Methods for assessing Giant Kelp (*Macrocystis pyrifera*) biomass sinking rates and decomposition for carbon dioxide removal applications

ARPA-E Seaweed CDR Project (UCSB, WHOI, UCLA)

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December 6th, 2023

This is a non-peer reviewed preprint submitted to EarthArXiv

Abstract

One promising carbon dioxide removal (CDR) strategy to achieve negative emissions of carbon dioxide is the sinking of farmed seaweed into the ocean where it may be sequestered. Methods to quantify the sinking rates, degradation rates and the release of degradation products from kelp is fundamental to understanding the durability of the strategy if applied to scale. The aim of this white paper is to describe the methods we plan to use to determine the sinking rates, quantify kelp degradation and the release of kelp degradation products such as particulate and dissolved organic carbon, as well as dissolved inorganic carbon. The methods design is guided by previously published methods and considers the type of packaging and conveyance of kelp biomass as well as, the necessary parameters needed to model this CDR strategy to scale. This white paper will show our planned approach includes the combination of both laboratory and field-based experiments to determine each of these parameters, and provide foundational guidelines in future work to test the durability of this CDR strategy.

1. Introduction

A promising marine carbon dioxide removal (CDR) strategy to reach net zero emissions of carbon dioxide in the atmosphere is the rapid placement of large quantities of farmed kelp biomass to the deep sea and/or marine sediments (National Academies of Sciences, 2021). Various methods to package and convey kelp (*Macrocystis pyrifera*) biomass to the seafloor are under consideration including natural sinking, mastication, pumping, and baling (Krause et al., 2023). The robustness and durability of any of the conveyance methods is dependent on the

ability to monitor, report, and verify (MRV) the sinking rates of harvest kelp biomass, the decomposition rate of the kelp biomass, and quantify the degradation products.

Detached or eroded kelp biomass, from either natural kelp forests or from artificial farms, can be transported long distances (~1000 km) and to great depths (i.e., 1000's meters) over reported time periods of months (Broch et al., 2022; Smale et al., 2022; Harrold et al., 1998; Hobday, 2000a). As kelp biomass is exported from its source, it breaks down through abiotic (e.g., mechanical damage, photodamage, and high temperature) and biotic (macro and micro – organism grazing) mechanisms (Rothä Usler et al., 2011) releasing particulate organic carbon (POC) and dissolved organic carbon (DOC). The oceanographic community historically defines POC as all combustible organic carbon that can be collected on filters. The most common filter pore sizes used to determine POC are 0.7, 0.8 or 1.0 μm (Kharbush et al., 2020). Here, we classify the POC release from kelp degradation into two forms: 1) small POC (SPOC) with a bracketed size range between 0.3 μm – 8.5 mm and 2) large POC (LPOC) that are > 8.5 mm (see details of select size range in section 4.1). DOC is historically characterized as organic carbon that is < 0.2 μm (Kharbush et al., 2020; Wagner et al., 2020; Halewood et al., 2022), which we have also adopted for our work to characterize DOC from kelp biomass degradation.

Released POC, due to kelp degradation, is an important food source for marine microbial communities which can further break down the larger POC fragments into other forms of POC and DOC. The more labile forms of kelp derived POC and DOC is further remineralized by heterotrophic microorganisms back to carbon dioxide (Feng et al., 2022), while the remaining POC and DOC can settle on the seafloor where it can be further degraded by the benthic food webs and/or buried and sequestered (Krumhansl and Scheibling, 2012; De Bettignies et al., 2020; Abdullah et al., 2017). Remineralization of POC and DOC that occurs within the shallower mixed layer has a greater chance of carbon dioxide re-equilibration with the atmosphere (Paine et al., 2021; Perkins et al., 2022; Legendre et al., 2015), while POC and DOC that sinks below the mixed layer has greater potential to be locked in the deep ocean from 100 to 1000 year time scales (Paine et al., 2021; Shen and Benner, 2018). Thus, as farmed kelp remains in the water column, the greater the chance of POC and DOC release and remineralization back to DIC where it may equilibrate with the atmosphere. It is, therefore, crucial to this CDR approach to measure the sinking rates of kelp biomass and quantify the major carbon pools that make up the decomposition products (i.e., POC, DOC, and DIC) as a function of the conveyance method in smaller scale laboratory and field experiments. Such experimental results will be important in the future as they can be translated to the model representation of conveyance. In this work we discuss the laboratory and field methods that we plan to use to determine the sinking rates as a function of its conveyance method, determine the rate of kelp decomposition, and quantify the released decomposition products.

2. Sinking rates

2.1. Conveyance of whole kelp fronds

One form of harvested kelp biomass conveyance is to immediately release the kelp at the surface and leave it to sink naturally without assistance (Krause et al 2023). Kelp fronds that detach from the holdfast can naturally bundle together to form kelp rafts, that average 2-3 meters in diameter, and can either wash up on beaches or remain floating in the open ocean (Hobday, 1998; Hobday, 2000a, b). Hobday, (2000b) reported *Macrocystis* kelp rafts can stay afloat in the

open ocean ranging from 69 to 109 days. Kelp rafts do sink when enough senescence occurs to change the buoyancy of the kelp biomass and/or when the gas-filled pneumatocysts rupture. To determine the sinking rates of whole kelp fronds we plan to conduct in-situ experiments. Hobday, (2000b) simulated kelp rafts by bundling kelp fronds together and tethering the bundled kelp fronds to a shallow marine moorings with a surface buoy and periodically determined the age of the rafts by monitoring the blade length over time. For this project we plan to make kelp rafts by bundling kelp fronds together which would then be tethered to either cinderblocks or to a dock within a lagoon on the UCSB campus. The UCSB lagoon is a prime location for this project as it is easily accessible for instigators to work, is a low energy environment to reduce loss of kelp bundles by unpredictable wave action and has constant marine inflow that keeps the salinity close to typical marine salinities. The age of the raft will be determined by measuring the kelp blade length over time. We plan to leave the kelp rafts out for several months and record when the rafts have sunk below the surface.

To simulate and determine the sinking rate of kelp fronds once it sinkings below the surface we plan to pre-compress intact kelp fronds to decrease the kelp's bouyancy. Preliminary laboratory experiments with pressurizing kelp biomass in a pressure chamber, led to the rupturing and/or compression of the gas filled pneumatocysts (Krause et al., 2023). The sinking rate of the pre-compressed kelp biomass can then be measured similar to the procedure detailed in Wernberg and Filbee-Dexter (2018) where the kelp biomass is sunk in shallow marine locations (e.g., semi-enclosed marinas, or off piers) next to transect tape and filmed by scuba divers. The sinking rates of the kelp fronds will be calculated by dividing the distance kelp sinks by the video recording time.

2.2. Conveyance of masticated kelp biomass

Another conveyance method under consideration is the release of masticated kelp biomass (Krause et al 2023). In this case, kelp would be masticated immediately after harvesting and either be released at the sea surface, similar to what is described in section 2.1, or pumped to a depth > 50m (short-depth pumping). The advantage of mastication is that the gas filled pneumatocysts will be ruptured, reducing time at the surface by decreasing the buoyancy of the kelp compared to intact kelp biomass.

To simulate the sinking of masticated kelp biomass either directly from the kelp farm at the sea surface or after it is pumped to a given depth, we plan to sink masticated kelp in laboratory settings using vertical cylinders, such as the one in Fig.1. The vertical cylinders can be made out of plastic and have been used in previous studies to determine sinking rates of masticated kelp biomass (Queirós et al., 2023; Wernberg and Filbee-Dexter, 2018). Vertical cylinders may be cheaply constructed and outfitted with adhesive measuring tape to mark the distance a particle has traveled. The relatively small size of the vertical cylinders makes it feasible to

Figure 1. Picture of a vertical cylinder used for masticated kelp sinking rates.

measure the sinking of smaller kelp components (i.e., stipe, pneumatocysts, and blades). Furthermore, the transparent plastic material offers a robust way to record the distance traveled using video cameras positioned outside the vertical cylinder. Sinking rates are calculated by dividing the sinking distance by video time.

2.3. Conveyance of kelp filled bales

The last conveyance strategy under consideration is to package kelp in bales, scaling up to industrial bales which can have a 1-ton capacity (Krause et al 2023). To measure sinking speeds for this method we will deploy in burlap sacks or in milk crates, and larger packages like slinky pots to simulate conveyance of bales, with varying size and surface area, in the field at predetermined moorings (see Krause et al., 2023, Figure 3). To determine the sinking rate of the various bale styles, pressure loggers such as the Sensus Ultra Pressure Logger will be equipped to record the pressure difference over time. Alternatively, for sinking rate deployments at greater depths (>100 meters), kelp bales will be equipped with depth sensors with higher depth ratings such as the miniature DST Centi Temp Depth Recorder (Star-Oddi). Both sensors are compact, considered negligible weight against the larger weight of the kelp biomass and will not affect the buoyancy of the kelp bales. Sinking rates in this instance will be calculated by dividing the difference in pressure (Sensus Ultra Pressure Logger) or change in depth (DST Centi Temp Depth Recorder) by the time it took to reach the seafloor as determined by the internal timers of the pressure/depth loggers.

3. Decomposition rate constants

3.1. Laboratory decomposition incubations

Decomposition rate constants (k) of kelp biomass can be determined through laboratory experiments. For laboratory-based kelp degradation experiments we are choosing to use transparent plastic (poly-nylon) heat-seal bags (Fig. 2). The poly-nylon heat seal bags are a potentially economical option as they can be purchased easily and cheaply from vendors such as Universal Meat Packaging Co. The poly-nylon bags, after proper cleaning with 1% HCl, are DOC clean and can hold kelp biomass in seawater, without leaks for extended periods of time. The bag transparency allows for visual observations of the changing kelp biomass as it degrades. Moreover, unlike ridged containers (e.g., glass serum vials), the flexibility of the plastic sample bag allows investigators to sample the seawater over time without balancing the volume with a gas or a liquid. This feature allows investigators to determine kelp degradation trends of a single replicate over the course of an incubation period with high precision.

To sample seawater easily and efficiently within the poly-nylon sample bags over time, custom made sampling ports are equipped to the bags (Fig. 2). The sampling ports are made of silicon-Teflon washers, silicon tubing, HDPE plastic barbed NPT, and plastic sample valves (Swagelok) which, after testing, produced negligible plastic derived DOC.

To determine the degradation rate constants of kelp components, fresh kelp fronds are first dissected into individual components (i. e., blades, pneumatocysts, and stipe). A known amount of kelp component biomass is added to individual poly-nylon bag and heat sealed prior to the addition of seawater (Fig. 2B, C, and D). The initial wet weight of the kelp component biomass is recorded before it begins to degrade. Residual air inside the sealed sample bag(s) is

vacuumed out through the sample port with a vacuum pump (Fig. 2A). The custom sampling port on the poly-nylon bag can easily be attached directly to niskin bottles in the field or in the lab to directly transfer in-situ seawater into the poly-nylon bags. The kelp degradation incubations are conducted at 4°C and in the dark to simulate kelp biomass degrading in dark and cold conditions.

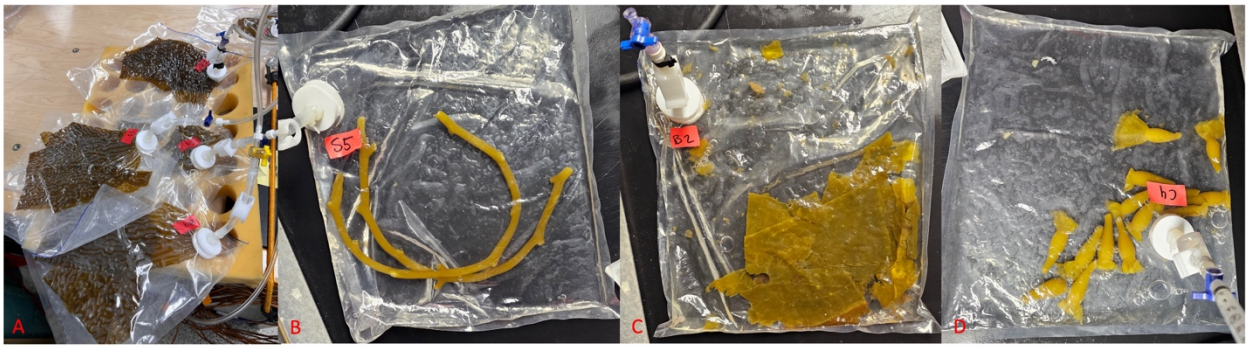

Figure 2. Preparation of kelp degradation experiments with poly-nylon sample bags equipped with custom sampling port (A) . Pictures of sample bags filled with in situ seawater and dissected kelp components stipe (B), blades (C), and pneumatocysts (D).

3.2. Field degradation incubations

Kelp decomposition incubations can also be conducted in the field. Krause et al., (2023) described the use of simple moorings that can be constructed out of neutrally buoyant line, a surface buoy and an anchor, and can be deployed at offshore locations. Packages filled with kelp (i.e., mesh bags, slinky pots and or bails) can be equipped to the bottom of moorins, deployed at various depths, and left in the environment for the kelp to degrade over time. The kelp packages can be recovered periodically to observe the state of the kelp and to measure the wet weight of the remaining kelp to determine the overall loss.

3.3. Degradation rate constant determinations

In either laboratory or field experiments, the decomposition rate constant of the kelp can be determined by the change in kelp wet weight over time. The decomposition rate constant (k) of the kelp biomass can be calculated using Equation 1 according to previous studies i.e., (Enríquez et al., 1993; Filbee-Dexter et al., 2022),

$$W_t = W_0\, e^{-kt} \qquad \text{[Eq. 1]},$$

where W_t is the wet weight of the kelp remaining at the end of the experiment, W_0 is the initial wet weight of the kelp, t is the duration of the experiment, k is the degradation rate in % loss per day.

3.4. Oxygen half saturation and temperature coefficients

In addition to the methods in 3.1-3.3 to assess kelp degradation, oxygen half saturation (K) and temperature coefficients (Q_{10}) are useful to parameterize the kelp degradation rates. The

K are defined as the substrate availability (i. e., dissolved oxygen) at which half the maximum process rate (i.e., kelp degradation) is reached (Mulder and Hendriks, 2014). In this case the *K* will help us understand what happens to the degradation rate at lower oxygen concentrations. The Q_{10} is the degree to which a reaction is dependent on temperature and usually defined as the rate ratio of a process occurring at temperature differences by 10 °C (Mundim et al., 2020).

The laboratory kelp degradation experiments described in 3.1 will be conducted at constant temperatures using either a temperature controlled cold room (4° C) located on the UCSB Marine Science Institute or in refrigerators that can be set to slightly warmer temperatures (14° C) closer to the average sea surface temperatures. The laboratory-based kelp degradation experiments will also be setup with seawater with a range of oxygen concentrations. To monitor the availability of dissolved oxygen while kelp is degrading in the sample bags, oxygen concentrations are measured with a FireSting optical oxygen meter (PyroScience) by drawing seawater out of the plastic sample bag through the custom sample port. The seawater is then pushed through a flow-through adaptor where the seawater passes through the optical source that measures the dissolved oxygen.

For the field kelp degradation experiments described in 3.2 dissolved oxygen and in situ temperatures loggers will be attached to the packages containing kelp. The sensors will monitor over time changes in both oxygen and temperature wherever the package is placed. The in-situ logger we are choosing to use for our field experiments are the miniDOT oxygen and temperature logger.

Eventually, with enough measurements of kelp degradation rates in the laboratory and in the field, we can estimate *K* for kelp degradation using Equation 2,

$$k = k_{max} \frac{DO}{DO+K} \quad \text{[Eq. 2]}$$

where *k* is the kelp degradation rate, k_{max} is the maximum kelp degradation rate, *DO* is the dissolved oxygen concentration, and *K* is the half saturation constant or the dissolved oxygen concentration at half the maximum kelp degradation rate.

The kelp degradation rates determined at two different temperatures during the laboratory and field experiments will then be used to estimate the Q_{10} according to Equation 3,

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\frac{10}{T_2-T_1}} \quad \text{[Eq. 3]}$$

Where Q_{10} is the temperature coefficient, *k* is the kelp degradation rate constant at temperature(s) *T*.

4. Decomposition product determinations

Experiments to quantify the release of kelp decomposition products (POC, DOC, and DIC) will be prepared similar to the kelp degradation experiment in section 3.1. by incubating kelp component biomass within in situ seawater sealed in the poly-nylon plastic sample bags (Fig. 2). Below, we outline the methods to determine the partitioning of kelp degradation products.

4.1. Particulate organic carbon (POC)

As the kelp biomass degrades in the poly-nylon sample bag(s) in seawater, the kelp begins to degrade into smaller pieces that do not fit through the largest opening of the custom sampling ports. As mentioned in section 1, we have decided to categorize kelp derived POC into two size fractions: 1) SPOC which are particulates that fit through the inner diameter of the sampling port (8.5 mm) and are collected onto a filter with a pore size of 0.3 µm, 2) LPOC which includes kelp that is still intact or as eroded floating particulates > 8.5 mm. To quantify the SPOC (particulates between 0.3 µm and 8.5 mm) released from degrading kelp, poly-nylon sample bag experiments are prepared as described in section 3.1. For SPOC sample collection, a known amount of seawater from the poly-nylon sample bags with degrading kelp biomass is filtered onto a 45 mm GF-75 (nominal pore size of 0.3 µm) pre-combusted filters (450 °C for 4.5 hours). SPOC samples are sent to the Marine Science Institute Analytical laboratory located at UCSB and prepared (including acidification) according to York et al., (2013) and analyzed with an Automated Organic Elemental Analyzer. Kelp particles that are >8.5 mm at the end of the incubations, are dried at 50°C for at least 3 days. The carbon content of the dry LPOC is estimated using mean mass-specific conversion factors for *Macrocystis pyrifera* (C = 30.6 ± 0.2% dry mass; Santa Barbara Coastal Long Term Ecological Research project [SBC LTER] unpublished data).

4.2. Dissolved organic carbon (DOC)

Methods for kelp derived DOC sample collection, storage, and analyses can be done similar to Halewood et al. (2022). Briefly, seawater is sampled from the poly-nylon sample bags through the sampling port and valve with a 60 mL rubber-free plastic syringe. Prior to sample collection, the 60 mL syringes are soaked in 10% HCL for >1 hour and rinsed well with MilliQ, to remove potential DOC contaminants. The seawater sample is filtered with Sterivex syringe filter (SVGP 0.22 um). Prior to sample filtration and collection, the Sterivex filters are flushed with at least 300 mL of MilliQ water to remove any potential manufacturer DOC contaminants within the Sterivex filter. The DOC sample is transferred to a pre-combusted (4.5 hours at 450 C) Environmental Protection Agency (EPA) 40 mL glass vial. Prior to DOC sample collection, the EPA vials are rinsed three times with approximately 5 mL of filtered sample seawater and discarded. Each filtered sample is diluted (1:5) with MilliQ water. The diluted DOC sample is preserved by adding 50 µL of 4N HCl using a pipette and non-autoclaved acid-cleaned pipette tips. All samples are capped with 1% HCL cleaned Polytetrafluoroethylene (PTFE) lined silicone caps, thoroughly mixed by inversion, and stored upright at 4ºC till future analysis in the laboratory. DOC samples are analyzed by the Carlson lab at UCSB by the high-temperature combustion method using a TOC-V or TOC-L analyzer (Shimadzu, Kyoto, Japan) with a 25 µmol per liter C detection limit as described in Halewood et al. (2022). DOC concentrations are corrected to account for the EPA vial dilution (1:5) and the background DOC that is in the MilliQ water used to dilute the sample at the given time point Equation 4.

$$DOC_t = \left(DOC_{Sample} * DF_{Sample}\right) - \left(DOC_{MQ} * DF_{MQ}\right) \quad \text{[Eq. 4],}$$

where DOC_t is the undiluted DOC concentration (in µmols C L^{-1}) at the time of sampling, DOC_{Sample} is the diluted DOC concentration of the sample, DF_{Sample} is the dilution factor for the sample at time *t*, DOC_{MQ} is the diluted DOC concentration of the MilliQ, and DF_{MQ} is the dilution factor for the no kelp seawater control that is diluted with MilliQ. The above concentration can be easily converted to µmol of DOC carbon (DOC µmol C) in the sample bag at a given time during the incubation, by multiplying the DOC_t in Equation 4 by the total volume of the sample bag at the time of sampling.

4.3. Dissolved inorganic carbon (DIC)

For DIC determinations, a peristaltic pump equipped with silicone tubing (Masterflex) directly draws seawater through the sampling port of the poly-nylon sample bags at each timepoint. Each seawater sample is transferred to a 20 mL glass crimp vial by placing the other end of the silicon tubing at the bottom of the glass vial. The glass vials are overflowed with ~20 mL of sample seawater to minimize ambient CO_2 exchange with the sample. Vials were immediately sealed with <1% total sample headspace volume using a grey butyl rubber stopper and aluminum crimp cap. Samples are preserved with 20 µL of saturated mercuric chloride (1g $HgCl_2$:10mL deionized water). Samples are stored in the dark at room temperature for future analysis. DIC samples are analyzed using an Autonomous Infra-Red Inorganic Carbon Analyzer (AIRICA) with 2 µmol C/L detection limit in the Carlson Lab at UCSB.

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