoza et al. (2018).

- The Grow More brand contains a nutrient concentration of 17 : 8 : 22 total nitrogen (N), available phosphate (P_2O_5), and soluble potash (K_2O).

- We used 0.14 gr, 0.28 gr, and 0.56 gr (1x, 2x, and 4x nutrient concentrations, respectively) of fertilizer per 64 oz of Reef Salt mix and purified water.

Bromeliad nutrients.

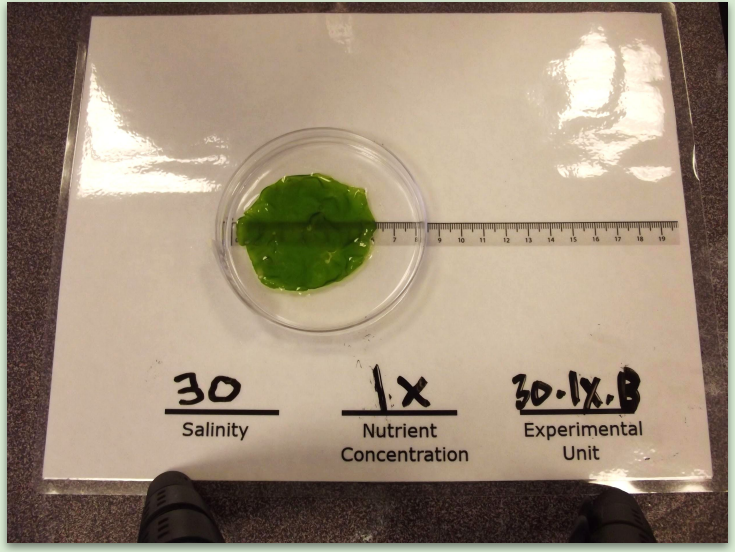
Seawater Mix Preparation

- Reef Salt mix was mixed at three different salinities: 25, 30, and 35 ppt.
- Salinity content was determined with a handheld salinity refractometer.
- Each of the three salinities of Reef Salt mix was measured into three 64 oz buckets (9 buckets total) and mixed with either a 1x, 2x, or 4x concentration of nutrients.

Reef Salt mix.

Filling the *Ulva* experimental units with the completed mixture.

Maintenance and Measurement Schedule



Ulva being measured.

- Experiment started on Saturday, February 08, 2020.
- We found there was as much as a 10% increase in salinity every three days, so we determined that we would change the water every Monday, Wednesday, and Friday to ensure that salinity levels did not increase too much.
- The diameter of the *Ulva* was also measured on these days by placing the clear petri dish containing the *Ulv*a experimental unit onto a laminated sheet with a ruler printed onto it.
- To ensure that each *Ulv*a experimental unit received as equal an amount of light as is possible during the experiment, they were always put back into the refrigerator in a random order.

Outcome of Project

- Experimental unit “Salinity 30 ppt | Nutrient 2x,” had the fastest growth rate, averaging an increase in diameter size of 2.97 cm, or 48.5% (Figs 3 and 4).

- Experimental units “Salinity 35 ppt | Nutrient 2x” and “Salinity 35 ppt | and Nutrient 1x” had the next best growth rates, averaging an increase in diameter of about 2.2 cm each, or an increase of 36.7% and 35.0%, respectively (Figs 3 and 4).

- The experimental unit with the slowest growth rates was unit “Salinity 25 ppt | Nutrient 4x,” averaging an increase in diameter size of just 0.47 cm, or 7.1% (Figs 3 and 4).

- Experimental unit “Salinity 30 ppt | Nutrient 4x” had the next slowest growth rate, averaging an increase in diameter of about 0.73 cm, or 12.2%, while “Salinity 25 ppt | Nutrient 2x” averaged an increase of 0.8 cm, or 14.2% (Figs 3 and 4).

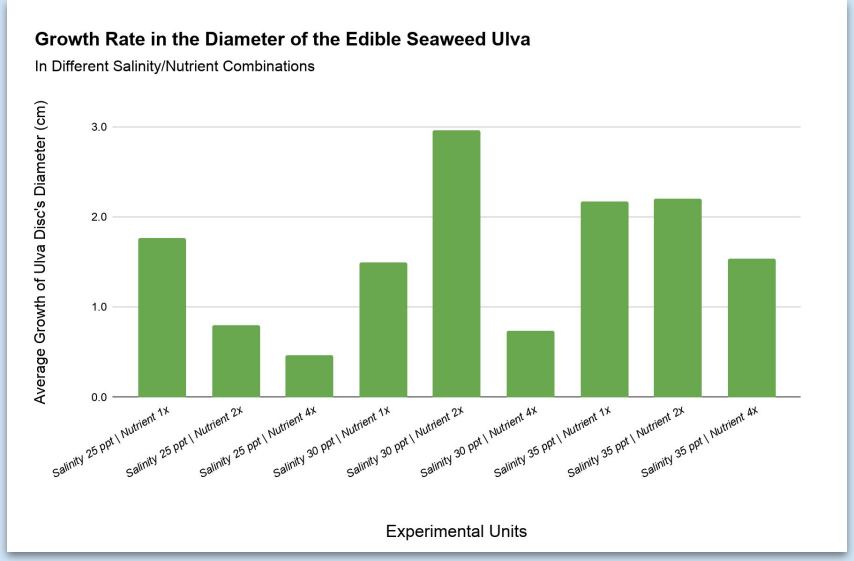


Figure 3: Shows the average growth rate in the diameter (cm) of our *Ulva* experimental units over time,making it easier to visualize the growth of the *Ulva* experimental units during the course of the experiment.

Outcome of Project

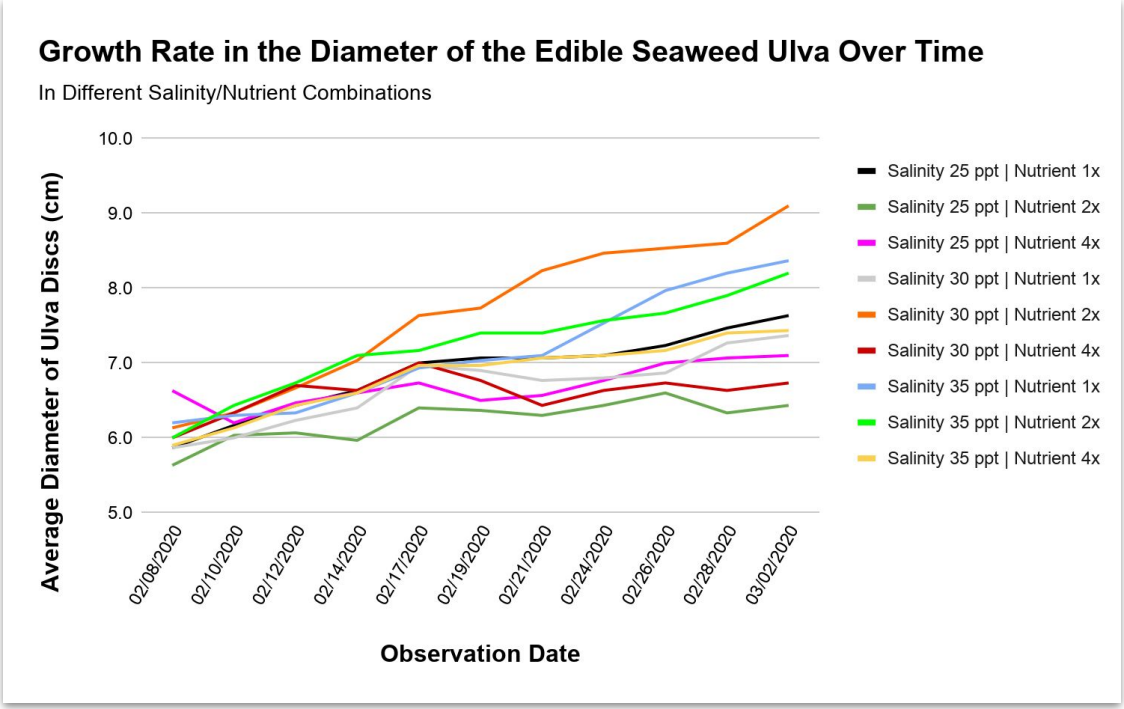


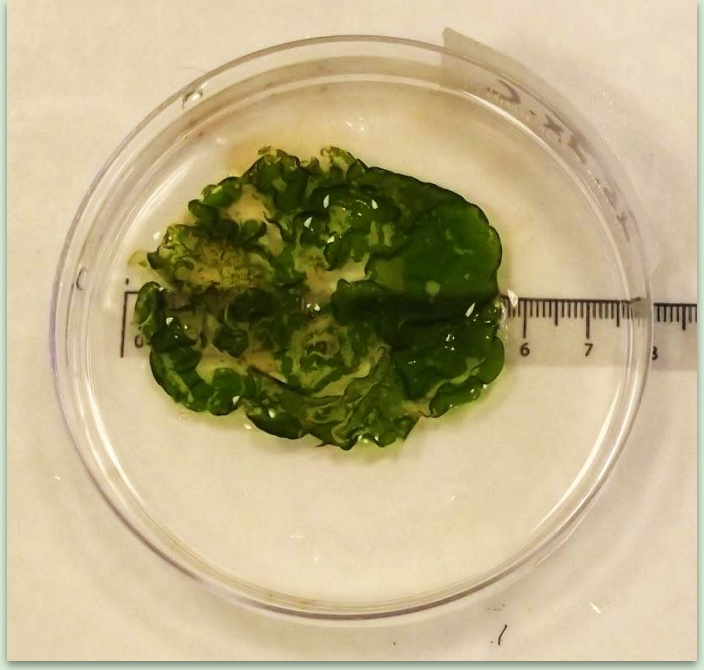
Figure 4: Shows the average growth rate in the diameter (cm) of our *Ulva* experimental units over time, making it easier to visualize the growth of the *Ulva* experimental units during the course of the experiment.

Analysis of Results

Mixed Results.

- We did observe some very fast growth amongst the *Ulva* experimental units, but our results were skewed by a few units becoming reproductive, possibly because of stress caused by a suspected bacterial/fungal growth on a few of our *Ulva* specimens.

- We were planning on having the *Ulva's* protein and carbohydrate content tested in a spectrophotometer, but unfortunately LCC was shut down due to COVID-19.



A reproductive unit of *Ulva*.

What I Personally Gained from this Experience.

- Working with my mentor, Edgar, on this research project was a great learning experience for me. He not only answered my many questions, but was also open to new ideas such as modifying the protocols of the experiment and helped me to find additional resources at LCC and maximize their potential.

- I was introduced to Rosie Kirwin, the Life Science Laboratory Coordinator at LCC. She was instrumental in obtaining the parts necessary to perform our protein analysis, as well as locating anything else we needed to run our experiment.

- It was also great having all of the resources of the Life Science laboratory, including its stockroom, at our disposal. I have learned that a campus is more than just a place I can go to learn, it is a tool I can utilize to accomplish my goals.

- It was also great learning how to write a research proposal, and it is a skill I plan on using a lot in the future.

- It was such an amazing feeling I experienced when I was out in the field collecting specimens of *Ulva*, using various tools to analyze the seawater. It made me feel like a legitimate researcher, and it is a feeling I won't soon forget.

Acknowledgements

I would like to thank:

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