



RUNNING TIDE TECHNOLOGIES OCEAN CARBON REMOVAL RESEARCH ROADMAP

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TABLE OF CONTENTS

PART 1 – PURPOSE AND OBJECTIVES	2
1.1 LONG TERM CARBON REMOVAL DEVELOPMENT GOAL	2
1.2 HIGH PRIORITY QUESTIONS	3
1.3 PLANNED PROGRAM OF RESEARCH	4
1.3.1 OBSERVATIONAL EXPERIMENTS IN COASTAL WATERS	5
1.3.2 LABORATORY-BASED EVALUATION OF MATERIAL	6
1.3.3 PILOT-SCALE EXPERIMENTS IN THE OPEN OCEAN	6
1.3.4 DEEP OCEAN BENTHIC EXPERIMENTS	8
PART 2 – EXECUTIVE SUMMARY OF ONGOING AND COMPLETED WORK	9
2.1 MACROALGAE EXPERIMENTATION AT FIXED COASTAL SITES	9
2.2 OPEN OCEAN TESTS OF INTEGRATED PROTOTYPES	10
2.3 MODELING & COMPUTATIONAL EXPERIMENTS	11
2.4 LAB BASED CARBON BUOY CHARACTERIZATION	12
2.5 SENSOR PILOT DEPLOYMENTS	13
2.6 ECOLOGICAL INVESTIGATIONS	14
2.7 MACROALGAE BIOTECHNOLOGY	15
2.8 ONGOING OF ITREACH & COLLABORATION DEVELOPMENT	16





PART 1 – PURPOSE AND OBJECTIVES

Running Tide develops technology focused on making positive interventions for restoring ocean health. Our Ocean Carbon Dioxide Removal (CDR) Platform is meant to operationalize several CDR pathways. Mechanistically and operationally, the platform functions via the placement of passive drifting floating organic material (hereafter, "carbon buoys") from an offshore vessel into open ocean currents. The carbon buoys are designed to passively float for a designated period of time on the ocean's surface, after which they will lose buoyancy and sink rapidly over a period of hours to the ocean floor.

Here we describe Running Tide's overall R&D roadmap designed to develop this platform into an earth system scale ocean based carbon removal effort.

1.1 LONG TERM CARBON REMOVAL DEVELOPMENT GOAL

Carbon Removal is the net movement of carbon from the <u>fast carbon cycle to the slow carbon</u> <u>cycle</u> on a fully accounted supply chain basis. Running Tide's ultimate goal is to provide a platform to amplify several adjacent natural pathways for carbon removal to the deep sea:

Ocean Transport Amplification: The carbon buoys are synthesized from organic material of natural origin, including carbon-rich forestry and agricultural residue materials. Strategic and targeted removal of this biomass from terrestrial systems amplifies the carbon drawdown of forestry and other land use projects. Use of this material in our carbon buoys results in the transfer of this material to the deep ocean, amplifying a substantial natural pathway removing carbon from ready flux within the fast carbon cycle.

Open Ocean Macroalgae Growth: While floating in the photic zone, the carbon buoys act as a growth substrate for macroalgae. We explore both preparing the flotation with macroalgae starting material as well as allowing the flotation to be passively colonized by macroalgae already in the water. When macroalgae perform photosynthetic carbon fixation, it removes carbon from the surface ocean and perturbs the equilibrium state of the carbonate system in the seawater. This induces carbon dioxide flux into the seawater which later mixes down into the deep ocean. The non-buoyant species of macroalgae sink intact to the sea floor, transporting the carbon fixed into their biomass to durable storage.





Ocean Alkalinity Enhancement: The carbon buoys contain an alkaline payload which, when introduced to surface waters, amplifies the transfer of atmospheric carbon dioxide to the durable reservoir of dissolved marine bicarbonate. This procedure is commonly known as ocean alkalinity enhancement. Ultimately, the contribution of alkalinity enhancement will be smaller than that of the other pathways enabled by our platform, but Running Tide's approach is to find ways to squeeze every ton of carbon out of our system.

1.2 HIGH PRIORITY QUESTIONS

Of course there are many open questions related to Running Tide's carbon removal pathways of interest, as well as for Ocean CDR more generally. The following are the topics we target with highest priority:

Ocean transport research: Physical advection at the ocean surface is a dynamical process with different phenomena dominating at different scales. How will the ocean evolve the spatial distribution of a large cohort of passively drifting carbon buoys? To what extent can modeling characterize carbon buoy cohort dispersion and aggregate motion? What is the statistical error associated with Running Tide's proposed method of integrating laboratory, model-based, and in situ data-based assessment of this motion and how will this error vary across the dynamics of different ocean basins and gyres? How do we best characterize and mitigate potential negative externalities arising from the purely physical perturbation of introducing drifting carbon buoys, such as navigational hazards or ecological trapping of fish?

Ecological perturbations of organic carbon sinking: Sinking terrestrial biomass, and eventually macroalgae biomass, will enhance the flux of organic matter to benthic ecosystems. What are the degradation rates associated with different forms of organic carbon? What is the impact of this food source on the species diversity, abundance, and successional dynamics of benthic ecosystems; how does this depend on the spatial density and flux of the food supply and how will this vary across different ocean biomes? How will an increase in metabolic activity arising from this food flux impact the oxygen content of the benthic boundary layer? How will an increase in remineralized dissolved inorganic carbon (DIC) arising from this metabolic activity impact the pH of the benthic boundary layer? How will this flux of organic matter change the composition of the underlying sediments?

Open ocean macroalgae cultivation: Most macroalgae species are coastal organisms adapted to thrive in environments with high nutrient concentrations and low wave energy relative to the open ocean. Running Tide focuses on species that will achieve intact sinking and does not cultivate





naturally pelagic macroalgae such as Sargassum. How do we adapt originally littoral macroalgae to be successful in this open ocean environment? What is the range of natural genetic and phenotypic variation within and across macroalgae populations; how similar are these across ocean basins? How does genomic variability impact nutrient uptake and utilization? How can macroalgae growth models, derived for use in coastal settings, similarly be ported to the open ocean? What polyculture interactions will provide robust open ocean microcommunities? How does the macroalgae microbiome contribute to nutrient utilization or other metrics of health, and how will these interactions change in open ocean conditions?

Pelagic Nutrient Competition: At Running Tide's current scale and spatial density, our macroalgae cultivation will have a *de minimis* impact on the nutrient availability for phytoplankton. Nonetheless this is a research focus for us, as nutrient competition will ultimately determine the carrying capacity, efficiency, and ecological impacts of open ocean macroalgae growth as a CDR solution. How does the C:N:P stoichiometry of macroalgae determine competition with phytoplankton both from the perspective of net primary production (NPP) and net carbon removal? What is the statistical error associated with Running Tide's methods of characterizing a macroalgae/phytoplankton tradeoff in an open ocean system? How does this perturbation impact not just gross phytoplankton accumulation but the seasonal and successional dynamics of phytoplankton functional groups? What are the consequences of perturbations to phytoplankton diversity and abundance on the greater pelagic ecology? How does competition spatially and temporally scale with increasingly larger perturbations?

Systems and methods of surface ocean alkalinity enhancement: Running Tide's research and development in surface ocean alkalinity enhancement is targeted to address ocean acidification and CO_2 dissolution, as well as offset any introduction of organic acid from the placement of biomass in seawater. What are the most effective techniques of delivering alkaline minerals to the open ocean such that they are dissolved in surface waters? How does the ocean saturation state of calcium carbonate impact the dissolution kinetics of cementitious materials? How do we characterize what fraction of alkaline agents dissolve in, versus sink through, the mixed layer? What are the impacts of ocean alkalinity enhancement on phytoplankton distribution and community structure, particularly when considering carbonate materials?

1.3 PLANNED PROGRAM OF RESEARCH

Running Tide has developed a research toolkit in the model of a nascent oceanography department. We continually invest in improving our capabilities for (1) engineering of novel sensor systems to collect physical and biogeochemical data from the open ocean; (2) laboratory methods





to replicate controlled properties of ocean seawater; (3) computational techniques that deploy trusted academic models within a tech-industry data engineering approach; (4) coastal and shallow water field work; and (5) open ocean research cruises.

In addition to these tools and methods, Running Tide has partnered with Ocean Visions to convene an <u>independent scientific advisory board</u>. This board provides advice to guide our research planning and execution.

Running Tide plans to conduct several complementary programs of experiments over the coming years to assess the efficacy of these carbon removal pathways and evaluate impacts on surface and benthic environments: (1) replicated observational experiments of biomass degradation in coastal and shallow waters; (2) laboratory based study of carbon buoys, macroalgae physiology, and alkaline material delivery; (3) replicated pilot-scale experiments in the open ocean; (4) replicated deep-sea benthic experiments on the abyssal plain; and (5) computational experiments within the framework of accepted biogeochemical models of the earth system.

Although these experiments are not capable of transferring sufficient carbon from the fast to the slow carbon cycle to materially rebalance the carbon cycle, they are essential for developing and testing some of the few natural pathways of carbon transfer that are scalable to a level that could ultimately contribute to stabilizing atmospheric carbon dioxide against the ongoing climate emergency.

1.3.1 OBSERVATIONAL EXPERIMENTS IN COASTAL WATERS

Running Tide performs observational experiments at fixed shallow water sites to observe the behavior and life cycle of carbon buoys in the ocean. These experiments have been performed in Casco Bay, Maine and are ongoing on the continental shelf of Iceland. These experiments are similar in design and method to our program of deep-sea benthic experimentation, but afford a higher frequency and quantity of direct water and sediment sampling in addition to method testing. Although the dynamics on the shelf will naturally differ from those in the deep-sea, Running Tide is conservatively conducting these coastal experiments to gain a more complete understanding of the potential sensitivity of benthic systems to the input of these materials, thereby informing the design of the company's deep-sea benthic experiment and allowing empirical refinement of the methodologies required in that experiment.





This program of coastal experimentation also affords Running Tide the opportunity to explore methods of seeding carbon buoys with macroalgae and observing and measuring macroalgae growth rates in an ocean setting. The technology developed in this setting will be ported to the open ocean to support the quantification of macroalgae biomass cultivated offshore.

1.3.2 LABORATORY-BASED EVALUATION OF MATERIAL

Physical evaluation: Running Tide assesses the physical behavior of its carbon buoys in lab-replicated ocean environments. We study the floating duration of various carbon buoy synthesis methods and form factors as they vary with ocean energy during controlled wave tank studies. We similarly observe the mechanical dissolution and degradation rate in these conditions. Some of these wave tank samples are pre-conditioned with UV exposure, freeze/thaw cycles due to outdoor storage and/or "tumble tests" to simulate handling during processing and loading. We further study the sinking rate of these materials, once negatively buoyant, within tubes of seawater monitored with cameras. This laboratory work forms the basis for our predictive modeling of expected carbon buoy transport behavior in the open ocean.

Chemical evaluation: Running Tide assesses the impact on the carbonate chemistry of seawater when exposed to our carbon buoys, as well as screening carbon buoy input materials for toxicology in the marine environment. We characterize the effect of organic acid leaching from wood and the offsetting alkalinizing effects of calcium carbonate and other alkaline mineral dissolution. These interactions are then incorporated into our predictive modeling of the perturbation to the ocean carbon cycle caused by our proposed interventions.

Biological evaluation: Running Tide uses laboratory methods to study the biology of macroalgae, as well as the biological interactions between our materials and ambient seawater. These experiments are designed such that our findings will inform predictions about dynamics and physiology in the open ocean. We study the bio-lability of woody organic carbon within the carbon buoy composition as well as that of organic carbon produced by macroalgae. We further study the effects of abiotic stress, such as nutrient limitation, on macroalgae growth and tissue carbon intensity.

1.3.3 PILOT-SCALE EXPERIMENTS IN THE OPEN OCEAN





To explore the efficacy and environmental impacts of the open ocean deployments, Running Tide plans to deploy carbon buoys in offshore waters at conservative rates of between 500 and 5,000 tons every two to five weeks throughout the course of a year. The carbon buoys are deployed alongside our custom instruments designed to measure their motion and behavior once in ocean currents. Each deployment represents one of a series of replicated field experiments to be conducted over the course of seasonal cycles.

This series of replicated experiments was intentionally designed to evaluate the behavior of carbon buoys throughout the range of conditions in ocean state that occur during an annual seasonal cycle. The experiment replicates will be gradually scaled along several parameters, beginning at a size needed to generate measurable results in the well-mixed surface ocean, while still small enough to limit the scope of secondary impacts to ecosystems. At each stage, observations of environmental exposures will be evaluated before increasing in scale. The scale parameters are:

- Deployment tonnage: Replicated experiments are scaled from 500 to 5,000 tons. Exposures to environmental risks will depend on the spatial and temporal density of carbon buoys in the ocean. We will observe how the physical dispersion of carbon buoys in the open ocean conforms to model based prediction of this dynamic, which dictates the relationship between deployment tonnage and density.
- **Floating duration**: Predictive modeling of carbon buoy trajectories, dispersion, and density evolution is a key method of environmental impact mitigation, and it must be validated in the open ocean. We begin with shorter floating durations of 10-14 days, which are easier to predict, and will iteratively lengthen this period as we study passive surface ocean transport. Ultimately we seek to develop carbon buoys that float for 6-9 months in order to support growth of brown macroalgae to maturity. This longer floating duration will also decrease carbon buoy density on the seafloor due to increased dispersion of buoy trajectories.
- Biomass composition: Woody biomass will conservatively be deployed as the primary biomass in initial open-ocean experiments because it is more refractory than macroalgae, and because the metabolic interaction between macroalgae and the pelagic ecology is under-studied. Our first priority is to rigorously evaluate the impacts of sinking woody biomass. Macroalgae will be introduced in subsequent years; in the meantime, Running Tide will proceed with fixed site experiments focused on growing and sinking of macroalgae biomass, studying these interactions for eventual incorporation into open ocean deployment. After this point, the ratio of macroalgae to woody biomass will be gradually increased, with the long-term (decadal) goal of achieving a 5:1 ratio of macroalgae yield to wood on a C basis.





- Non-biological material composition: The non-biological materials used to synthesize carbon buoys also represent a complexity parameter that will be gradually increased over the course of study. Initially, the biomass in carbon buoys will be bound by calcium carbonate. This mineral is abundant in the ocean and likely to be stable in the surface ocean, given its saturation state in those waters. We may observe slow dissolution, enabling this pathway for alkalinity enhancement. Once the degradation rate of the carbon buoys in the open ocean environment is better understood, we will explore the incorporation of more readily soluble alkalis such as calcium oxide.

Surface water conditions will be monitored and sequentially sampled behind the carbon buoy pathway during the serial deployments. Because the concentration of the carbon buoy population will be diluted over a large spatial area, we do not expect to be able to directly measure any perturbation we cause to the biogeochemistry of the surface ocean. Instead, our surface water sampling regime is oriented toward establishing a solid empirical baseline, such as to initialize the modeling work which will be performed to analyze the impact of our intervention. On the other hand, direct observation of a carbon chemistry perturbation and how it evolves in time may in fact be possible, so we still consider it important to try to detect a signal.

The water samples will be analyzed for key parameters related to CO₂ removal, including pH, salinity, dissolved inorganic carbon, and total alkalinity. They will additionally be analyzed for factors which will determine the biogeochemical carbon cycle feedback of macroalgae cultivation, such as nutrient (nitrate, silicate, phosphate) and trace element (e.g. iron) concentrations and the eDNA signatures of phytoplankton functional groups. Subsamples of these water samples shall also be preserved as a permanent archive of the surface deployments that can be retroactively analyzed at any time. The sensor instruments deployed alongside the carbon buoys track their trajectory via GPS, log seawater temperature and wave height, and obtain and transmit images of the carbon buoys prior to their sinking.

Running Tide has performed probabilistic predictive modeling of the fate of carbon buoys as they disperse through the ocean, and empirical sensor data from these field replicates will be used to validate and refine this modeling approach. In addition to the sensors that we currently use, we are evaluating the addition of sensors for biogeochemical properties including pH, alkalinity, and chlorophyll-a.

1.3.4 DEEP OCEAN BENTHIC EXPERIMENTS





Since it is not feasible to repeatedly assess the impacts of the serial open ocean deployments on the deep-sea benthic environment and the deep-sea benthic environment will not experience the same degree of annual variation in key parameters (pH, salinity, total alkalinity, dissolved inorganic carbon, temperature, wave energy) as the surface ocean, we plan to conduct intensive, long-term benthic experiments in the deep ocean. The purpose of these experiments is to quantify the ecological and geochemical impacts and rates of degradation of Running Tide's carbon buoys and macroalgae biomass in the deep sea, as well as to quantify potential associated changes in the abundance and distribution of macroinvertebrates, vertebrates, and microorganisms.

Running Tide is currently exploring a research partnership with Ocean Networks Canada (ONC), a non-profit ocean research organization affiliated with the University of Victoria. In this proposed program of experimentation, ONC will place treatments of carbon buoy materials at their established deep-sea observation stations in the Cascadia Basin off the west coast of Canada. ONC will then conduct one-year benthic monitoring of these treatments. The evolution of the carbon buoys shall be observed with cameras and environmental sensors (salinity, temperature, dissolved oxygen) throughout the duration of the experiment. Water samples and sediment cores shall also be obtained throughout the experiment by remotely operated vehicles and/or submersibles to allow observation of carbon flux into the sediment, as well as eDNA characterization of changes to the microbial community composition in response to the presence of carbon buoys.

PART 2 – EXECUTIVE SUMMARY OF ONGOING AND COMPLETED WORK

The Running Tide team and our collaborators have been hard at work developing further insights into the marine carbon cycle and the behavior of our climate solutions. This section is meant to provide a survey of work that is ongoing or completed thus far.

We have begun to consolidate our results and find ways to share them with the broader scientific community. This includes posting on our <u>website</u>, presenting methods and findings at conferences and, where appropriate, collaborating with academic researchers to submit papers for peer review and publication. Running Tide continues to engage through our scientific partners to improve the sophistication of our research planning and to disclose our findings in a transparent and accessible manner.





2.1 MACROALGAE EXPERIMENTATION AT FIXED COASTAL SITES

Running Tide has performed a series of coastal macroalgae growth experiments in Casco Bay, Maine:

Coastal Exp 1, Nov 2020 - April 2021: Established technical ability to replicate techniques of macroalgae aquaculture and to apply those techniques to novel architectures (e.g. vertical long lines). 1600 vertical farm lines were grown to maturity at a range of sites within Casco Bay, exposed to a range of wave energy and nutrient availability conditions. Macroalgae tissue samples were collected for elemental analysis to validate the C:N stoichiometry for macroalgae metabolism. This stoichiometry was found to be invariant across the growing environments in the experiment.

Coastal Exp 2, Dec 2021 - June 2022: Biological replicates of macroalgae farms were grown within a smaller Casco Bay site ("Goose Island") to better understand environmental exposures. Established technical ability to build image-based phenotyping data sets as time series macroalgae measurements were taken for fresh weight and tissue carbon, corresponding to regularized photographs of the samples. Experiment also included time series measurements of environmental conditions at the grow site: temperature, pH, salinity, light intensity at depth, photosynthetic active radiation (PAR) at depth, and chlorophyll-a content of the ambient water.

Coastal Exp 3, ongoing: Compared to prior experiments, genetic variation in macroalgae farms was reduced with most biological replicates limited to a single population, and a subset of experimental units was limited to spores from a single parent. The goal is to establish a robust data set to evaluate the correspondence between genetics and the environment (GxE) with time series measurements of fresh weight, volume (from continued image-based phenotyping), tissue carbon content, and regularized photography. We expanded the environmental time series data collection from Coastal Exp 2 to now include fluorescent dissolved organic matter (fDOM), ammonium, dissolved oxygen, nitrate, turbidity, and subsurface ocean currents.

2.2 OPEN OCEAN TESTS OF INTEGRATED PROTOTYPES

Running Tide has performed offshore prototype tests of its verification hardware. These tests involved placing macroalgae seed on instrumented drifters in the open ocean.

North Atlantic 1, March 2021 tested off-the-shelf GPS and remote imaging systems for the purpose of observing open ocean macroalgae seed recruitment. The basic functionality of these Work Product of Running Tide Technologies

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systems was validated, as was the ability to maintain viable macroalgae seed on an open ocean vessel.

Labrador Sea 1, July 2021 improved the method of deployment for macroalgae seedline into the ocean. Custom-designed imaging prototypes were tested and certain failure modes were revealed. This deployment also allowed us to explore drifting dynamics in a location with less dispersive ocean gyre properties.

North Atlantic 2, Dec 2021 successfully tested the electronics and software systems for custom imaging systems and explored the failure modes of several mechanical architectures. **Recruitment and growth of macroalgae was imaged in the open ocean!**



Tiny little (we estimate 3-5 cm) sugar kelp blades grew from juvenile sporophytes in the open ocean. This image was sent back to us from 45.569 N, 41.125 W. These yields will not balance the carbon cycle, but they demonstrate that open ocean kelp cultivation is in principle possible.

2.3 MODELING & COMPUTATIONAL EXPERIMENTS

Running Tide continues to develop capabilities to perform predictive modeling of our system behavior:

2021 MOU with Centre for Climate Repair at Cambridge: Running Tide worked closely with John Taylor's research group at Cambridge University in their work to run a sugar kelp growth model for kelp growing at densely distributed locations across the North Atlantic. This modeling work bounded the carrying capacity of ocean afforestation with sugar kelp as a carbon removal *Work Product of Running Tide Technologies*

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pathway, and explored depths beyond which light attenuation would limit growth potential in various regions of the North Atlantic.

Advection Modeling: Running Tide has adapted the Ocean Parcels framework for lagrangian advection modeling to create predictive analysis for the ocean transport of its system across various ocean basins. Our analysis has integrated HYCOM global ocean circulation data with ECMWF reanalysis data for Stoke's drift. In addition, we integrated CMEMS ocean state forecasts for predictions of deployments. Running Tide continues to explore the application of numerical techniques such as the addition of stochastic noise in improving the correlation between model predictions and in situ trajectory data.

Biogeochemical Modeling: Running Tide has begun to adapt the <u>ECCO-Darwin</u> ocean biogeochemistry model to 1) demonstrate the impact and scalability of our future CDRs on the coupled earth system and 2) to optimize efficiency of our CDR interventions. This includes studying the impact of surface-ocean carbon uptake or alkalinity addition on the air-sea gas exchange of carbon dioxide, the vertical transport of dissolved inorganic and organic carbon from the sunlit upper-ocean to the seafloor, and the potential nutrient and trace metal competition between proposed macroalgae afforestation projects and the endemic phytoplankton ecology. In addition, open-source biogeochemical datasets have been incorporated into the advection model platform to pair observational and operational model data to material trajectories including data from CMEMS and Aqua/MODIS.

ECCO-Darwin Collaboration: Running Tide has engaged in a collaboration with scientists from the ECCO-Darwin project at NASA's Jet Propulsion Laboratory and Moss Landing Marine Laboratories (San José State University) with the goal of building robust ocean CDR modeling packages into this existing data-constrained ocean biogeochemistry modeling tool, as well as designing a suite of easy-to-use analysis tools for end users.

2.4 LAB BASED CARBON BUOY CHARACTERIZATION

Running Tide has performed laboratory based characterization of its CDR placement material.

Physical studies: Running Tide has assessed the physical behavior of its carbon buoys in lab-replicated ocean environments. Using controlled wave tank studies with a range of ocean energy conditions, we studied the floating period, mechanical dissolution, and mechanical degradation rate of varied carbon buoy synthesis methods and form factors. We studied the





sinking rate of these materials, once negatively buoyant, within instrumented tubes of seawater. This laboratory work forms the basis for our predictive modeling of expected transport behavior in the open ocean.

Chemical studies: Running Tide has developed capabilities to assess the impact to seawater chemistry when exposed to our carbon buoys. Our dissolution reactors continuously log temperature, pH, salinity of water and pCO2 of air at the surface. Alkalinity is monitored with an auto titrator and water samples are routinely sent away for additional elemental analysis. We ran preliminary experiments to characterize the effect of organic acid leaching from wood and the mitigation of this by alkaline mineral dissolution, namely lime kiln dust. These interactions are then incorporated into our predictive modeling of the perturbation to the ocean carbon cycle caused by our proposed interventions. Additional planned experiments include further acid leaching and alkaline material testing in Ocean Hub (see below) as well as dissolved organic carbon degradation studies in partnership with Northeastern via Dr. Aron Stubbins lab group.

We are currently adding the capability to measure DIC in house and building out a more sophisticated test center we call our "Ocean Hub" at 1004 Congress St in Portland, Maine. Tours are available to members of the scientific community by appointment – come visit!

2.5 SENSOR PILOT DEPLOYMENTS

Dec 2022 - Feb 2023: Running Tide performed IS-SD-1 and IS-SD-2 sensor pilots for our proposed deployment activity in Iceland. In each pilot, we placed a suite of sensors into the open ocean southeast of Iceland. The sensors consisted of our custom designed remote imaging instruments, as well as GPS-enabled trajectory instruments and environmental sensors (wave energy, chlorophyll-a, and temperature) designed to study the region of the ocean in which we operate. When accompanied by an actual deployment of carbon buoys, these sensor suites will provide the data needed to achieve verification of the ocean transport of our materials and the total carbon removal impact of a deployment project. These sensor-only deployments provided an opportunity to test and parameterize our surface transport models against in-situ data and tune our methodology for quantifying carbon removal interventions.

The sensors were exposed to a wide range of weather conditions, including a severe storm with wind speeds up to 70 mph and waves as high as 9 meters. This provided an opportunity for Running Tide to stress test our designs in real world conditions.





IS-DS-1 had a two week sensor success rate of 87% for the imaging instruments and 56% for the trajectory instruments. After troubleshooting on the basis of our diagnostics, IS-DS-2 had an improved success rate of 100% for imaging instruments and 79% for the trajectory instruments.

Running Tide's hardware team is investigating how to extend our open ocean sampling capabilities to include parameters of the carbonate chemistry of seawater. Creating low cost and low touch sensing instruments for these measurements is an active area of interest for the entire ocean community.

2.6 ECOLOGICAL INVESTIGATIONS

Running Tide has developed a program of research to study the exposure to ecological risk associated with our proposed CDR methods:

2021-2022 Ocean Visions Advising and Evaluation Team: Running Tide executives and scientists worked closely with an expert panel convened by Ocean Visions to study and comment on the research plan, potential environmental impacts, and governance needs of our specific system design.

2022 Ocean Visions working group: Running Tide's research director participated in a year-long working group to support the production of a <u>research framework</u> to guide investigation of the efficacy and impacts of sinking marine biomass into the deep sea for carbon sequestration.

Ongoing Stubbins Lab Research: Beginning in 2022, Running Tide has funded work through a research group at Northeastern University to study the bio-lability of dissolved and particulate organic carbon generated by macroalgae. The outcome of this research will inform questions posed by the ocean visions working group considering the degradation rate of macroalgae tissue in the benthos and algal-derived DOC in the water column.

RADI Modeling: Running Tide has advised researchers at Utrecht University as they perform biogeochemical modeling of the impact of sinking organic carbon to the deep ocean. This research explores the consequences of enhanced organic carbon delivery to the seafloor on oxygen, dissolved carbon and alkalinity cycling in the deep ocean.

Benthic Pilot - Aug 2022: Running Tide conducted a pilot study in Casco Bay, ME, USA to develop methods to observe carbon biomass on the seafloor, and the impact on species abundance and diversity during their degradation lifecycle. Two 20x20 m plots were placed 60m





apart, one as a control with no carbon biomass and the other containing half a ton of carbon biomass. The carbon biomass was an assemblage of raw materials (woody biomass [50%], limestone dust [20%], lime kiln dust [15%], and cement [15%]) placed into netting and anchored to the seafloor. Both plots were subdivided into transects with data collection occurring via visual observation and video recordings to understand species abundance and diversity. As an outcome of this study Running Tide developed a standard operating procedure to implement benthic studies, which can be scaled up for larger research opportunities.

Benthic Experiment - Q1 2023: Running Tide has planned and is preparing to deploy a shallow water fixed site experiment on the Icelandic shield to observe carbon buoys during their degradation lifecycle on the seafloor, their impact on species abundance and diversity, and to monitor for changes in the sediment carbon concentrations. The experiment, in Hvalfjörður fjord, consists of one control site with no carbon buoys present and one experimental site with five replicates of 100 kg of carbon buoys in mesh nets. Due to the ease of accessing the shallow water location when compared to the deep ocean, a more intensive monitoring plan will be used to sample water on a weekly basis and sediment and substrate on a monthly basis. In-situ image capture and microscopy of samples will be used to identify larger organisms and eDNA will be used to identify microorganisms. Experts at the Southwest Iceland Nature Research Center will perform visual identification, while NatureMetrics will be used for eDNA analysis. In addition, continuous data loggers for temperature, pH, salinity, and dissolved oxygen will measure these environmental conditions.

2023 Deep Ocean Studies: Running Tide is engaging in a research partnership with Ocean Networks Canada (ONC) to perform deep ocean (> 1000m depth) observations of carbon buoys during their degradation lifecycle on the seafloor and their impact on species abundance and diversity. This work is to be performed in Cascadia Basin in the Canadian Pacific.

Alfred Wegener Institute Helmholtz Center for Polar and Marine Research (AWI): Running Tide is engaged in the planning of a sinking seaweed and carbon buoy substrate experiment with researchers at AWI. They will perform deep sea observational experiments in the deep sea (4000m) of the Fram Strait area of the Arctic Ocean. The goal of this work is to understand how different seaweed species (Kelp, Sargassum and Ulva) degrade at 4000 m depth and replicate our observation of carbon buoy degradation in the deep sea, in a different marine biome.





2.7 MACROALGAE BIOTECHNOLOGY

Running Tide has performed foundational work on macroalgae genomics and lifecycle.

Foundational bench work: Running Tide has performed DNA extractions on kelps and ulvas. We believe we are the first to successfully extract high molecular weight DNA from sugar kelp as well as from various *Ulva* species. We have performed isolation and banking of various stages of the macroalgae lifecycle across these species.

Genome building: Running Tide is partnering with researchers at Los Alamos National Laboratories to build macroalgae genomes and cross species pan-genomes. This collaboration will lead to the first published long-read genomes for *Ulva lactuca* and *Saccharina latissima*.

Natural variation surveys: Running Tide has performed genotype and phenotype data collection for a variety of macroalgae species in western Iceland. We have engaged in a collaboration with the University of Iceland to analyze and publish this population level data.

Foundational microbiome work: Running Tide is engaged in a contract with <u>Ginkgo Bioworks</u> to identify and characterize macroalgal microbiomes across geography and seasonality.

2.8 ONGOING OUTREACH & COLLABORATION DEVELOPMENT

Running Tide continues to pursue collaborations to engage in transparent scientific work:

NOC: Running Tide is engaged in exploratory conversation with United Kingdom's <u>National</u> <u>Oceanography Centre (NOC)</u> to perform in-situ observations of our open ocean deployments then assess their impact on the local carbon cycle and ecological dynamics.