RUNNING TIDE

Macroalgae at Alda
2023 Annual Report

Authors:
Dr. Ingólfur Bragi Gunnarsson
Dr. Sigurður Trausti Karvelsson
Dr. Matthew Stephen Haynsen

Date of publishing: March 7th, 2024

The story

Running Tide started operations in Iceland in the spring of 2022. The company is an ocean carbon dioxide removal (CDR) company, and one of the company's most promising technologies is the deployment of macroalgae seeded carbon buoys. Running Tide holds the world's first ocean CDR permit that allows the company to deploy macroalgae seeded material into the ocean for CO_2 removal. To be able to execute our vision, we set out to build a pilot scale macroalgae research and production facility in Akranes, Iceland.

In the summer of 2022, a shipping container outfitted as a macroalgae hatchery arrived on-site at Breið in Akranes. The purpose of the portable macroalgae hatchery was to quickly establish macroalgae cultivation capabilities on-site, while the larger research and production facility, Alda, was being designed, built and brought on-line. This gave the company a six month head start of retrieving macroalgae from the ocean around Akranes, growing it and having a selection of candidate species to cultivate inside Alda when the facility was ready.

The initial macroalgae species selected by the Running Tide macroalgae program management team were *Ulva fenestrata* (Northern Atlantic green algae species closely related to *Ulva lactuca*) and *Saccharina latissima*, commonly known as sugar kelp. Reasoning behind the selection of these two species was mainly their availability in Iceland, their life cycle being relatively well understood, compatibility with our cultivation systems and scalability.

On May 3, 2023, the macroalgae research and production facility Alda was inaugurated by The Icelandic Minister of Foreign Affairs, Þórdís Kolbrún R. Gylfadóttir, and has been in operation since, filling its ranks with a team of scientists and technicians that have been scaling macroalgae production while conducting informative methodological research.

The team

Dr. Ingólfur Bragi Gunnarsson, Director of Applied Biotechnology

Ph.D. in Biotechnology. Background in biochemical production, bioprocess design, biorefinery, genetic engineering accompanied with extensive experience of working with bacteria, yeast and photosynthetic organisms on lab-, pilot- and industrial scale.

Dr. Sigurdur Trausti Karvelsson, Cultivation Scientist

Ph.D in biomedical science. Background in systems biology, biochemistry and molecular biology. Experienced in mass spectrometry, omics and data science. Previous work includes process modeling and optimization in the pharmaceutical industry.

Dr. Matthew Stephen Haynsen, Cultivation Scientist

Ph.D. in biology, M.S. in biology, B.S. in biology and B.A. in English and creative writing. Background in population genetics, evolutionary biology, plant biology, and molecular ecology. Experienced in programming, data science, plant taxonomy and next generation sequencing.

Estrela Ramos Abelleira, Cultivation Technician

Aquaculture Technician. Background in fish, mollusc and microalgae farming and research. Experienced in hatchery and laboratory operations, with emphasis on biosecurity and procedure optimization and standardization.

Kristófer S.A.P. Júlíusson, Cultivation Technician

Background in biotechnology. Experienced in pharmaceuticals, mammalian cell cultures, small and large-scale cell cultivation, working in a GMP environment, GDP, MBR, and SOP creation.

Cultivation setup

The cultivation strategies and equipment are quite different between the two species cultivated at Alda. Therefore, this section will be divided into two, one for each species.

Saccharina latissima gametophytes

Between September 2023 and November 2023, the Alda team sourced good quality sugar kelp sporophytes from the western coasts of Akranes and Seltjarnarnes, Iceland, that contained sorus tissue (reproductive tissue containing spores). By sporulating the sorus tissue in the lab, under clean and controlled conditions, we were able to develop spores into gametophytes inside our specially outfitted and temperature controlled gametophyte grow room (see below, **Figure 1**).

The temperature controlled room is maintained at 10°C and is exclusively used to cultivate our *S. latissima* gametophytes. In addition to the temperature, the light spectrum, intensity, and duration is rigidly controlled to maintain the gametophyte life stage, specifically permitting only red light between 60 and 100 PAR (photosynthetic active radiation) on a 16h:8h schedule.

A range of culture volumes (0.5L, 1L and 5L) are actively maintained and the best performing gametophyte cultures are scaled-up into larger volumes. By the end of 2023 our largest gametophyte cultures were 5L photobioreactors (PBRs), with multiple such cultures active. However, three 20L PBRs were ordered at the end of 2023, significantly increasing our cultivation capacity for 2024. Additionally, over 100 individual gametophytes have been microscopically isolated for future research and sex-specific cultivations.

Compared to other algae, *S. latissima* gametophytes grow slowly, and that fact makes the cultures highly susceptible to contamination. Therefore the macroalgae team have designed the gametophyte culture maintenance process to be as risk free as possible. Precautions include, but are not limited to: segregation of gametophyte specific tools and equipment, frequent and regular sterilizations of tools and equipment, regular microscopic inspections, and immediate isolation and culling of contaminated cultures.

Ulva fenestrata

In May 2023, when Alda became operational, the total cultivation capacity of Ulva was approximately 100L. Most of that capacity was housed in 4L PBRs on our cultivation shelves (**Figure 2**). In the following months, cultures were expanded into the rest of our operational PBRs and by December 2023, the cultivation capacity had increased to 3500L by adding four 100L PBRs, two 500L PBRs and two 1000L PBRs (**Figures 2, 3** and **4**). Additional PBR capacity is available at Alda when needed, as additional PBRs are already on-site and the total capacity of Alda could be doubled when required.

Figure 2 *- 4L PBRs on culture shelves (left) and 100L PBRs (right)*

Figure 3 *- 500L PBRs (left) and 1000L PBRs (right)*

Manipulation and optimization

To successfully grow our target macroalgae species a significant amount of life cycle manipulation of the organisms was necessary, followed by extensive optimization which is still ongoing.

S. latissima gametophytes

As shown in the figure below (**Figure 5**), spores develop into male/female gametophytes which get fertilized and a sporophyte develops; however, by manipulating environmental factors the life cycle can be halted in the gametophyte stage and, subsequently, this stage can be prolonged for months, if not years. As described in the **Cultivation setup** section, our gametophytes are maintained at 10°C and exclusively exposed to red light, preventing them from forming gametes and undergoing fertilization. Our oldest gametophytes have been cultivated under these conditions since April 2023, steadily increasing their sizes from microscopic to macroscopic. As the gametophytes grow they can be fragmented into numerous clonal copies of themselves. Through fragmentation, the densities and overall volumes of the gametophytes are increased, building up a collection of cultures that can be used in a breeding program.

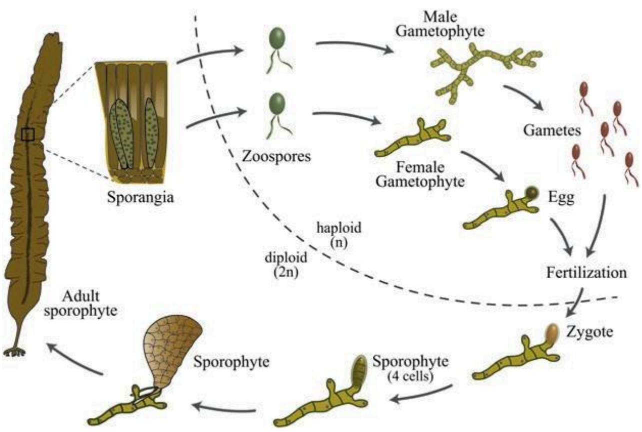

***Figure 5** - Saccharina latissima (sugar kelp) life cycle*

When the gametophytes are eventually removed from the cultivation room they will continue their life cycles: gametogenesis, fertilization, and sporophyte formation. In December 2023, we sprayed a hank with gametophytes that had been cultivated since September 2023. The seeding treatment proved successful, as evidenced by the presence of *S. latissima* sporophytes in January 2024 (see left, **Figure 6**). As the volumes of our gametophyte cultures keep increasing, so do the potential seeding and deployment experiments Running Tide can use this material for.

Ulva fenestrata

Biomass renewal

From May 2023 to September 2023, we experienced numerous random Ulva sporulation events in all our operational PBR systems. This is characterized by an unplanned release of spores from the cultivated biomass. When unplanned sporulations happen in the scale-up, it yields the biomass unusable for further cultivation in PBRs. What we found was that the older the Ulva biomass was, the more likely it was to randomly sporulate. Moreover, we found that the sporulation events seemed to follow the lunar cycle.

To gain better control over when to release spores from the Ulva biomass, the team throughout the summer and autumn of 2023 developed and experimented with a new sporulation protocol that would yield consistent results and viable spore release from Ulva biomass. After developing this protocol we can now generate spores on-demand for substrate seeding or for biomass stock renewal. As mentioned earlier, renewing Ulva biomass during the scale-up is important in order to maintain control over when the biomass sporulates; therefore, the team regularly seeds metal plates with freshly prepared spore solution. These seeded metal plates are then cultivated for four to five weeks. By that time, Ulva seedlings are growing on the plates (**Figure 7**) and are easily harvested by scraping the plates and cultivating the seedlings in 4L PBRs on the cultivation shelves. This freshly renewed Ulva material replaces older algae cultures in the scale-up, thereby ensuring that the scale-up can be effectively run for the next few months. This process is repeated every three months, enabling us to generate repeatable and consistent results.

Figure 7 *- Ulva seedlings growing on steel plate*

Cultivation improvements

<u>*Specific growth rates and CO_2*</u>

Monitoring the specific growth rate and consistency of Ulva thallus serves as crucial indicators of cultivation progress. A steady specific growth rate reflects optimal conditions, while consistency in thallus morphology indicates stability and uniformity in cultivation. Observing higher growth rates alongside consistent Ulva morphology suggests improvement in the cultivation process, signaling successful adjustments to maintain optimal conditions for enhanced productivity and sustainability.

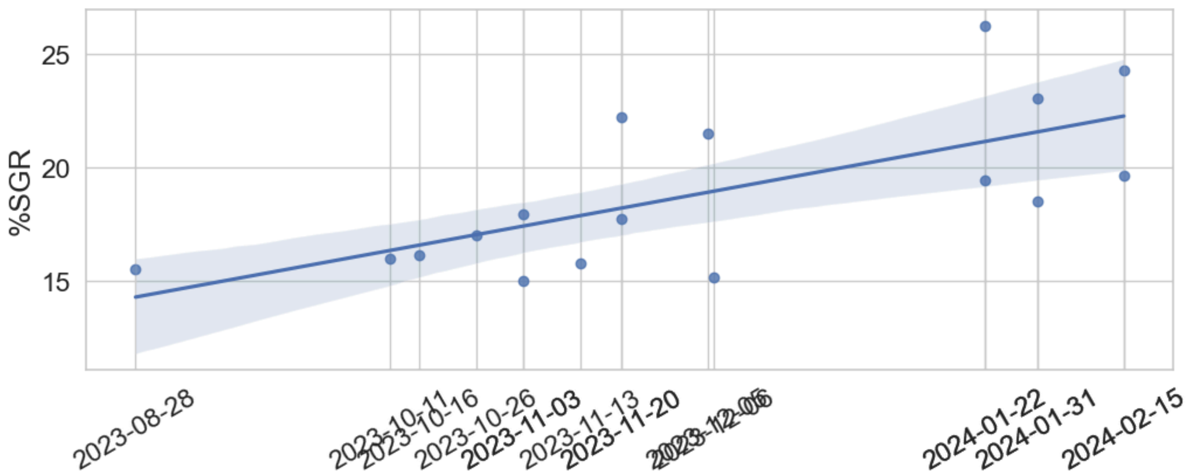

Figure 8 *- Specific growth rates (%SGR) of Ulva in 500L PBRs in Alda from August 2023 to February 2024. The regression line depicts the linear relationship between time and %SGR , indicating the increase in growth rates over time.*

The growth rates of Ulva In Alda have been increasing steadily since its inauguration. For the 500L systems, the average specific growth rate (%SGR) in August 2023 was 14-15% (**Figure 8**). In early 2024, it had increased to an average of 22%. The most significant increase in growth rates was observed when we added controlled CO_2 injections into our PBRs to maintain a steady pH throughout the cultivation period. On average, the Ulva growth rate increased by 33% when CO_2 was added to the PBRs (**Figure 9**). To put this into perspective, this means that in one week we can grow 60% more biomass just by injecting CO_2. Therefore, it would clearly be beneficial for the productivity of Alda cultivation systems to supply all of the larger PBRs with CO_2, as such modification is a low cost and low effort way to increase production capacity, while also gaining increased pH control in cultures.

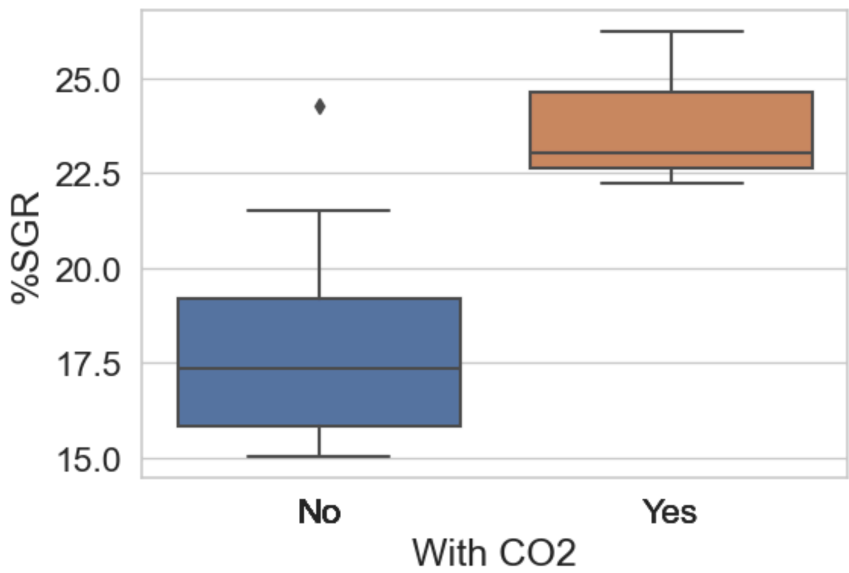

Figure 9 *- Specific growth rates (%SGR) of Ulva with and without CO_2 injection in 500L PBRs in Alda from August 2023 to February 2024.*

PBR carrying capacity

The carrying capacity of PBRs, indicating the maximum sustainable density of cultivated macroalgae, is pivotal for optimizing cultivation. Increasing the carrying capacity allows for greater biomass production within the system, enhancing productivity while minimizing costs associated with resource allocation.

The team has been spending a considerable effort in investigating how much density of Ulva we can achieve in the larger sized PBRs in Alda without having the cultures negatively impacted. The carrying capacity of the PBRs in Alda has been slowly increasing since August 2023. In 2023, the maximum density of Ulva in any of our large PBR systems was just under 0.8g/L. However, in February 2024 we managed to operate one of our 1000L PBRs until the Ulva reached a density of 1.5g/L (**Figure 10**).

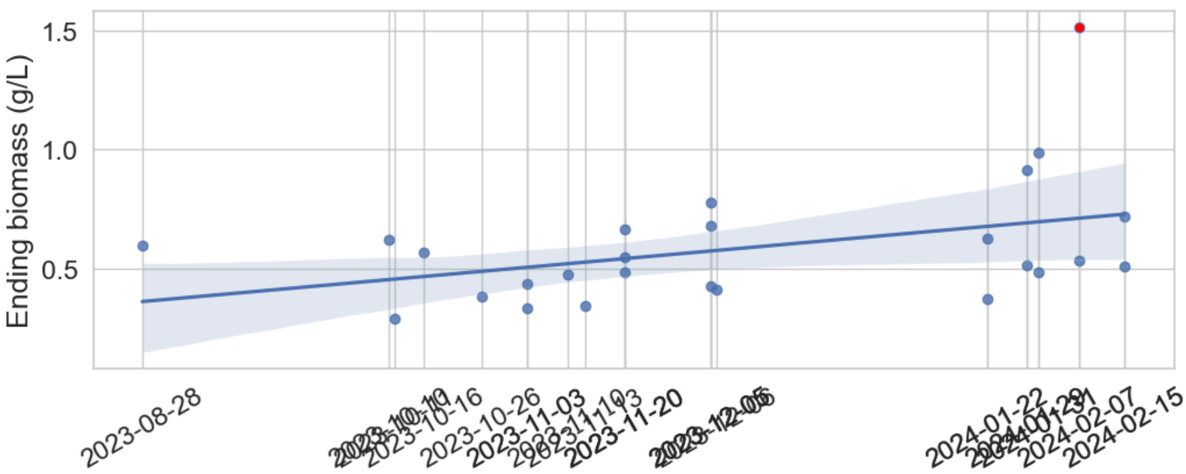

Figure 10 *- Ending biomass density of Ulva in 500L and 1000L PBRs in Alda from August 2023 to February 2024. The regression line depicts the linear relationship between time and the end biomass density in the PBRs, indicating the increase in PBR carrying capacity over time. The red dot at the top right represents the batch with the highest ending density (1.5g/L), which was in a 1000L PBR in February 2024.*

Continuing this investigation involves studying how to optimize biomass density and quality, light intensity, nutrient uptake and availability, CO_2 availability, all together to create the best conditions for high performance of Ulva biomass. The team believes that with further optimization, we can continue the positive trend of increasing biomass densities in our PBRs, and will continue to explore the upper limit of the carrying capacity in 2024.

Controlled sporulation

During Q3 and Q4 of 2023 a considerable effort was made to develop our Ulva sporulation protocol. The work started with an extensive scientific literature review followed by months of experimentation. We found that what gave us the most consistent spore release from Ulva was a combination of: fragmentation of the biomass into fragments in the 3-7mm range, washing away sporulation inhibitors in fresh water and sporulating in room temperature rather than in the cooling rooms.

When developing the sporulation protocol it was a requirement that the process would be scalable, since this potentially would be the last processing step performed in Alda prior to transporting spore solution off-site to seed substrate/buoys. In Q4, SOPs for both small and large scale sporulations were written and published.

Our large scale sporulations are conducted using at least 1 kg, fresh weight, of Ulva biomass that is harvested from the 1000L PBRs and processed according to the sporulation protocol. Then we start the spore release in our 1000L sporulation tank (**Figure 11**).

Figure 11 *- Sporulation of Ulva in 1000L tank*

The efficiency of the sporulation is determined by the total number of spores in the spore solution in relation to the weight of Ulva biomass used. To determine this the team takes samples from the sporulation and manually counts spores using a hemocytometer according to our in-house SOP.

Before the end of Q4 2023 we were able to sporulate our first couple of large batches. These resulted in total spore counts in the range of 50-200 billion spores. That number in itself is difficult to convey without a reference to seeding substrate and the amount of seedable substrate from that spore amount. Therefore, there will be a separate report published during Q1 2024 where the main goal is to give better clarity around exactly this and the implications on scalability and more.

This being a novel process and technology the team is developing, there is still great room for improvements and optimization. The team expects to spend a considerable effort during 2024 increasing sporulation efficiency, as well as on the methodology for spore attachment to substrate.

Spore attachment and growth

When Ulva biomass has been freshly sporulated, with both spore density and spore motility at acceptable levels, the spore solution can be used to seed almost any type of substrate. In most cases the team is focusing on seeding substrate that is a carbon buoy candidate. The two main types of carbon buoys that the team has been focusing on seeding are different types of wood, as well as formed buoys that are being developed in cooperation with Icelandic company BM Vallá (**Figure 12**). Each type of carbon buoy can have their pros and cons, but our task is to demonstrate they are seedable, develop the seeding methodology and expand the company's knowledge on how that process can be further improved and ensure that the process is scalable.

For most of 2023 the Alda team focused on using submersion type seeding of substrate. Submersion seeding utilizes the natural ability of Ulva spores to "swim" and attach to whatever substrate that is available for them to attach to. The spore solution is diluted down to the target spore density with seawater and the substrate of interest is submerged in the spore solution for a designated amount of time. After the designated time of seeding the substrate is removed from the spore solution and placed into cultivation. In 2023 we demonstrated that we can effectively seed the following types of substrates: Wood, BM Vallá formed bricks, cotton and spool twine. Spool twine and cotton serves as a control substrate that helps establish a baseline for attachment and algal growth.

Figure 12 *- Ulva growing on BM Vallá brick*

When growing substrate seeded with Ulva in the lab the plantlet size gets limited by the space/volume of the cultivation. In the figure above the attachment and blade coverage on the BM Vallá brick is very good, but since the Ulva is confined in the relatively small volume of the cultivation setup, the growth of the plantlets gets inhibited by space and competition. However, when increasing the volume of cultivation the Ulva plantlets can grow larger in size as shown in **Figure 13**. The dynamics of where on the surface of substrate/buoys spores attach and develop into Ulva plantlets are complicated and not fully understood. This of course is partially dependent on the light source and where in relation to the substrate the light source is.

Figure 13 *- Long Ulva plantlets*

Though formed substrates such as BM Vallá bricks display great attachment and coverage, they still are lacking in float time which needs to be further improved over time, but certainly should be achievable through substrate development and testing.

As for spore attachment to wooden substrate, in Q4 the Alda team started seeding wooden substrate. In **Figure 14** Ulva is shown growing on wood, while further seeding of a variety of wooden substrates is planned in 2024. It's still important to note that in 2023 the bulk of substrate seeding and attachment experimentation was conducted at Running Tide's Ocean Hub, Portland, Maine.

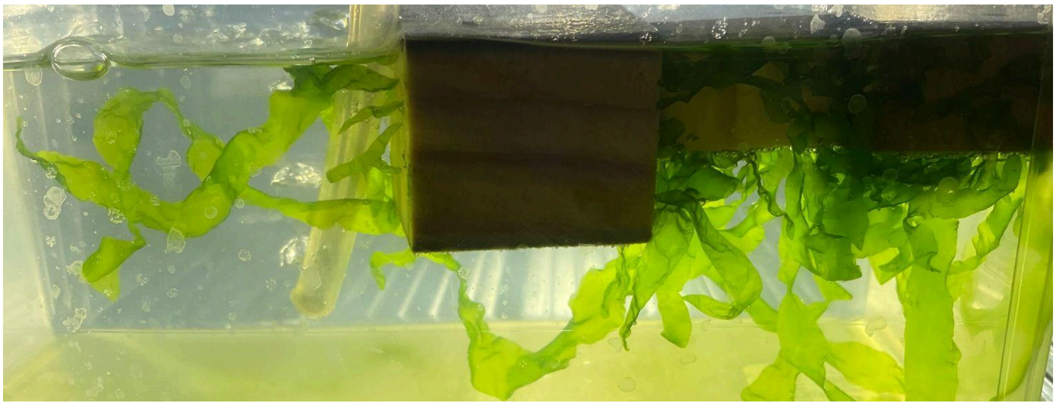

Figure 14 *- Ulva growing on wooden substrate at Alda*

Deployments

In the summer of 2023, Running Tide deployed two open ocean growth experiments (OOGEs) from Iceland to learn and iterate upon our macroalgae research efforts. Both experiments used local *Ulva fenestrata*, cultivated, sporulated and seeded onto cotton substrate inside Alda. One of the highlights of 2023 was the image data that we received from our camera buoys containing these cotton substrates (**Figure 15**). These deployments were conducted in cooperation with Eimskip (Iceland based shipping company) with the goal of developing operational and scientific capability from inoculation to deployment to verification that can inform full-scale macroalgae deployments in future years.

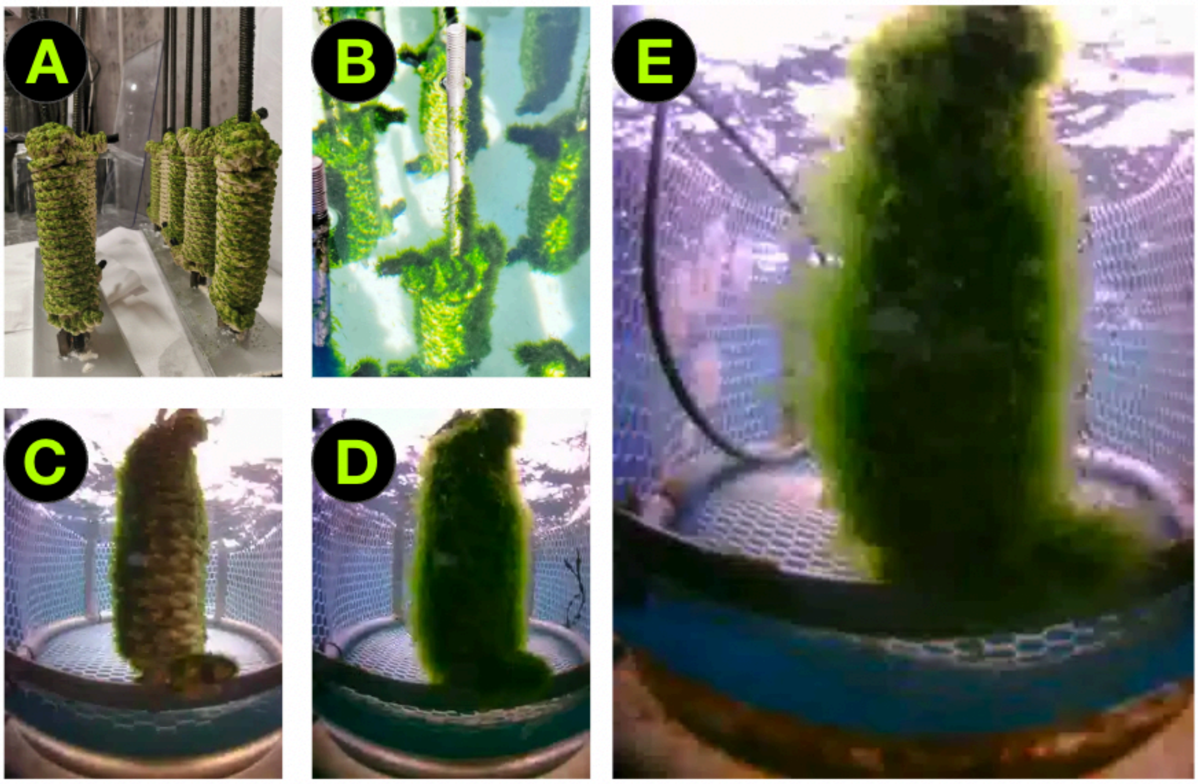

Figure 15 *- Time series of the inoculated cotton hanks (OOGE-3). The dates from left to right are (A) All inoculated cotton hanks, three weeks after inoculation (July 10th). (B) All inoculated cotton hanks, day of deployment (July 19th), (C) camera buoy 2.1, camera 2 one day after deployment (July 20th), (D) camera buoy 2.1, camera 2 three weeks later (Aug. 12th), (E; the large photo) camera buoy 2.1, camera three weeks later (Sept. 5th).*

Image data was received through satellite, showing a visual increase in macroalgae. Quantifying the macroalgae growth in relation to blade area or weight however proved difficult. Overall, we can confidently say that we observed increases of macroalgae coverage on a cotton hank deployed in the open ocean, but can not yet quantify growth and/or carbon content. This marked, to our knowledge, the first time *Ulva fenestrata* or *Ulva lactuca* has been proven to successfully grow in the open ocean.

These successful research deployments establish a baseline performance of our macroalgae product (macroalgae + substrate + operational logistics) in a given oceanic location over time, setting an important starting point in an iterative, multi-year product development cycle and uncovering opportunities for improvement in our system design.

Areas for development at Alda in 2024

Substrate seeding with increased focus on scalability

Scaling up the seeding method for both Ulva and sugar kelp is crucial for the efficiency and sustainability of production. With the need to seed thousands of tonnes of substrate, relying on current methods would be impractical and labor-intensive. Therefore, scalable seeding methods involving automation or mechanization are essential to avoid manual labor and ensure consistent, cost-effective production.

To be able to use automated/mechanized methods of seeding, we need to know how the Ulva spores as well as gametophytes respond to certain stress factors such as heat, dryness, different seeding surfaces etc. This knowledge can only be gained through delicate experimentation. In 2024, the team in Alda will focus on investigating how the spores and gametophytes respond to the following: Usage of binder, extended periods out of IO (torture testing), choice of seeding method (submersion vs. spray-on vs. dipping), and substrate pre-treatment.

Alda capacity increase through biomass cold storage

We estimate that every week, we should be able to produce 6 kg (fresh weight) of Ulva biomass at the Alda facility using four 1000L PBRs. According to our calculations, that should be enough to seed between 444 and 1326 tonnes of substrate every week (**see "Current and predicted macroalgae seeding capacity" report for further detail**). However, we are not deploying substrate every week, and therefore we have been investigating approaches where we could store Ulva biomass (for 1-4 weeks) that is not used for spore production upon harvesting from the 1000L PBRs. Furthermore, we are investigating whether the stored biomass maintains its ability to produce healthy, motile spores, and subsequently whether the spores maintain their ability to attach, germinate and grow into adult Ulva thallus. If successful, this would allow us to accumulate biomass over time and induce the sporulation of 12 to 24 kg of Ulva biomass at the same time, and multiply our capacity 2-4x.

Preliminary results have shown that the spore density resulting from induced sporulation of the stored biomass does not decrease over time. However, we remain to see the effects on the attachment and viability of the spores.

Improving CO2 injection approach

The CO_2 injections in our PBRs were performed manually. In brief, we adjusted the CO_2 injections according to offline measurements of pH in the system. The reference pH was 8.2, and if the pH was rising too fast, we increased the CO_2 injection, and if it was lowering too fast, we decreased the CO_2 injection. Obviously, this approach is very simple and limited in its ability to regulate the pH. We often see the pH drop below 7, which is undesirable. The pH regulation can be improved significantly by introducing a control system (e.g. Agrowtek) that does the CO_2 injection automatically. This way, the pH could be maintained within a relatively small range (8.1 to 8.3) and we would possibly see an even greater improvement in growth rates.

Determination of PBR carrying capacity

As previously mentioned, we aim to further investigate the Ulva carrying capacity of the PBRs in Alda. We suspect that by increasing the light intensity, coupled with improved regulation of pH through CO_2 injection, we can achieve higher densities.

The team continues to work on expanding our sugar kelp gametophyte culture capacity, thereby also increasing the substrate seeding capabilities, however those efforts don't scale as fast as those of Ulva spores. Equipment for scaling gametophyte cultures to 1000L scale is on-site in Alda. Reaching the 1000L scale is a goal for the team, but whether that is achievable in the year 2024 remains to be seen.

Modeling cultivation process for optimization

In January 2024, we developed a method in our UV-Vis spectrophotometer to accurately quantify nitrate concentrations in our PBRs. As we gather more data on the interaction dynamics of biomass, nutrient concentration, pH and light intensity in the coming months, we will have the opportunity to develop a process model. This process model will be capable of describing the Ulva biomass production in our PBRs, given a predetermined starting density, feeding regime and cultivation duration. We could determine the process design in which biomass production is maximized. Work has already started on this model.

Conclusion

Looking back at the year 2023 at Alda and reviewing the work that took place, the team was able to execute a difficult task of building, starting up and running a pilot scale macroalgae production and research facility, successfully. The team encountered unforeseen issues and roadblocks, but was able to overcome those challenges, and gained an increased in-depth understanding of the biological processes at hand. There are still many unanswered questions regarding important aspects of the macroalgae technology Running Tide is developing and optimizing the processes within it. However, now that Alda can effectively supply seedstock for open ocean macroalgae deployments at larger scale than previously available at Running Tide, the focus for the year 2024 will shift towards how Running Tide can utilize these capabilities effectively at scale.
During Q4 2023/Q1 2024 the Alda team produced numerous SOPs in an effort to standardize the work conducted at Alda (**see Appendix I**).