**ARPA-E Quarterly Technical Report**
**Award - DE-AR0001559**
**Quarter 3 (February 1, 2023 – April 30, 2023)**

**Project Title: Quantifying the Potential and Risks of Large-Scale Macrophyte Cultivation and Purposeful Sequestration as a Viable CO2 Reduction (CDR) Strategy (SeaweedCDR)**

This is the third quarterly report for the SeaweedCDR project, covering the first three months of the project timeline (February 1 – April 30, 2023). We have milestones and issues to report. We have an issue with completing SOPO 2.1 (Design & Implement Seaweed Packaging) as planned due to the lack of giant kelp biomass available for purchase (as discussed in our email on April 14, 2023). We can work on small samples (several kg) that are diver collected, but larger bales (> 100 kg) are not available. In the meantime, kelp abundance is rebounding quickly and we expect it to be sufficiently recovered to start implementation in summer 2023.
All other work is being conducted successfully as planned.

## Task 2 – Quantification of Seaweed Biomass Fates
### M2.1: Design and Implementation of Seaweed Packaging - 75% completion

Task M2.1 is to develop and implement at least 4 packaging and conveyance methods to deliver giant kelp biomass to the seafloor where it has an increased chance to be sequestered. During the winter 2023, a series of major storms hit the west coast of United States, and associated wave disturbance greatly reduced the density of giant kelp beds in the Santa Barbara region to very small and sparse patches. Reports from UCSB scientific divers and the kelp harvesters employed by The Cultured Abalone (our source of large quantities of kelp), have corroborated these observations. Thus, the implementation component to this task is temporarily impacted, as we are unable to perform large scale field experiments without adequate amounts of available kelp. Nevertheless, we have designed 4 packaging techniques that will be either conducted in the laboratory or field, or a combination of both.

These designs have been described and their potential efficacy vis-à-vis conveyance (kelp vertical transport) and durability, are discussed in detail in our draft white paper. We intend to finalize and submit the white paper to an online preprint server (likely EarthARXiv.org) in the next quarter. The publication of the white paper in a preprint server will give our white papers a Digital Object Identifier (DOI) and because of that will be searchable on publication archives (such as Google Scholar). The white papers will serve as frameworks for manuscripts that will be submitted to peer reviewed journals in the near future. The contents of the white paper are briefly summarized below.

The Conveyance white paper evaluates four methods (Table 1), both in the laboratory and in the field, for conveying harvested giant kelp biomass to the deep ocean: 1. Natural sinking - floating biomass is released and allowed to sink on its own, 2. Mastication – biomass is shredded and released to sink according to its buoyancy, 3. Short-depth pumping – kelp is pumped to a shallow depth (50 - 100 m) where rapid increase of hydrostatic pressure leads to negative buoyancy, and 4. Baling - harvested kelp is compressed, baled, and placed on the seafloor.

The optimal sinking method will minimize decomposition rate and release of DOC in the upper water column while maximizing sinking speed (and eventually minimize processing cost and handling time). Importantly, careful consideration must be placed in the quality of MRV for each method. The measured sinking rates, DOC release rates, and decomposition rates will serve as parameters for the simulated conveyance by each of the different sinking methods.

**Table 1.** Experimental matrix showing how we will investigate DOC release (mmol C $m^{-3}$ $s^{-1}$), sinking rate (m $s^{-1}$), and decomposition rate of kelp biomass (mmol C $m^{-3}$ $s^{-1}$) for each sinking method. Lab means laboratory and/or mesocosm experiments. The translation between measured rates and model parameters will be coordinated based on the experimental design.

| Sinking Method | DOC Release Rate | Sinking Rate | Decomposition Rate |
|---|---|---|---|
| **Natural Sinking** | Lab | Lab | Lab |
| **Mastication** | Lab | Lab | Lab |
| **Short Depth Pumping** | Lab | Lab/Field | Lab/Field |
| **Baling** | Lab | Field | Field |

The white paper details the potential advantages and disadvantages of each method type. Due to the challenges of tracking the transport, DOC release, and decomposition rates of floating (*natural sinking*) or masticated kelp biomass in the field, we will conduct experiments to determine these rates, and the environmental variables that drive them, in a laboratory or mesocosm setting. However, kelp baling (and some aspects of short depth pumping) have the ability to be assessed in the field. Kelp bales will be weighted to enhance rapid conveyance to depth and minimize DOC release into the shallower water column and be equipped with various sensors (e.g. dissolved oxygen, pressure loggers) to monitor degradation, sinking rates to validate the strategy.

As previously introduced in earlier quarterly reports, we are planning to sink a larger volume/weight of kelp in the marine environment using slinky traps as the representative kelp bale. For this we have completed the construction of a mooring array that consists of a surface sparge buoy with a GPS tracker, a surface buoy, approximately 150 meters of line, slinky trap equipped with dissolved oxygen sensors and pressure logger, and steel rebar (30 lbs each) as the anchor (**Figure 1**). The mooring array can be expanded to reach deeper depths if needed for future deployments. While we await the recovery of local kelp beds for harvesting, we plan to deploy the mooring array without kelp in the slinky trap, to test and fine-tune the setup and deployment of the mooring and adjust our strategy if needed before future deployments with kelp.

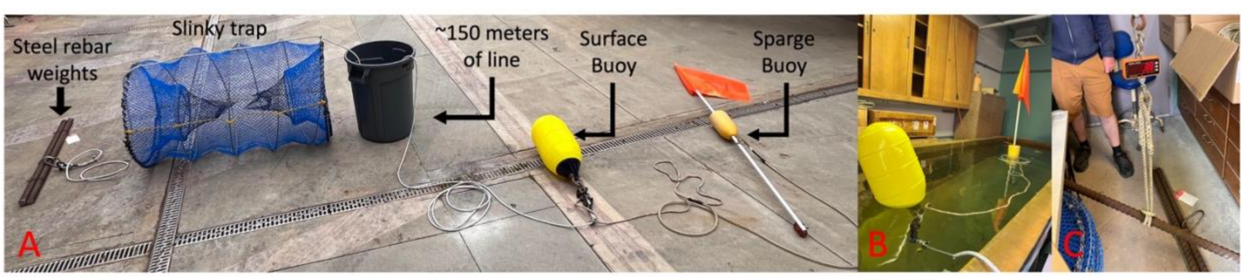


**Figure 1.** A) Picture of the constructed mooring array that consist of the steel rebar anchor, slinky pot, 150 meters of line, a surface buoy, and a sparge buoy; B) Testing of surface and sparge buoy in seawater tank; C) Weight of an individual steel rebar segment that will be used to weigh down/anchor the kelp package.

## Current and planned activities

While awaiting the recovery of the local giant kelp beds, we are directing efforts towards other subtasks (M2.2, M2.3, M2.5, and M2.6). As part of M2.2 (Seaweed Biomass Fates Methods Development) we are developing and documenting methods for upcoming laboratory experiments to assess kelp degradation rates and the production of other biogeochemical biproducts. The experimental conditions will also assess the degradation of the kelp biomass in oxic vs. anoxic conditions, incubated at ambient pressure (1 atm) and under hydrostatic pressure (~100 atm), and in the presence of sediment and sediment microbial communities (M2.3, M2.5 and M2.6).

For M2.2, M2.3, M2.4 and M2.5 we will be using Kapak plastic (trilaminate polyester/polyethylene) sample bags (Fig. 2). These transparent plastic bags allow researchers to inspect any changes of the kelp and seawater over time (Fig. 2A), and will be equipped with a stopcock valve, allowing for samples to be acquired throughout the duration of the incubation without rebalancing the removed liquid with a volume of gas (Fig. 2B). These bags are also ideal for future pressure chamber experiments that we plan to conduct as described in previous quarterly reports. Recent tests with Kapak sample bags, containing $N_2$ sparged water or sulfidic water (without kelp), indicate the sample bags are impermeable to gas exchange and are clean from any DOC contaminants and are thus ideal tools for conducting experiments for M2.4, M2.5 and M2.6.

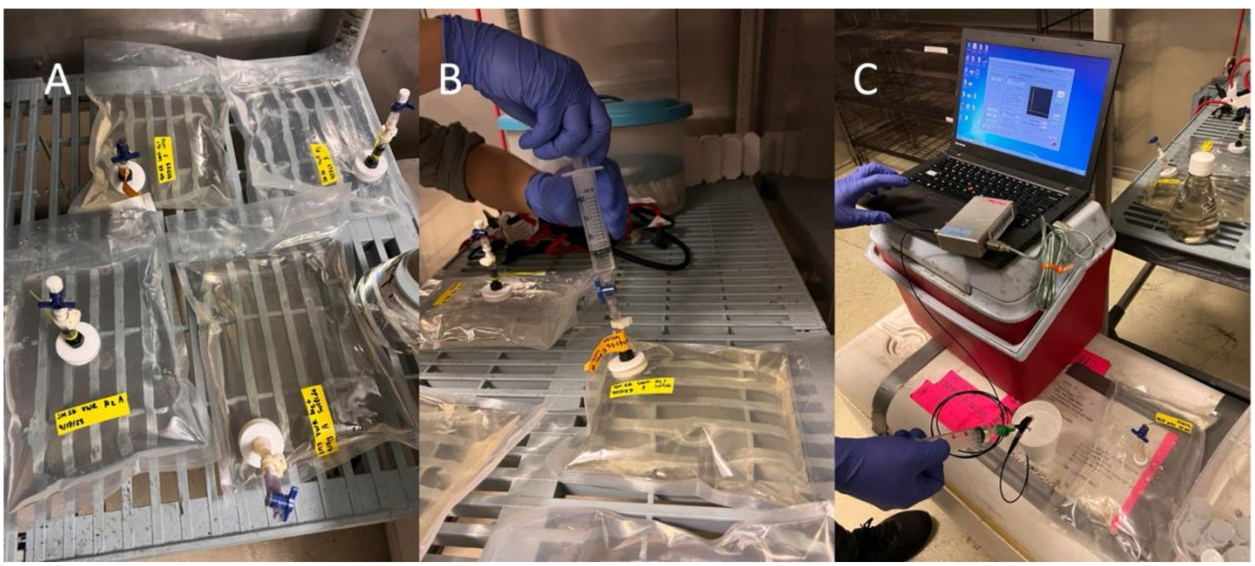


**Figure 2.** A) Photo of heat-sealable Kapak plastic sample bags, equipped with a stopcock valve for sampling liquids; B) Photo demonstrating the sampling of bags with plastic syringe; C) Photo of dissolved oxygen measurements using a Pyrosense dissolved oxygen logger, equipped with a flow-throw optode.

Although the current status of local giant kelp beds have limited our ability to do larger-scale work, we are able to obtain smaller quantities (~15-20 lbs) of giant kelp from UCSB divers. These smaller quantities of giant kelp will be sufficient to conduct smaller scale laboratory experiments with the Kapak sample bags. Over the next quarter, we will begin sample bag incubations with kelp components (stipe, blade and pneumatocysts) and monitor the kelp degradation rates and production of other biogeochemical biproducts. *In situ* seawater will be obtained from seawater intake lines that collect seawater offshore of UCSB at Campus Point from ~20 meters depth. This raw seawater will be used to measure kelp decomposition under oxic conditions.

Recently we have been offered a unique opportunity to sample the anoxic seawater from below the sill depth within the Santa Barbara Basin (>475 meters), by joining a previously funded research cruise on board the *R/V Atlantis*, which will be equipped with *HOV Alvin*. That cruise will be conducted within the Santa Barbara Basin this upcoming summer (June 22$^{nd}$ – July 12$^{th}$ 2023) (**Figure 3**). On this expedition we plan to collect *in situ* anoxic seawater using the CTD rosette on board the *R/V Atlantis*. Prior to the research cruise we will harvest small quantities of giant kelp and transport it to the *R/V Atlantis*. We will keep the giant kelp fresh during the cruise, on the deck of the *R/V Atlantis* in an open cooler equipped with a flow-through seawater line similar to what is shown in Figure 3B. We will then dissect kelp fronds as needed for our sample bag incubations which will be prepared and monitored on board similar to the sample bag preparation described earlier.

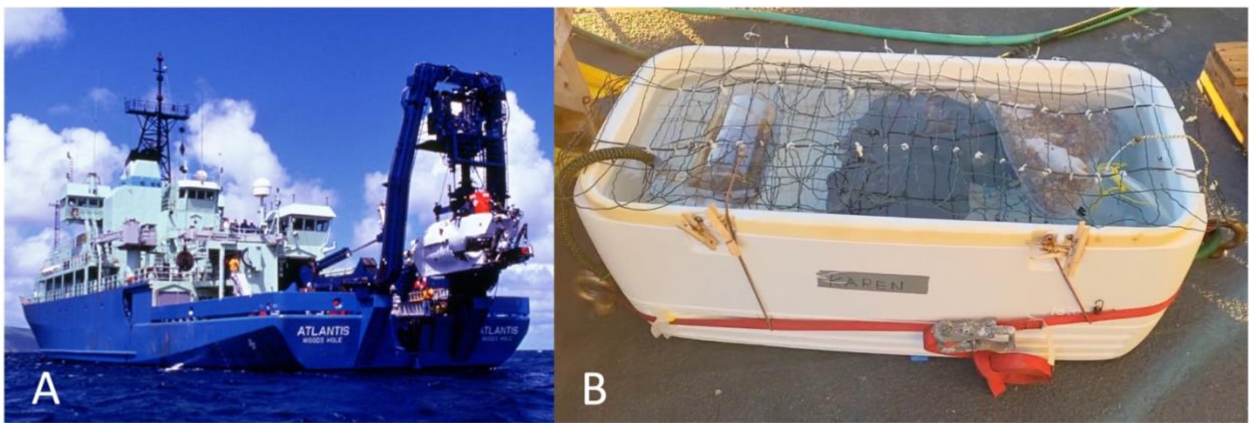

**Figure 3.** A) Picture of the *R/V Atlantis* during *HOV Alvin* operations, photo credit: Woods Hole Oceanographic Institute. B) Example of open cooler equipped with hoses delivering and draining seawater to keep live kelp during a previous research cruise, photo credit: Dani Cox, PhD candidate in Valentine lab, UCSB.

In addition to the sample bag experiments on board, if the giant kelp beds recover before the beginning of the summer research cruise, we will attempt to deploy larger kelp bales in the slinky traps shown in **Figure 1** at locations within the Santa Barbara Basin that are near the targeted sampling stations. Ideally, we would deploy up to two kelp bales in water depths between 475 and 585 meters, 10-15 days prior to the cruise. We would then use *HOV Alvin* to find the kelp bale(s), take video recordings to assess the condition of the remaining kelp, record and document macrofaunal interactions and document and sample the surrounding seafloor characteristics (M2.5). *HOV Alvin* would also be used to aid in kelp bale recovery back onto the deck of the *R/V Atlantis* for sample collection and further assessment of kelp degradation including biomass and carbon loss. Logistics of the operations with *R/V Atlantis* and *HOV Alvin* are currently in progress, and we will be convening with the *HOV Alvin* team to discuss our proposed kelp bale experiments.

**Task 3 – Quantification of Seaweed DOM Fates**
**M3.1: Seaweed DOC Fates Methods Development**
Completion level - 100%

We have completed our DOC fates measurement protocols white paper. It will be published shortly on an online preprint server (likely EarthARXiv.org) similar to the Conveyance document described previously. In the following, we summarize these our protocols to measure and model the release rate of DOC, its composition and remineralization rate. Additionally, we report on preliminary results showing these protocols meet the criteria defined in SOPO 3.2. Release rates from 6 replicate blades show a significant positive relationship with light and primary production. The composition of DOC release from 8 blades show that carbohydrates and polyphenols are 54% of the DOC. Lastly, we define methods to determine the sequestration potential of seaweed DOC based on its molecular properties, community composition and environmental variables (nutrients, temperature, light).

*Seaweed Incubation Design for DOC Release Rate Determinations* - Giant kelp is too large to be entirely enclosed for incubation, therefore single blade incubations are used to measure DOC release. Blades are clipped where the pneumatocyst meets the stipe to prevent damage to the lamina. Work with other laminarin seaweeds demonstrate no significant difference in DOC release rates when using clipped blades versus whole plants (Weigel and Pfister 2021). Similarly, our preliminary work with mature, clipped giant kelp blades show DOC release rates from 1.7 – 14 µmolC $g_{DW}$ $hr^{-1}$, comparable to rates observed by (Reed et al. 2015) (0 – 12.5 µmolC $g_{DW}$ $hr^{-1}$) who used sleeved incubations of giant kelp blades without clipping. Mature blades (2m from the growing tip of a frond) are collected from Mohawk Reef in Santa Barbara, California and transported back to a nearshore laboratory in surface seawater and placed in 7L acrylic incubation tanks with 0.2 µm filtered seawater and magnetic stir bars to maintain flow. Incubations are conducted in triplicate at six different light levels for two hours. Blades are allowed 20 minutes to acclimate to the incubation in order to prevent sampling of exudation driven by rapid changes to light, temperature or salinity (Carlson and Carlson 1984; Zhao et al. 2023).

*DOC and DON Analyses* - Detailed information on the best practices for DOC and DON sample collection, storage and analyses can be found in Halewood et al. (2022). Briefly, duplicate samples for DOC and DON are taken from the beginning and end of each incubation. Samples are filtered through pre-combusted 25 mm GF-75 (nominal pore size of 0.3 µm) into pre-combusted 40 ml EPA vials with PTFE lined caps and acidified to pH < 3 with the addition of 60 µl of DOC clean 4N HCl. At least 20 ml of sample should be flushed through the GF-75 filter and vials should be rinsed three times with sample before being filled ~¾ full. DOC concentrations are determined by the high temperature combustion method using a Shimadzu TOC-V or TOC-L. Total dissolved nitrogen (TDN) is determined on the same instruments but with a detector for NO by chemiluminescence. DON is calculated as the difference between TDN and dissolved inorganic nitrogen so measurements for nitrate + nitrate are required.

DOC & DON exudation rates are calculated as follows:

$$\mu molC/N \; g_{DW} \; hr^{-1} = \frac{[DOC/N]_t - [DOC/N]_0 * V}{T * M} \qquad (1)$$

where $[DOC/N]_t$ and $[DOC/N]_0$ are the DOC or DON concentrations in µmol C or N $L^{-1}$ at the end and beginning of each incubation, respectively. V is the volume (liters) of seawater during the incubation, T is the incubation time (hours) and M is the dry weight (grams) of the seaweed tissue used in the incubation.

*Photosynthetic Rate Determinations* - Samples are obtained simultaneously with DOC during incubations by overflowing a 125 ml glass serum bottle with seawater at least twice using silicon tubing placed at the bottom of the bottle. Samples are preserved with 100µl of saturated $HgCl_2$ (1g $HgCl_2$:10ml deionized water). The sample is sealed with a small headspace (<1% total sample volume) with a rubber stopper and aluminum crimp seal and stored at room temperature until analysis. DIC samples are analyzed using and automated infrared inorganic carbon analyzer (AIRICA) designed by Marine Analytics and Data (MARIANDA). Briefly, 1.75 ml of sample is injected into a stripper where it is then acidified with 10% $H_3PO_4$. Liberated $CO_2$ is then carried using an inert reference gas ($N_2$) into a LICOR-7000 that measures $pCO_2$ against a reference cell. A condenser, nafion drying tube and magnesium perchlorate trap are used to remove moisture from the sample before it enters the LICOR cell. Samples are injected 4 times and an average of 3 are used to determine the concentration. The average $pCO_2$ peak area is converted to µmol C $L^{-1}$ using a coefficient calculated from a certified reference material (Dickson et al., 2007). Reference materials are run every 12 samples to check for analytical stability throughout a run. Photosynthetic rates are determined as follows:

$$\mu\text{molC } g_{DW}\ hr^{-1} = \frac{[DIC]_0 - [DIC]_t * V}{T * M} \qquad (2)$$

where $[DIC]_0$ and $[DIC]_t$ are the DIC concentrations (µmolC $L^{-1}$) at the beginning and end of each incubation, respectively. V is the volume (liters) of seawater during the incubation, T is the incubation time (hours) and M is the dry mass (grams) of the seaweed tissue used in the incubation.

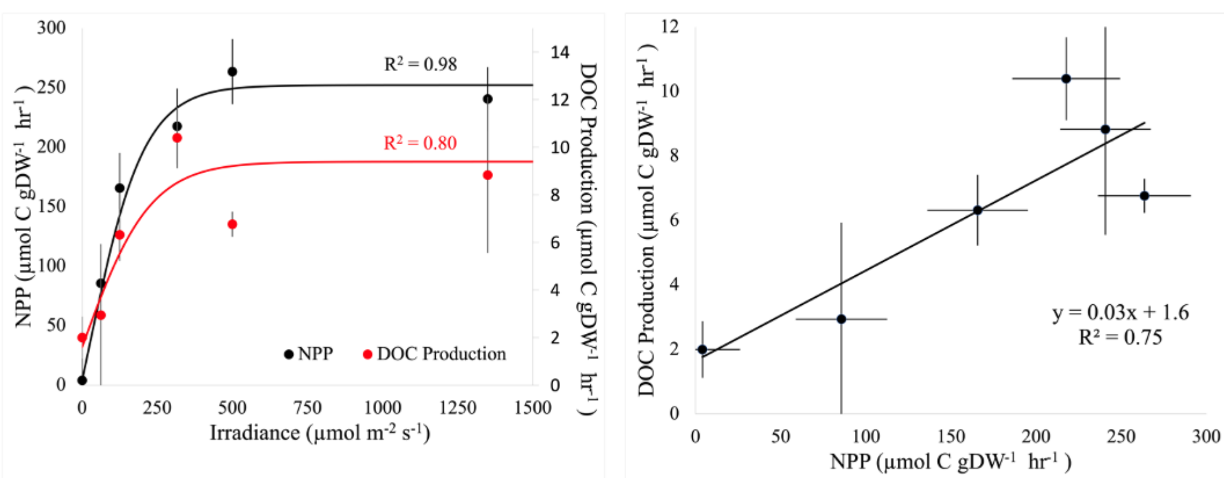


**Figure 4.** (a) DOC production and NPP light curves. Solid lines are modeled NPP and DOC production using a hyperbolic tangent fit. $R^2$ values represent how well the model fit compares to measured rates (solid circles). (b) The relationship between NPP and DOC release. Inset is results of model II linear regression.

*Preliminary Incubator Results* - Our incubation design captures a typical relationship between NPP and light intensity and that DOC release rates show a similar relationship to light as NPP (Figure 1a). DOC release increased linearly with NPP, but proportion of NPP released as DOC was consistent (~3 ± 0.8%) across all light levels when irradiance > 0. Lastly DOC release continued when irradiance was 0. Future work will evaluate whether the fraction of NPP released as DOC is regulated by nutrient concentrations and blade stoichiometry and age.

*Seaweed DOC Composition* - We are focusing on two classes of compounds: Carbohydrates and Polyphenols which are large components of seaweed DOC (Jennings and Steinberg 1994; Abdullah and Fredriksen 2004; Wada et al. 2007; Nelson 2013; Powers et al. 2019). Total carbohydrates are measured using the TPTZ method (Myklestad et al. 1997) and individual sugar monomers are measured by HPAEC-PAD (Engel and Händel 2011). Total phenol content is measured using the Folin-Ciocâlteu method (Box 1983; Takeda et al. 2013; Powers et al. 2019).

Using the incubations described above, accumulated DOC are collected and characterized using High Performance Anion Exchange Chromatography with Pulsed amperometric detection (HPAEC-PAD) and the Folin-Ciocâlteu colorimetric method for neutral sugars and polyphenols, respectively. From the incubations of 8 blades, neutral sugars and polyphenols were responsible for 30 ± 21% and 24 ± 16% (54% total) of the accumulated DOC. Specific sugars are dominated by fucose and galactose and has been linked to fucoidan (Buck-Wiese et al. 2023). HPAEC-PAD is not capable of detecting the entire carbohydrate pool and is likely an underestimate (Dean Pakulski and Benner 1992; Engel and Händel 2011). The TPTZ method will likely increase the amount of DOC characterized. Our work with *Sargassum*, another brown macroalgae show that carbohydrates detected by HPAEC-PAD are 50±10% of total carbohydrates detected by the TPTZ method.

*Remineralization Bioassays* - Remineralization bioassays are essential to quantify the fraction of accumulated seaweed DOC that is bioavailable to microbial remineralization at surface versus how much survives degradation and is available for export during physical mixing (i.e. export potential). In addition, microbial remineralization experiments with mesopelagic microbes (>100m) and accumulated DOM will investigate its bioavailability to mesopelagic bacterioplankton which are thought to specialize in degrading recalcitrant DOM (Carlson et al. 2004; Landry et al. 2017; Saw et al. 2020; Liu et al. 2020). Remineralization culture experiments are set up as seawater dilution cultures in which a source microbial assemblage (1.2 µm filtrate) is inoculated into a naturally occurring seawater media (0.2 µm filtrate) (Carlson et al. 2004). The cultures are incubated at *in situ* temperatures (maintained in upright incubators) in the dark for one year. Samples monitoring changes in bacterioplankton abundance, DOM concentrations, total carbohydrates, sugar concentrations and total phenol content are collected from incubations in periods from days to one year. Changes during the initial growth phase will be used to determine bacterial growth efficiency (BGE), obtained from concomitant changes in DOC concentration and bacterial carbon (Wear et al. 2015; Stephens et al. 2023). DNA samples are also collected to assess changes in microbial community composition. Changes in DOC composition will determine what compounds produced by seaweed may contribute to sequestration on longer time scales.

*Photomineralization* - A handful of studies demonstrate that a fraction of accumulated seaweed DOC is rapidly oxidized when exposed to light (Shank et al. 2010; Wada et al. 2015). This photooxidation occurs alongside the degradation of seaweed DOC optical properties indicating polyphenols may be degraded when exposed to light (Powers 2020), limiting their potential to contribute to sequestration. Photooxidation experiments should be conducted to determine the fraction of DOC that is rapidly oxidized in the surface ocean. Additionally, the production of partial photooxidation products such as CO should be monitored as they it can affect atmospheric redox chemistry (Valentine and Zepp 1993; Conte et al. 2019; Ossola et al. 2022). Photomineralization experiments are conducted in 15-20 ml quartz vials using a LS1000W Solar Simulator (Solar Light CO. Inc). Briefly, seaweed DOC is collected and filled into quartz vials with PTFE lined caps. Experiments should be conducted at seawater salinity and pH. Samples are placed in a circulating water bath to maintain temperature and irradiated for 48 hours. Vials are sampled sacrificially at 0, 24 and 48 hours to resolve the timescale of photooxidation and are subsampled for DOC concentrations, optical properties (CDOM), total phenol content, dissolved inorganic carbon (DIC) and carbon monoxide. DOC, CDOM, TPC and DIC are measured as described above. Carbon monoxide is measured following Ossola et al. 2022 in which samples are irradiated in custom 20 ml quartz serum vials without headspace and closed with Teflon lined rubber seals. CO is measured by transferring 6ml of sample to a $N_2$ flushed serum vial using a disposable syringe. Samples are shaken for 20 minutes and allowed to come to room temperature before analysis on an SRI 8610C gas chromatograph and calibrated using a CO standard balanced with $N_2$.

**Task 4 – Modeling the environmental impacts of seaweed cultivation and sequestration**

There are no modeling SOPOs due this quarter. We continue parallel development of an offline MAG model (Task 4.3) and the online coupled ROMS-BEC-MAG modeling system (Task 4.4). For the former, we have developed a MATLAB code that updates and simplifies aspects of the original MAG model (Frieder et al. 2022). For the latter, we are developing a prototype idealized channel-flow simulation with prescribed nutrient fluxes. Our initial target (in collaboration with MARINER) is a paper that introduces ROMS-BEC-MAG with a demonstration of modeling capabilities (simulate C conveyance and resolve upper-ocean interactions between kelp, currents, and biogeochemistry) and a quantification of upper-ocean farm impacts and C conveyance in the idealized configuration.

We have simplified and updated the MAG model of Frieder et al. 2022 (Task 4.3), in coordination with C. Frieder, K. Davis, I. Arzeno-Soltero, and others. A primary simplification is the elimination of frond tracking (initiation, senescence), which is computationally cumbersome and dynamically non-conservative (relative to its coupling with BEC). The growth and mortality rates associated with the frond tracking will now be parameterized in the bulk growth and mortality terms in MAG (see Frieder et al. 2022). We have also tested various choices for the vertical distribution of biomass. Preliminary sensitivity tests (with constant nutrients, light, currents, and waves) indicate a marginal sensitivity to this choice. We have provisionally settled on an inverse linear power law shape function for the vertical biomass distribution. We are coordinating with C. Frieder and SBC LTER (N. Eegholm, R. Miller, T. Bell, D. Siegel) to further inform this choice based on observations of giant kelp biomass as a function of its height.

The updates to MAG described above are also being implemented in the online coupled, fortran code (ROMS-BEC-MAG). The test-bed for the coupled code is an idealized channel-flow simulation. The idealized configuration consists of a ~ 76 km x 76 km x 1500 m domain, with ~300 m horizontal resolution with a ~10 km x 10 km farm in the middle of the domain. We prescribe a surface layer (0 - 200 m), geostrophically balanced velocity (~0.1 m/s, representative of a typical mesoscale surface current) and surface wind stress. The latter will sustain the parameterized boundary layer turbulence. The next steps for the development of this idealized simulation include: defining the nutrient initial condition and boundary fluxes and implementing a preliminary vertical conveyance model. We will represent kelp DOC as an additional tracer in ROMS-BEC-MAG with a prescribed rates of sinking, decomposition, and remineralization (ultimately informed by experiments from Tasks 2 and 3).

**Task 6 – Tech to Market**
**M6.4: Website – 90% completion**

The project website (seaweed.eri.ucsb.edu) is complete and is undergoing final checks by the IT team at ERI. The website will go live during the week of May 1, 2023. The website currently contains four pages: a detailed 'About' page that summarizes the project (Fig. 5), a 'Team' page that provides biographies for all team member, a 'Projects' page that details the goal and plan for each task, and a 'Publications' page that currently hosts our approved quarterly reports and will soon host white papers and peer-reviewed publications for the project.

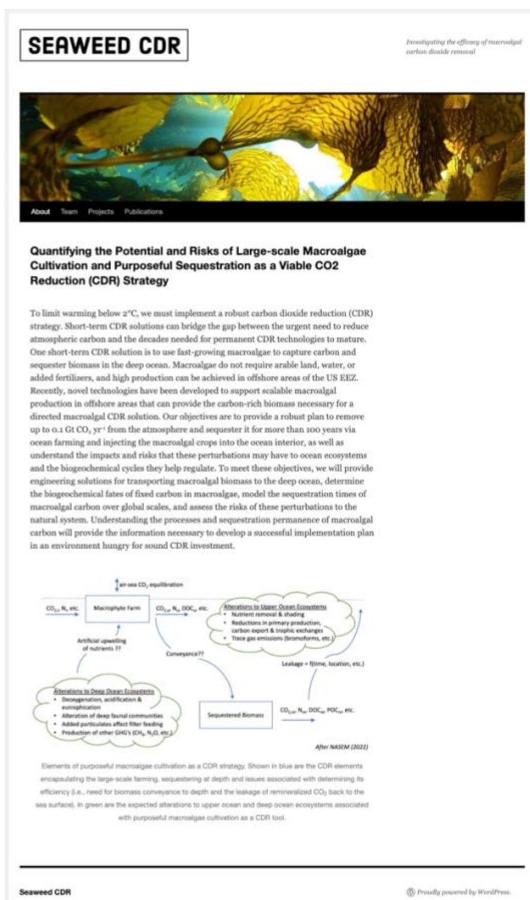


**Figure 5.** Landing page of the Seaweed CDR project website.

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