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NYSG Completion Report

Please include the following information for your project. The text of this report should be at least 5-8 pages and be composed for an audience of your peers. Other formats, or reports with incomplete sections, will not be accepted. The expectation is that information or material will be provided under each section.

Report Written By: Michael Doall, Chris Gobler Date: October, 2023

A. Project Number and Title:

R/ATD-15-NYCT, entitled: ***Quantifying the ability of seaweed aquaculture in Long Island Sound to remove nitrogen, combat ocean acidification, improve water quality, and benefit bivalves***

B. Project Personnel:

Chris Gobler, PI

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C. Project Results:

Complete the following sub-sections to discuss your results as they relate to the project's objectives:

C1. Meeting the Objectives:

Specifically, the objectives of this study were to:

1) Grow multiple species of cold-water (*Saccharina latissima*) and warm-water (*Gracilaria tikvahiae*; *Ulva* spp.) seaweeds in multiple locations and at multiple scales in Long Island Sound and quantify net nitrogen (N) and carbon (C) removal rates as well as quantify and

map changes in dissolved oxygen and ocean acidification (pH) in regions with seaweed aquaculture.

2) Quantify the growth and survival, by Eastern oysters and blue mussels grown with and without seaweeds in multiple locations in NY and CT waters.

3) Write a Guidance Document and host a workshop sharing best practices for maximizing growth of seaweeds, bioextraction of N, performance of bivalves, and improvements to water quality.

2. Methods

2a. Overview

Three species of seaweed were cultivated for this study, including one cold water (winter-spring) species (*Saccharina latissima*, or sugar kelp), and two warm-water (summer-fall) species, *Ulva spp*. (green seaweed) and *Gracilaria spp*. (red seaweed). The seaweeds were cultivated in two contrasting LIS harbors along along the north shore of Long Island that represent a strong contrast in eutrophication but are also representative of many NY-LIS harbors and bays: Northport Harbor and Mount Sinai Harbor. Seven years of weekly water quality monitoring data (2014-2020) by the Gobler Lab has revealed that Mount Sinai Harbor is well-flushed, rarely hypoxic, and does not experience HABs, attributes it shares with several other central and eastern LIS harbors including Port Jefferson Harbor, Stony Brook Harbor, and the Mattituck Inlet (LIMMN, 2014-2020). In contrast, Northport Harbor experiences annual HABs caused by multiple species (Hattenrath-Lehman et al., 2015A&B, 2018) and extended bouts of hypoxia, attributes it shares with Huntington Harbor, Cold Spring Harbor, and Hempstead Harbor (LIMMN, 2014-2020). As such these two sites offered an excellent opportunity to understand how different seaweeds grown via different approaches perform under differing water quality conditions that are prevalent across the entire southern shore of LIS and how they, in turn, may benefit bivalves and water quality.

In cultivation experiments with all three seaweed species, bivalves were cultivated in close proximity to the seaweeds and far from the seaweeds, and bivalve growth with and without seaweeds was compared. Two different bivalve species were used in experiments, including eastern oysters (*Crassostrea virginica*) and blue mussels (*Mytilus edulis*), with blue mussels co-cultivated with kelp only, and oysters co-cultivated with all three seaweed species. For each experiment, continuous pH and dissolved oxygen data was collected inside seaweed cultivation areas and outside seaweed cultivation areas to evaluate the impacts of seaweed cultivation on localized water quality.

Finally, in an aditional effort to evaluate the ability of seaweeds to modify water chemistry, high-resolution surface mapping cruises were conducted in and around the largest seaweed farm in Long Island Sound, the 9-acre Thimble Island seaweed farm. This approach was designed to specifically identify the sphere of influence that seaweed aquaculture has on water quality.

2b. Winter/spring seaweed experiments - *Saccharina latissimi* (sugar kelp)

2b.1 Study Sites

Sugar kelp was cultivated along horizontal longlines in shallow (2-3 ft MLW), near-shore waters in Northport and Mount Sinai Harbors for the entire duration of the kelp growing season, which began in December 2021 when kelp lines were seeded and lasted through late May/early June 2022 when kelp lines were harvested. The growth and nitrogen content of kelp tissue along the experimental lines was monitored monthly, and results were compared with that from parallel studies of kelp growth and bioextraction by the Gobler Lab in other Long Island estuaries during the 2022 kelp growing season. In total, kelp was cultivated along horizontal longlines at eight experimental sites across Long Island during the 2022 kelp growing season (Figure 2b.1, Table 2b.1). These sites were spread across the three main estuarine systems surrounding Long Island, including the Long Island Sound to the north (3 sites), the Peconic Estuary to the east (4 sites), and the South Shore Estuary to the south (1 site; Figure 2b.1). The sites spanned a gradient in water quality that improved from west to east, from the heavily impacted waters of the East River in the far western portion of LIS, to the cleaner waters of the central Peconic Estuary on the east end of Long Island. In addition, these locations spanned a range of water depths from 2 to 30 ft (MLW), and included sites within shallow enclosed bays as well as more open and deeper estuarine waters (Table 2b.1). The comparative analysis among sites provided a relative context for evaluating the effectiveness of sugar kelp farming as a nitrogen mitigation strategy in LIS and across Long Island. In addition, it provided insight into the environmental factors impacting kelp growth, information that will help guide site selection and cultivation techniques for future bioextraction projects across Long Island Sound.

Within the Long Island Sound, kelp was cultivated in three sites within the western half of the Sound. From west to east, these sites included the East River, Northport Harbor, and Mt. Sinai Harbor (Figure 2b.1). The East River cultivation site was in deep (~30 ft MLW) waters near the Throgs Neck Bridge, while the two harbor cultivation sites were in shallow (~ 2-3 ft MLW) near-shore waters. While kelp farming has been traditionally performed in open, deep-water regions typically greater than 20 ft water depth, it is the shallow, semi-enclosed harbors and bays that directly receive the largest nutrient loads (SCSWP, 2020) and are most in need of water quality improvement. The Mt. Sinai and Northport Harbor sites within Long Island Sound were selected for this study specifically for this reason.

Within the Peconic Estuary, all four kelp cultivation sites were in deep waters that ranged from 15 to 23 ft at MLW (Table 1). Within the South Shore Estuary, the cultivation site in Moriches Bay was in shallow (~2ft MLW) near-shore waters, similar to Mt. Sinai and Northport Harbors (Table 2b.1).

2b.2. Cultivation Methods

Installation of kelp lines. Kelp was cultivated along horizontal ropes (three to four lines per site) using one of two general methods depending on water depth, including the 1) 'suspended' line method in deep waters, and 2) 'staked' line method in shallow (i.e., < 4 ft MLW) waters (Table 2b.1). In deep waters (~15-30 ft MLW), which included all four sites in the Peconic estuary and the East River site in Long Island Sound, kelp was cultivated along ‘suspended’ horizontal longlines following standard industry methods (Flavin et al 2013). The suspended longlines were anchored by moorings on either end and suspended ~4 ft below the surface using buoys (Figure 2b.2). Suspended kelp lines were approximately 150 ft in length from mooring buoy to mooring buoy, which included 130 ft of ½” rope (i.e. the kelp line) and a 10-ft ‘pigtail’ at either end to which the kelp line was tied (Figure 2b.2). The mooring systems were anchored with either 4-ft screw anchors (PE2, PE3, PE5), or 300-lb mushroom anchors (PE4, East River). For moorings with screw anchors, ¾” rope was used for mooring lines, with the rope attached to the anchor with a thimble and shackle and tied to the buoy on the other end with a bowline knot. For moorings with mushroom anchors, ½” chain was used for mooring lines to help keep the mooring lines from wrapping around the stem of the mushroom anchor. Mooring lines ran diagonally from anchor to buoy, with about a 2:1 scope in the mooring lines (i.e. mooring line length to water depth) to increase the holding power of the anchors. The screw anchors and mooring lines at PE2, PE3, and PE5 were installed by hand by SCUBA divers, and the mushroom anchors at PE4 and the East River were installed from boats using either a winch or crane to lower the anchors into place.

In shallow waters (<4 ft MLW), which included the two harbor sites in LIS (i.e., Northport and Mt. Sinai Harbor) and the site in Moriches Bay in the South Shore Estuary, kelp was cultivated along ‘staked’ horizontal longlines. This staked-line method was developed in 2018 by Doall & Gobler (in prep.), and has been used to produce high kelp crop yields in Moriches Bay by the Gobler Lab for five years in a row. Kelp lines (~ 100 ft long each) were staked a fixed distance above the bottom using screw-down anchors on either end of each line (Figure 2b.3). The four-foot-long screw anchors were screwed approximately three feet into the bottom so that kelp lines were elevated about one foot off the bottom. A 5-ft section of ½” rope with an eyesplice, referred to as a ‘pigtail’, was tied to each anchor to provide a connection point for the kelp line that can be lifted out of the water (Figure 2b.3). After seeding the line, the line was pulled taught with a trucker’s hitch, so that the entire length of line was tight and elevated a fixed distance (~ 1 ft) above the bottom. As opposed to suspended lines, staked lines remain in a fixed position above the bottom and do not rise and fall with the tides.

Seeding kelp lines. At each cultivation site, kelp lines were seeded in December using spools of hatchery-produced ‘seedstring’. Three different seedstock ‘strains’ were used to seed the lines at each site, with each seedstock strain seeded along a separate line (Table 2b.2). The three seedstock strains were derived from reproductive tissue of local, wild kelp collected by divers in three different locations in eastern Long Island Sound and Block Island Sound: 1) Montauk (MTK) 2) Black Ledge (BL), and 3) Fishers Island (Figure 2b.4). Two of the three different seedstock strains (MTK and BL) were produced by the Gobler Lab at the Stony Brook Southampton kelp hatchery, and the third strain (RP) was produced at the kelp hatchery in West Sayville, NY run by Hart Lobster company (Figure 2b.4; Table 2b.2).

Similar hatchery procedures and husbandry techniques were used at the two hatcheries (Figure 2b.5). Following collection, reproductive kelp blades were immediately brought back to the lab where the sorus tissue was excised, cleaned, and desiccated overnight in a refrigerator. The following day, the prepared tissue was placed in 10 °C filtered seawater to induce spore release, and the spores were set onto spools of string in aquarium tanks at known setting densities. The spools of seedstring were grown in aquarium in f/2 nutrient enriched sterilized seawater under controlled environmental conditions (10 °C; 12:12 light-dark cycle), with weekly water changes performed until the juvenile sporophytes were large enough (~2 mm) to seed kelp lines at the field sites.

One difference in procedure between the hatcheries, however, was the density of spores that were set on the seedstring (i.e., spore setting density). Spores were set onto seedstring at a low spore density at the Stony Brook hatchery (~1,333 – 1,800 spores ml^{-1}), and at high spore densities (~7,000 – 7,500 spores ml^{-1}) at the Hart Lobster hatchery (Table 2b.2). Altogether, the three seedstock batches differed in three variables that could potentially impact growth, including: 1) the source location from where parental reproductive tissue (i.e., sorus tissue) was collected; 2) the density of spores that were set on the seedstring (i.e., spore setting density); and 3) the specific hatchery conditions of each hatchery such as seawater source and quality (Table 2b.2).

Bivalve co-cultivation. Two species of bivalves were co-cultivated with kelp, including eastern oysters (*Crassostrea virginica*) and blue mussels (*Mytilus edulis*). In both Northport and Mount Sinai Harbors, oyster seed (initial shell height = 23.86 ± 0.16 mm) and mussel seed (initial shell height = 27.45 ± 0.29 mm) were cultivated in bottom cages placed between kelp lines which were spaced ~6 ft apart. Control oysters and mussels with no surrounding kelp were grown in separate cages positioned ~50 meters away from the kelp lines. In both locations, oysters and mussels were placed in triplicate 4-mm mesh bags (46 cm x 25 cm x 8 cm) that were housed and elevated above the bottom in wire cages (Figure 2b.6). Each replicate bag was stocked with either 100 mussels or 50 oysters. Mussels were cultivated within and outside kelps lines in each harbor for approximately 2 months (early April to early June), and oysters were cultivated for one month (early May to early June). In an additional co-cultivation experiment, blue mussels (initial shell height = 25.38 ± 0.31 mm) were also deployed at Greenwave's Thimble Island kelp farm in mussel socks that hung vertically from horizontal kelp lines within the center of the farm (Figure 2b.7). Control mussel socks were placed on vertical lines that hung from buoys outside the farm. The experimental and control mussels were cultivated for ~2.5 months at the Thimble Island farm, from 3/30/22 to 6/15/22.

2b.3. Bivalve growth monitoring

The average weight and height (i.e. longest shell dimension) of oysters and mussels in each replicate grow-out bag (or mussel sock in the case of the Thimble Island mussel experiment) was measured at the start and conclusion of each cultivation experiment, and weight and height based growth rates were calculated in g d^{-1} and mm d^{-1}, respectively. Growth inside and outside of kelp lines was compared using Student t-tests.

2b.4. Kelp monitoring

To monitor kelp growth, samples of kelp were collected from each site monthly until harvest in late May-early June. On each sample date, triplicate tissue samples were collected from each line of kelp at each site. Each sample included all kelp biomass from a 15-cm section of line. Samples were carefully removed from each line to keep kelp as intact as possible, with blade, stipe, and holdfast. Kelp samples were brought back to the lab, excess water was removed with a salad spinner, and the 'wet' weight of each sample was measured. Ten kelp blades were randomly selected from each sample for measurement of blade length, blade width, and stipe length. Samples were then placed in a drying oven at 60 °C until the weight stopped decreasing (several days), and dry weight measurements were obtained. Kelp growth was evaluated in terms of weight per unit length of line (i.e., kg m^{-1}), which was referred to as line yield.

The end of the growing season was characterized by a deterioration of the kelp caused by biofouling, grazing, and blade senescence as waters warmed during spring. The optimal harvest time was considered to occur just prior to the onset of kelp deterioration and biofouling when line yields were at or near maximum. One-way ANOVAs and Tukey HSD tests were used to compare the optimal line yields among sites, and among seedstock strains within sites.

Triplicate samples of kelp were collected from each kelp line at each site on each sample date to monitor changes in the nitrogen content of kelp tissue through the growing season. For each sample, all kelp biomass was collected from a 15cm section of line. The samples were weighed and dried at 60 °C immediately after collection. After complete drying, each sample was ground into powder to homogenize the tissue, and 3-6 mg was weighed out for N analysis. Samples were run on a combustion elemental analyzer to determine N content. Nitrogen content was expressed as a percentage of tissue biomass (dry weight).

2b.5. Quantifying nitrogen bioextraction

To quantify the amount of nitrogen sequestered by kelp, line yields (kg m^{-1}) were first converted from wet weight to dry weight yields, using the average ratio of dry to wet weight obtained from 227 kelp samples collected over the course of the growing season. The dry weight yields were then multiplied by the nitrogen percentage in the dry kelp tissue, as determined from the N analysis of dried tissue samples described above. The result expressed nitrogen sequestration in terms of kilograms of nitrogen per meter of kelp line. This calculation was done for each sample date at each site using the line yield and tissue nitrogen concentration measured on that date. The optimal harvest date for peak nitrogen bioextraction, or removal of nitrogen from the estuary, was then determined.

2b.6. Water quality monitoring

Continuous pH and dissolved oxygen data was collected during the cultivation experiments at each site using Onset® HOBO® devices. In addition, water temperature, salinity, and dissolved oxygen were measured at each site on each sample date with a YSI handheld multiparameter meter. Water samples also were collected for dissolved inorganic nutrient analysis (NO_x, NH_3, oPO_4) on each sample date. Water was collected approximately 2-3 feet below the surface directly next to kelp lines at each site. Immediately following collection, the water samples were filtered through a 0.22 um syringe filter, and the filtered samples were then stored frozen until analysis on an Lachat auto-analyzer.

2c. Summer/fall seaweed experiments - *Gracilaria* spp. and *Ulva* spp.

2c.1. Cultivation Methods

Cultivation trials with the red branching macroalgae *Gracilaria* sp. and the green macroalgae *Ulva* sp. (i.e., sea lettuce) were conducted in Northport Harbor and Mt. Sinai Harbor during the summer and fall of 2021 and 2022 (Figure 2b.1). Both seaweeds were grown in shallow, near-shore waters (~ 2-3 ft MLW) using floating mesh bags that are typically used for shallow-water oyster farming (Figure 2c.1). Each floating bag was composed of a rectangular oyster grow-out bag (36” x 18” x 3”) made of semi-rigid polyethylene mesh, with plastic, air-filled, cylindrical floats (3” diameter) attached to each long side with 4 plastic ties (Figure 2c.1). Two longline clips were strapped on one side of each bag to attach the bags to horizontal ropes. Bags were opened and closed on one end using stainless steel clips. To control biofouling, the bags were flipped over once every one to two weeks to expose the underside to sun and air and kill biofouling organisms.

The floating bags of seaweed were deployed in an array consisting of five closely spaced rows of five bags each (Figure 2c.2). The entire 25-bag array was constructed as a single unit, with 8-ft long wood studs (2” x 4”) acting as spreader bars on each end to space four parallel horizontal lines, each 20-ft long, and spaced approximately 2-ft apart. Five bags were attached to one side of each of the lines, and an additional row of 5 bags was added to the other side of one of the end lines. The floating array was anchored at either end with 4-ft screw-down anchors. The grow-out bags were deployed in this dense array in order to maximize any impacts of the seaweeds on the surrounding water quality.

Two size classes (i.e., small and large) of oyster seed were co-cultivated with the seaweeds in the 25-bag floating arrays, except for the 2021 *Gracilaria* experiments in which only one size class of oysters was deployed (i.e., large). Across all experiments for both seaweed species in both locations, the starting size of the small oyster size class ranged between 11-14 mm (shell height), and the starting size of the large oyster size class ranged between 23-51 mm (Table 2c.1). Within each 25-bag array, three bags contained large oysters with seaweed, three bags contained small oysters with seaweed, three bags contained a mix of large and small oysters without seaweeds, and the remaining 16 bags conatined seaweed only (Figure 2c.3). The nine bags containing oysters were positioned within the center of the array, with all 16 perimeter bags containing seaweed only (Figure 2c.3). The three bags with oysters only were positioned in the very center of the array. Oysters were positioned within the center of the array and surrounded by bags of seaweed so that any impacts of seaweed co-cultivation on oyster growth would be maximized. Control oysters with no surrounding seaweeds were grown in a separate, smaller array positioned 50 meters away from the experimental array. The control array consisted of a single line holding 6 floating bags, three with small oysters and three with large oysters.

Both *Ulva* and *Gracilaria* were cultivated through vegetative propagation, with each bag filled with a known starting quantity of parental tissue collected from Shinnecock Bay, NY (Table 2c.1). For *Ulva* cultivation, starting quantities of 10g and 20g of parental tissue were added to each grow-out bag in the 2021 and 2022 cultivation experiments, respectively. For *Gracilaria* cultivation, starting quantities of 200g or 1,500g of parental tissue were added to grow-out bags in 2021, with 200g used in bags with oysters, and 1,500g used in the peripheral bags without oysters. In 2022, a starting quantity of 20g of *Gracilaria* was added to each grow-out bag. Cultivation experiments were conducted between late June and mid-August for *Ulva*, and between late July and early October for *Gracilaria* (Table 2c.1).

2c.2. Summer seaweed monitoring

In the 2021 *Gracilaria* and *Ulva* experiments, seaweeds were weighed weekly over the course of each experiment to monitor growth and quality, and determine when 'peak biomass' in the grow-out bags was reached. In order to determine when peak biomass was reached, experiments in 2021 were terminated at the time point following peak biomass, when growth declined and macroalgae biomass began to deteriorate, often resulting in loss of biomss. The 2021 growth data was used to determine the optimal cultivation period for each species (i.e., number of days until macroalgae biomass should be harvested to yield peak biomass). The duration of the 2022 cultivation trials for *Ulva* and *Gracilaria* was based on the optimal cultivation periods identified for each species in 2021, and in 2022 only final weights was measured at the end of each experiment.

At the end of each cultivation trial, seaweeds were removed from bags and weighed (fouled wet weight), cleaned of biofouling, spun in a salad spinner to remove excess water, and weighed again after cleaning (cleaned wet weight). Subsamples of the cleaned seaweed were weighed and dried in a drying oven at 60 °C. After complete drying, each sample was ground into powder to homogenize the tissue, and 3-6 mg was weighed out for N analysis. Samples were run on a combustion elemental analyzer at the Center for Clean Water Technology to determine N content, which was expressed as a percentage of tissue biomass (dry weight).

The amount of seaweed biomass grown in a bag over the course of an experiment, referred to as 'bag yield', was computed as the difference between final and initial wet weights (kg bag^{-1}). Since daily growth is a percentage of the starting amount of biomass, the absolute amount of biomass grown over a given period of time is dependent on the initial starting quantity of seaweed in each bag. To compare growth rates among sites and seaweed species, daily growth rates were therefore computed as a percentage per day using the following equation:

$$GR = (10^{(\log (F/I)/t)} – 1) \times 100$$

where: GR = growth rate (% per day)
F = final wet weight
I = initial wet weight
T = experiment duration (days)

2c.3. Quantifying nitrogen bioextraction

To quantify the amount of nitrogen sequestered by *Ulva and Gracilaria*, bag yields (kg bag^{-1}) were first converted from wet weight to dry weight yields, using the average ratio of dry to wet weight obtained or each species from samples dried over the course of the experiment. The dry weight yields were then multiplied by the nitrogen concentration in the seaweed tissue, as determined from the N analysis of dried tissue samples described above. The result expressed nitrogen bioextraction in terms of kilograms of nitrogen extracted per bag.

2c.4. Water quality monitoring

Continuous pH and dissolved oxygen data was collected for the field experiments using Onset® HOBO® devices to track environmental changes and trends at each site.

2d. Mapping water quality around a LIS kelp farm

GreenWave's Thimble Island Ocean Farm is one of the largest sugar kelp farms in Long Island Sound. To assess the impact that this large grow-out of kelp in LIS has had on surrounding water quality, multiple cruises were performed between March and June of 2021 to the Thimble Island Farm and its surroundings. These cruises utilized a continuous flow system developed to measure surface water characteristics at a high spatial resolution (< 1 m), drawing water from a rigid intake affixed to the small resear5ch vessel ~0.5m below the air-sea interface. The water was delivered via laminar flow into a custom-built flow cell without bubbling. This cell contained a comprehensive sensor package, including a YSI EXO3 multi-parameter sonde, a Contros HydroC™ CO_2 sensor, and a SeaFET pH sensor. This setup provided continuous measurement of pCO_2, dissolved oxygen, pH, and temperature. In addition to this, discrete water samples were taken at 4 stations within the kelp lines and 4 stations ~150 m outside of the kelp lines to quantify and compare concentrations of dissolved nitrogen species (NO_3^- and NH_4^+). Using discrete station water samples, chlorophyll *a* was also analyzed by filtering samples onto glass fiber filters and using a Trilogy Fluorometer (Turner Designs™) using standard, non-acidification methods.

Four cruises were performed – on 3/30/21, 4/13/21, 5/18/21, and 6/9/21.

3. Results

3a. Growth and bioextraction - *Saccharina latissimi* (sugar kelp)

3a.1. Sugar kelp growth in Long Island Sound

Kelp lines were seeded in December at all sites, and the kelp was grown and monitored through late May-early June, at which time all the kelp was harvested. Growth was most rapid at all sites from early April to early/mid-May, at which point growth began to decline, particularly at the shallow harbor sites (Figure 3a.1). This slowing in growth was accompanied by increased biofouling and deterioration of kelp blades at the two shallow harbor sites, while kelp in the deeper East River showed little deterioration or biofouling through the final harvest on 5/24/22. Biofouling was heaviest at the shallow Northport site, where eastern mud snails (*Ilyanassa obsoleta*) began utilizing the kelp lines in early May as a breeding ground and substrate to deposit their eggs (Figure 3a.2a). This infestation by mud snails and accompanying deterioration of kelp tissue rendered the kelp commercially useless for food markets by early/Mid-May. Biofouling was less problematic in Mt. Sinai where mud snails did not infest kelp lines, but here too kelp blades began to deteriorate in early/mid-May, particularly at the blade tips which began to lose pigments and break apart (Figure 3a.2b). Optimal harvest times at the two harbor sites, therefore, were considered to occur in early to mid-May, just prior to the onset of deterioration when biomass yields were at or near their maximum.

Within each of the three LIS sites, kelp growth differed among lines of different seedstock over the course of the growing season (Figure 3a.1). Line yields in early May significantly differed among seedstocks in the East River (one-way ANOVA, F=5.49, $p<0.05$; Figure 3a.3A), Northport Harbor (one-way ANOVA, F=5.74, $p<0.05$; Figure 3a.3B), and Mt. Sinai Harbor (one-way ANOVA, F=9.48, $p<0.01$; Figure 3a.3C). At all sites, line yields were highest on lines with seedstock SB-MTK-low, with SB-MTK-low producing significantly higher yields than both SB-BL-low and H-RP-high in the East River (one-way ANOVA and Tukey HSD, $p<0.05$ all; Figure 3a.3A), significantly higher yields than SB-BL-low in Northport Harbor (one-way ANOVA and Tukey HSD, $p<0.05$; Figure 3a.3B), and significantly higher yields than H-RP-high in Mt. Sinai Harbor (one-way ANOVA and Tukey HSD, $p<0.05$; Figure 3a.3C). No significant differences were found among replicate lines of the same seedstock within the East River and Northport Harbor (one-way ANOVAs and Tukey HSD, n.s. all; Figure 3a.3A-B). These within-site comparisons demonstrated that kelp growth (and hence the resulting bioextraction) is, in part, a function of the seedstock used. Controlling for seedstock is therefore important when making comparisons of kelp growth among sites.

Kelp growth over the growing season differed among the three Long Island Sound study sites for each of the three seedstocks deployed (Figure 3a.4). Line yields at optimal biomass significantly differed among sites for seedstocks SB-MTK-low (one-way ANOVA, F=385.15, $p<0.0001$; Figure 3a.5A), SB-BL-low (one-way ANOVA, F=8.82, $p<0.05$; Figure 3a.5B), and H-RP-low (one-way ANOVA, F=102.80, $p<0.0001$; Figure 3a.5C). For each seedstock, line yields at optimal biomass were significantly higher in the East River than in Northport and Mt. Sinai Harbors (one-way ANOVAs and Tukey HSD, $p<0.05$ all; Figure 3a.5). Line yields in Northport and Mt. Sinai Harbor only differed for one of the three seedstocks (SB-MTK-low), with greater yields obtained in Mt. Sinai Harbor (one-way ANOVAs and Tukey HSD, $p<0.05$; Figure 3a.5A). The best yielding seedstock, SB-MTK-low, produced line yields of 11.8 kg m^{-1} in the East River (5/17/22), 1.04 kg m^{-1} in Northport Harbor (5/5/22), and 2.24 kg m^{-1} in Mt. Sinai Harbor (5/6/22).

3a.2. Inter-estuary comparison of kelp growth

Sugar kelp was successfully cultivated along horizontal longlines in all estuaries surrounding Long Island, NY, including the Long Island Sound, the South Shore Estuary, and the Peconic Estuary (Figures 3a.6 & 3a.7). There was, however, a significant difference in line yield at peak biomass among the eight sites where kelp was grown (one-way ANOVA, F=69.88, $p<0.0001$; Figure 3a.8, Table). The highest line yields were obtained in the East River (11.8 kg m^{-1}) and in Moriches Bay (10.7 kg m^{-1}), the deepest and the shallowest of the eight locations, respectively (Figure 3a.8). Line yields at these two sites did not significantly differ from each other, and both were significantly higher than all other sites (one-way ANOVA and Tukey HSD; $p < 0.01$ all; Figure 3a.8). The next highest line yields were found in Mt. Sinai Harbor (2.2 kg m^{-1}), followed by Northport Harbor (1.0 kg m^{-1}), and then all four sites in the Peconic Estuary (0 to 0.063 kg m^{-1}), but differences among these six sites were not significant (one-way ANOVA and Tukey HSD;

n.s. all; Figure 3a.8). On average, dry tissue weight was 11.2 ± 2.1 percent of the wet tissue weight, with fresh kelp losing nearly 90% of its weight when dried (Figure 3a.9).

3a.3. Nitrogen content in kelp tissue

Across sites and dates through the growing season, the nitrogen content of kelp tissue ranged from 0.75% to 4.76% of tissue biomass (dry weight), an over six-fold difference between minimum and maximum values (Figure 3a.10). At all sites, with the exception of the Peconic Estuary, the nitrogen content of kelp tissue declined through the growing season, with the highest values found early in the growing season (i.e., late January to early February) when the sporophytes were still very small, and the lowest values late in the growing season (May) when the kelp lines reached their peak yields (Figure 3a.10). At the Peconic Estuary site, the nitrogen content of kelp tissue remained relatively low throughout the growing season, peaking in April before declining in May.

Consistent differences in kelp nitrogen content were observed among sites throughout the growing season with few exceptions, with the highest nitrogen content found in kelp from the East River, followed by Moriches Bay, Northport Harbor, Mt. Sinai Harbor, and the Peconic Estuary (Figure 3a.10). At the time of peak biomass yields in May, the nitrogen content of kelp tissue ranged from 0.85 to 2.66 percent of tissue biomass (dry weight) across sites, and significantly differed among sites (one-way ANOVA, $F=46.07$, $p<0.0001$; Figure 3a.11, Table 3a.1). The nitrogen content of kelp tissue in the East River was significantly greater than that at all other sites (one-way ANOVA and Tukey HSD, $p<0.05$ all; Figure 3a.11). Kelp nitrogen content in Moriches Bay and Northport Harbor, the next two highest sites, did not significantly differ, and both of these sites had significantly greater nitrogen content than Mt. Sinai Harbor and the Peconic Estuary, which did not significantly differ from each other (one-way ANOVA and Tukey HSD, $p<0.01$ for all significant differences; Figure 3a.11).

3a.4. Nitrogen bioextraction through kelp farming

The amount of nitrogen sequestered per meter of kelp line increased through the growing season as the kelp grew and biomass yields increased, and maximum nitrogen bioextraction (i.e., removal of nitrogen when kelp is harvested) was obtainable at peak line biomass in May at all sites (Figure 3a.12). Among the eight study sites, the highest nitrogen bioextraction per meter of kelp line was obtained in the East River (35.3 g m^{-1}), followed by Moriches Bay (24.2 g m^{-1}), Mt. Sinai Harbor (2.3 g m^{-1}), Northport Harbor (1.9 g m^{-1}), and the Peconic Estuary (0.6 g m^{-1}; Figure 3a.13A, Table 3a.1). Nitrogen bioextraction in the East River and Moriches Bay far exceeded that of all other study sites due to the substantially higher line yields achieved at these sites (Figure 3a.8, Table 3a.1), as well as the higher nitrogen content in kelp tissue (Figure 3a.11, Table 4). Nitrogen bioextraction per meter of line was ~45% higher in the East River than Moriches Bay despite just a ~10% difference in line yields, with the difference driven by the significantly higher nitrogen concentration in kelp tissue from the East River (2.66% N) than Moriches Bay (2.01% N). Nitrogen bioextraction was nearly equal between Mt. Sinai Harbor and Northport Harbor, with higher line yields in Mt. Sinai Harbor offset by lower nitrogen concentrations in the kelp tissue.

To convert nitrogen bioextraction from N per meter of line to N per acre, it is necessary to make assumptions about the amount of kelp line that could be installed per acre. In this study, experimental kelp lines were spaced ~1.5 meters apart in shallow waters (Moriches Bay, Northport Harbor, Mt. Sinai Harbor), and at least six meters apart in deeper waters (East River, Peconic Estuary). Kelp lines were spaced further apart in deep waters so that a boat could be navigated between the lines, while in shallow waters lines could be spaced closer together because it was possible to walk between and access the lines at low tide. The actual spacing required between kelp lines in large-scale deployments will depend on a variety of factors, including the type of boat used, harvesting techniques, and site-specific conditions such as water depth. In addition, recent advances in kelp farming methods, such as the use of spreader bars to deploy multiline arrays of kelp in deep water, can substantially increase the number of kelp lines deployed per acre. For the purposes of the calculations here, however, we assume single lines of kelp spaced 1.5 and 6 meters apart in shallow and deep waters, respectively, as was deployed in this study. At 1.5-meter line spacing in shallow waters, approximately 2,400 linear meters of kelp line can be deployed (40 kelp lines, 60 meters long each; Table 3a.1). At 6-meter line spacing in deep waters, approximately 600 linear meters of kelp line can be deployed (10 kelp lines, 60 meters long each; Table 3a.1).

Despite higher nitrogen bioextraction per meter of line in the East River, the highest nitrogen bioextraction on a per acre basis was achieved in Moriches Bay (Figure 3a.13B, Table 4). This was because kelp lines were spaced closer together in the shallow Moriches Bay site than the deeper East River site, allowing four times more linear meters of kelp to be deployed in Moriches Bay (Table 3a.1).

3a.5. Water quality monitoring

Water temperatures followed a similar pattern among all sites during the growing season, rising from lows around 1-2 °C in January to highs ranging from ~16-20 °C in the end of May (Figure 3a.14A). Over the course of the growing season, temperatures were typically highest in Moriches Bay and Northport Harbor, and lowest in the East River (Figure 3a.14A). Salinity varied among sites and through time within sites during the 2022 kelp growing season, ranging from a low of 21.2 ppt (Northport Harbor on 2/9/22) to a high of 30.9 ppt (Moriches Bay on 12/30/21; Figure 3a.14B). Over the course of the growing season, salinities were typically highest in the Peconic Estuary and Moriches Bay, and lowest in the East River and Northport Harbor (Figure 3a.14B). Concentrations of dissolved oxygen followed a similar pattern among all sites during the growing season, dropping from highs ranging between 13-16 mg l^{-1} in January/February to lows ranging between 8-9 mg l^{-1} in the end of May (Figure 3a.14C). At all sites on all dates concentrations of dissolved oxygen were above threshold levels (3 mg l^{-1}) of concern that can impact marine life.

Concentrations of dissolved nitrate/nitrite (NO_x) varied substantially among sites and through time within sites (Figure 3a.15). Averaged over the course of the kelp growing season, NO_x concentrations were highest in Northport Harbor, followed by the East River, Moriches Bay, Mt. Sinai Harbor, and the Peconic Estuary (Figure 3a.16). Average NOx concentration significantly differed among sites (ANOVA, $F=3.73$, $P=0.01$), with the only significant difference in pairwise comparisons found between Northport Harbor and the Peconic Estuary, where NO_x concentrations were highest and lowest, respectively (ANOVA and Tukey HSD, p<0.01).

3b. Growth and bioextraction - *Ulva spp.*

In the 2021 cultivation experiments in both Northport Harbor and Mt. Sinai Harbor, *Ulva* grew for approximately five weeks (32-38 days), after which point growth declined and the macroalgae biomass began to deteriorate, often resulting in a loss of biomass (Figure 3b.1). These results indicate that, when using a floating bag cultivation system, *Ulva* should be harvested and replaced approximately every four to five weeks in order to maximize production and nutrient removal.

Overall, *Ulva* growth did not differ between grow-out bags in which *Ulva* was co-cultivated with oysters and grow-out bags in which *Ulva* was cultivated independently (Figure 3b.2). Only one significant difference in *Ulva* growth between treatments with and without oysters was found across the three experiments (Northport 2021, ANOVA, F=6.92, p<0.05, Figure 3b.2). Otherwise, no significant differences were found between shellfish treatments. Averaging all treatments together within each experiment, *Ulva* growth rates ranged from 8.5 to 14.2 percent per day across, and significantly differed among the three experiments (ANOVA, F=12.16, p<0.001, Figure 3b.3). *Ulva* growth was significantly higher in Northport Harbor than Mount Sinai Harbor in 2021 (ANOVA and Tukey HSD, p<0.01), and *Ulva* growth was significantly higher in Northport Harbor in 2021 than 2022 (ANOVA and Tukey HSD, p<0.05).

To estimate the potential biomass yields and nitrogen bioextraction obtainable through *Ulva* cultivation and harvest, we used the range of *Ulva* growth rates found in the three field experiments, and assumed that grow-out bags are inoculated with 20g of *Ulva* tissue and harvested after four weeks (the optimal cultivation period found in this study). Under these assumptions, we calculated that harvest yields per bag (i.e., final weight minus initial weight) ranged from 176 to 811 g, in terms of wet weight biomass (Table 3b.1). On average, the dry weight of *Ulva* tissue was 16.2 ± 1.7 percent of the wet tissue weight (Figure 3b.4). Thus, dry weight bag yields ranged from 29 to 131 g bag^{-1} (Table 3b.1).

To estimate the amount of *Ulva* biomass that can be cultivated per acre using the floating bag cultivation method, we assumed that a one-acre (63.6 m x 63.6 m) farm has the capacity to hold 1,600 floating bags, configured in 32 parallel rows spaced 2 meters apart, with 50 bags in each row. This assumption is based on actual floating bag densities used on shallow-water oyster farms on Long Island. This bag density would produce 281 to 1,297 kg of *Ulva* per acre every four weeks. Based on field observations of wild *Ulva* abundance, we estimate a 16-week growing season for *Ulva* from mid-May through mid-September. This would allow for four harvests per year, producing 1,126 to 5,189 kg wet weight, or 182 to 841 kg dry weight, of *Ulva* per acre per year (Table 3b.1).

Ulva from Shinnecock Bay, the parental source used to inoculate experimental grow-out bags in Northport and Mount Sinai Harbors, was found to contain 2.2% nitrogen and 28.1% carbon in the dry tissue biomass. Using these values, and the harvest estimates above, we estimate that 4.1 to 18.9 kg of N and 53.1 to 244.5 kg of C can be extracted per acre per year through *Ulva* cultivation in Long Island Sound harbors similar to Northport and Mount Sinai Harbors (Table 3b.1).

3c. Growth and bioextraction - *Gracilaria spp.*

In the 2021 cultivation experiments in both Northport Harbor and Mt. Sinai Harbor, *Gracilaria* grew for approximately two to three weeks, after which point growth declined and the macroalgae biomass began to deteriorate, often resulting in a loss of biomass (Figure 3c.1). These results indicate that, when using a floating bag cultivation system, *Gracilaria* should be harvested and replaced approximately every 2.5 weeks (~18 days) in order to maximize production and nutrient removal.

Within each of the four *Gracilaria* cultivation experiments conducted (two in 2021 and two in 2022), no significant differences were found in *Gracilaria* growth rates among treatments with and without oysters (ANOVAs, n.s.; Figure 3c.2). Averaging all treatments together, *Gracilaria* growth rates ranged from 0.2 to 5.4 percent per day across the four experiments. *Gracilaria* growth rates significantly differed among the four experiments (ANOVA, $F=16.51$, $p<0.001$, Figure 3c.3). *Gracilaria* growth was significantly lower in Northport Harbor in 2022 than in each of the other three experiments, which did not significantly differ from each other (ANOVA and Tukey HSD, $p<0.01$ for significant differences). The poor *Gracilaria* growth in Northport Harbor in 2022 thus appeared to be an outlier as growth in the other three experiments (Northport 2021, Mt. Sinai 2021, Mt. Sinai 2022) fell within a tighter range between 4.29 and 5.35 percent per day.

To estimate the potential biomass yields and nitrogen bioextraction obtainable through *Gracilaria* cultivation and harvest, we used the range of *Gracilaria* growth rates found in the field experiments, and assumed that grow-out bags are inoculated with 20g of *Gracilaria* tissue and harvested after 2.5 weeks (the optimal cultivation period found in this study). Under these assumptions, we calculated that harvest yields per bag (i.e., final weight minus initial weight) ranged from 0.8 to 31.1 g, in terms of wet weight biomass (Table 3c.1). On average, the dry weight of *Gracilaria* tissue was 12.8 ± 1.0 percent of the wet tissue weight (Figure 3c.4). Thus, dry weight bag yields ranged from 0.1 to 4.0 g bag^{-1} (Table 3c.1).

To estimate the amount of *Gracilaria* biomass that can be cultivated per acre using the floating bag cultivation method, we again assumed that a one-acre (63.6 m x 63.6 m) farm has the capacity to hold 1,600 floating bags, configured in 32 parallel rows spaced 2 meters apart, with 50 bags in each row. This bag density would produce 1.3 to 49.8 kg of *Gracilaria* per acre every 18 days. Based on field observations of wild *Gracilaria* abundance, we estimate a 16-week growing season for *Gracilaria* from mid-June through mid-October. This would allow for six harvests per year, producing 7.7 to 298.7 kg wet weight, or 1.0 to 38.2 kg dry weight, of *Gracilaria* per acre per year (Table 3c.1).

Gracilaria cultivated in Northport Harbor had significantly higher tissue nitrogen content than *Gracilaria* cultivated in Mount Sinai Harbor ($t=6.17$; $p<0.001$; Figure 3c.5A), but tissue carbon content did not differ between the two harbors ($t=1.72$; n.s.; Figure 3c.5B). *Gracilaria* cultivated in Northport Harbor contained 3.87% nitrogen and 23.48% carbon in dry tissue biomass, and

Gracilaria cultivated in Mount Sinai Harbor contained 3.34% nitrogen and 22.53% carbon in the dry tissue biomass. Using these values, and the harvest estimates above, we estimate that 0.4 to 1.48 kg of N and 0.23 to 8.98 kg of C can be extracted per acre per year through *Gracilaria* cultivation in Long Island Sound harbors similar to Northport and Mount Sinai Harbors (Table 3c.1).

3d. Seaweed impacts on bivalve growth and water quality

3d.1. Kelp-bivalve co-cultivation experiments

Northport Harbor: In Northport Harbor, the height-based growth rate of mussels was slightly higher within kelp lines than outside kelp lines, but the difference was not significant ($t=0.75$, n.s., Figure 3d.1A). The weight-based growth rate of mussels also was slightly higher within kelp lines than outside kelp lines, but the difference also was not significant ($t=-0.77$, n.s., Figure 3d.1B). In regards to oysters, the height-based growth rate of oysters was slightly higher within kelp lines than outside kelp lines, but the difference was not significant ($t=-0.27$, n.s., Figure 3d.1C). The weight-based growth rate of oysters also was slightly higher within kelp lines than outside kelp lines, but the difference also was not significant ($t=-1.24$, n.s., Figure 3d.1D). Seawater pH and dissolved oxygen were elevated inside the kelp cultivation area compared to outside the kelp cultivation area in Northport Harbor (Figure 3d.2).

Mount Sinai Harbor: In Mount Sinai Harbor, the height-based growth rate of mussels was slightly higher within kelp lines than outside kelp lines, but the difference was not significant ($t= -2.53$, n.s., Figure 3d.3A). The weight-based growth rate of mussels, however, was significantly higher inside than outside of kelp lines ($t = -5.25$, $p<0.01$, Figure 3d.3B). In regards to oysters, the height-based growth rate of oysters was slightly higher within kelp lines than outside kelp lines, but the difference was not significant ($t= -0.61$, n.s., Figure 3d.3C). The weight-based growth rate of oysters, however, was slightly higher within kelp lines than outside kelp lines ($t=-5.54$, $p<0.01$, Figure 3d.3D). Seawater pH and dissolved oxygen were similar inside the kelp cultivation area and outside the kelp cultivation area in Mount Sinai Harbor (Figure 3d.4).

Thimble Island Ocean Farm: At Greenwave’s Thimble Island kelp farm, the height-based growth rate of mussels was slightly higher within kelp lines than outside kelp lines, but the difference was not significant ($t= -0.95$, n.s., Figure 3d.5).

3d.2. Ulva-oyster co-cultivation experiments

Across the three *Ulva* cultivation experiments conducted, including in Northport Harbor in 2021 and 2022 and Mount Sinai Harbor in 2021, co-cultivation with or near *Ulva* had little effect on the growth rate of oysters compared to control oysters cultivated without *Ulva* (Figure 3d.6). Height-based growth rates of small oysters did not significantly differ among *Ulva* treatments in any of the three cultivation experiments conducted, and only significantly differed for large oysters in one of the three experiments (ANOVAs, Figure 3d.6). Seawater pH and dissolved oxygen were similar inside and outside *Ulva* cultivation areas in Northport Harbor in 2022 (Figure 3d.7).

3d.3. Gracilaria-oyster co-cultivation experiments

In the 2021 *Gracilaria* experiments, significant differences were found in oyster growth among co-cultivation treatments in both Northport Harbor and Mount Sinai Harbor, but the differences were not consistent across the two harbors. In Northport Harbor, height-based growth was significantly higher in the treatment with *Gracilaria* than in treatments near *Gracilaria* and away from *Gracilaria* (control), but weight-based growth did not differ among treatments (Figures 3d.8A-B). In Mount Sinai Harbor, both height-based and weight-based growth was significantly higher in the treatment near *Gracilaria* than in the control treatment away from *Gracilaria*, but the treatment with *Gracilaria* did not significantly differ from the control (Figures 3d.8C-D).

In the 2022 *Gracilaria* experiments, oyster growth overall tended to be higher in treatments with *Gracilaria* than in control treatments away from *Gracilaria*, but differences were only significant in some cases (Figures 3d.9-3d.10). In Northport Harbor, height-based growth was significantly greater for small oyster seed that was co-cultivated with *Gracilaria* than small oyster seed grown separately, but no difference was observed in the growth of large oyster seed among treatments (Figure 3d.9A). In Mount Sinai Harbor, height-based growth was significantly greater for both small and large oyster seed in co-cultivation treatments with *Gracilaria* (Figure 3d.10A). Seawater pH and dissolved oxygen were similar inside and outside *Gracilaria* cultivation areas in Northport Harbor (Figure 3d.9C-D) and Mount Sinai Harbor (Figure 3d.10C-D) in 2022.

3e. Mapping water quality around a LIS kelp farm

3e.1. Discrete sample analysis

On all dates besides 4/13, both nitrate and ammonia concentrations were lower within the kelp field than the perimeter, though these results were not statistically significant (Figures 3e.1 & 3e.2). This could be due to the low number of samples, and this data could be improved via the implementation of a nitrate sensor for the flow-through system.

On the first two cruises, there did not seem to be a correlation between chlorophyll *a* levels and kelp presence (Figure 3e.3A-B); however, in the later two cruises (5/18 and 6/9), there tended to be lower chlorophyll *a* concentrations within the kelp farm compared to the perimeter (Figure 3e.3C-D). This trend was statistically significant in June, reducing chl *a* by 8.6% relative to the perimeter ($p = 0.04$, Figure 3e.3D).

3e.2. Flow-through data analysis

Flow-through data was time-mapped to data within the kelp field, and data for the perimeter of the kelp field, and was then displayed for relevant parameters in a boxplot (see Figures 3e.4-3e.7) and analyzed using a two-sample *t*-test. For the 3/30, 4/13, and 5/18 cruises, pH data was obtained from the SeaFET sensor; for the 6/9 cruise, this sensor was malfunctioning, so the probe for the YSI EXO3 was used instead. The temperature and dissolved oxygen data was obtained from the YSI EXO3 sonde for all cruises. The pCO_2 data was obtained from the Contros HydroC sensor, which was unavailable for the 5/18 cruise.

On 3/30, pH was significantly higher within the kelp (kelp field – 8.095, perimeter – 8.091; Fig. 3e.4A), and pCO_2 was significantly lower (kelp field – 326.97 μatm, perimeter – 328.19 μatm;

Fig. 3e.4B). DO was significantly lower within the kelp field as well (kelp field – 9.938 mg L^{-1}, perimeter – 9.951 mg L^{-1}; Fig. 3e.4C), and temperature was significantly higher (kelp field – 7.15°C, perimeter – 7.09°C; Fig. 3e.4D).

On 4/13, pH was significantly higher within the kelp (kelp field – 8.031, perimeter – 8.028; Fig. 3e.5A), and there were no significant pCO_2 trends (kelp field – 365.96 µatm, perimeter – 365.75 µatm; Fig. 3e.5B). DO was significantly higher within the kelp field (kelp field – 9.985 mg L^{-1}, perimeter – 9.963 mg L^{-1}; Fig. 3e.5C), and temperature was significantly lower (kelp field – 8.53°C, perimeter – 8.72°C; Fig. 3e.5D).

On 5/18, pH was significantly lower within the kelp (kelp field – 7.928, perimeter – 7.949; Fig. 3e.6A), DO was significantly higher within the kelp field (kelp field – 9.053 mg L^{-1}, perimeter – 9.026 mg L^{-1}; Fig. 3e.6B), and temperature was significantly lower (kelp field – 13.51°C, perimeter – 13.61°C; Fig. 3e.6C).

Finally, on 6/9, pH was significantly higher within the kelp (kelp field – 7.90, perimeter – 7.88; Fig. 3e.7A), pCO_2 was significantly lower within the kelp field (kelp field – 555.34 µatm, perimeter – 565.86 µatm; Fig. 3e.7B). DO was significantly higher within the kelp field (kelp field – 8.28 mg L^{-1}, perimeter – 8.20 mg L^{-1}; Fig. 3e.7C), and there were no significant temperature trends (kelp field – 17.47°C, perimeter – 17.46°C; Fig. 3e.7D).

3e.3 Mapping water quality around a LIS Ulva deployment

A 25-bag Ulva array was deployed in Northport Harbor as depicted in Figure 2C2 and the Hycat autonomous surface vehicle equipped with a YSI EXO3 sonde was used to map surface pH and DO levels. This mapping effort demonstrated that during the day the levels of DO and pH around the Ulva array were elevated relative to background levels (Fig 3e8).

FIGURES AND TABLES

Figure 2b.1. Map of eight locations around long Island where kelp cultivation studies were conducted during the 2022 kelp growing season (December 2021 to May/June 2022). The locations are labeled as follows: NP = Northport Harbor, MS = Mount Sinai Harbor, ER = East River, MB = Moriches Bay, and PE2 to PE5 = Peconic Estuary sites. The summer seaweeds *Gracilaria* sp. and *Ulva* sp. were cultivated at Northport Harbor (NP) and Mount Sinai Harbor (MS) in Long Island Sound during the summer and fall of 2021 and 2022.

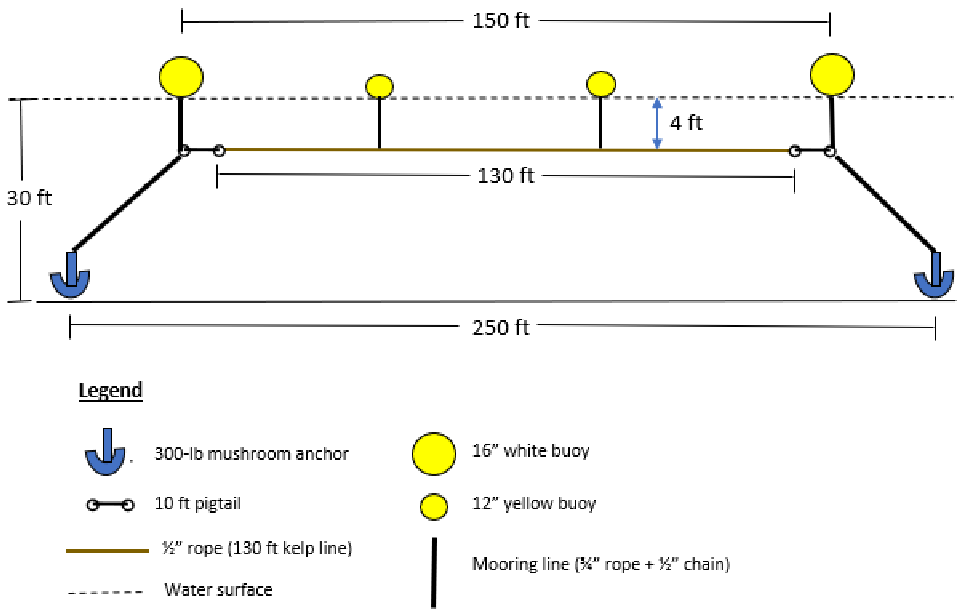


Figure 2b.2. Side-view diagram of suspended kelp line installations in deep waters (~30 m deep) at the East River site (not drawn to scale).

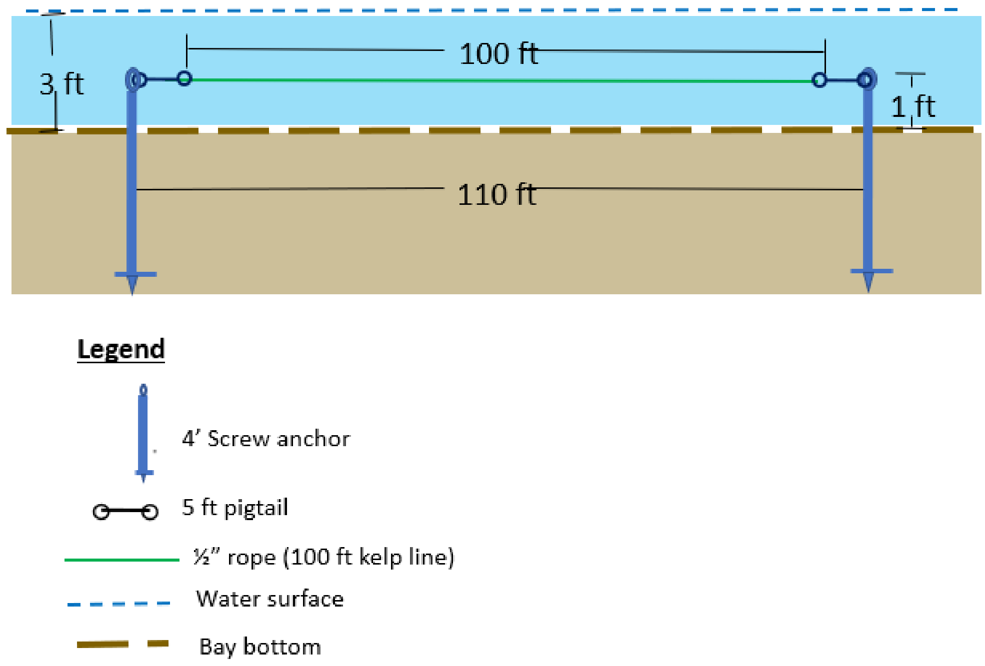


Figure 2b.3. Side-view diagram of staked kelp line installations in shallow waters (< 4 ft MLW) at the Northport Harbor and Mt. Sinai Harbor sites (not drawn to scale).

Figure 2b.4. Map showing 1) the three locations where reproductive kelp tissue was collected from wild populations to produce kelp seedstock (yellow pins), and 2) the two hatcheries that produced kelp seedstock for the 2022 kelp cultivation experiments in Long Island Sound (orange pins).

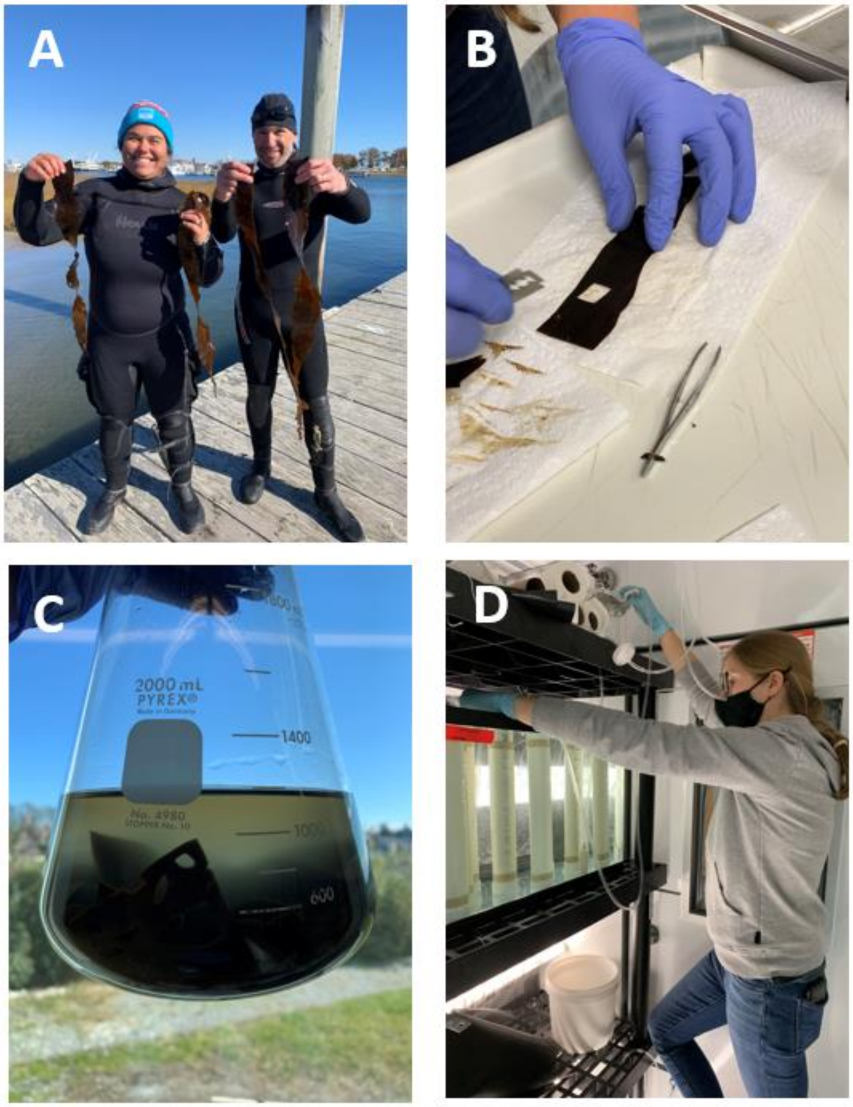


Figure 2b.5. Photos outlining steps in the process of making kelp seedstock, including (A) collecting reproductive tissue (i.e., sorus tissue) from local wild populations of sugar kelp, (B) preparing the sorus tissue for spore release, (C) releasing spores from the sorus tissue following overnight desiccation, and (D) adding the spores to aquaria containing with spools of string.

Figure 2b.6. Photos showing the wire cage used to house replicate mesh bags of oysters and blue mussels inside and outside of kelp lines at Northport Harbor and and Mt. Sinai Harbor. This photo was taken at the Mount Sinai Harbor site.

Figure 2b.7. Photos illustrating mussel cultivation along vertical lines at the Thimble Island farm in CT, including (A) wild-collected mussel seed that was counted and measured in the lab before field deployment, (B) placing the mussel seed into mussel socks, (C) attaching the mussel socks to horizontal longlines, with the mussel socks hanging vertically from the horizontal longlines (D) harvesting the vertical mussel lines at the conclusion of the experiment, 2.5 months after the mussel seed was deployed.

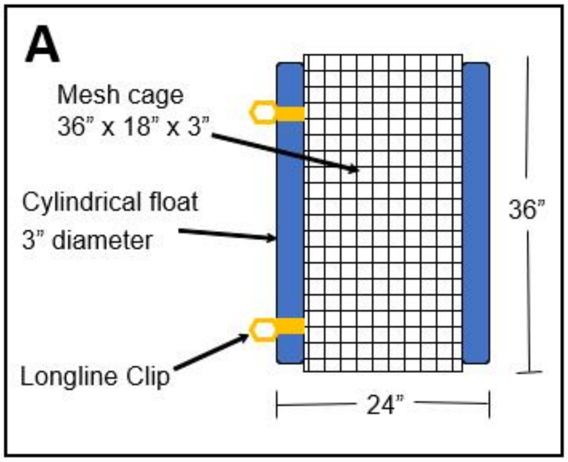


Figure 2c.1. **(A)** Diagram and **(B)** photo of floating grow-out bag used to cultivate *Gracilaria* sp and *Ulva* sp. The bag is commonly used to cultivate oysters, particularly in shallow waters.

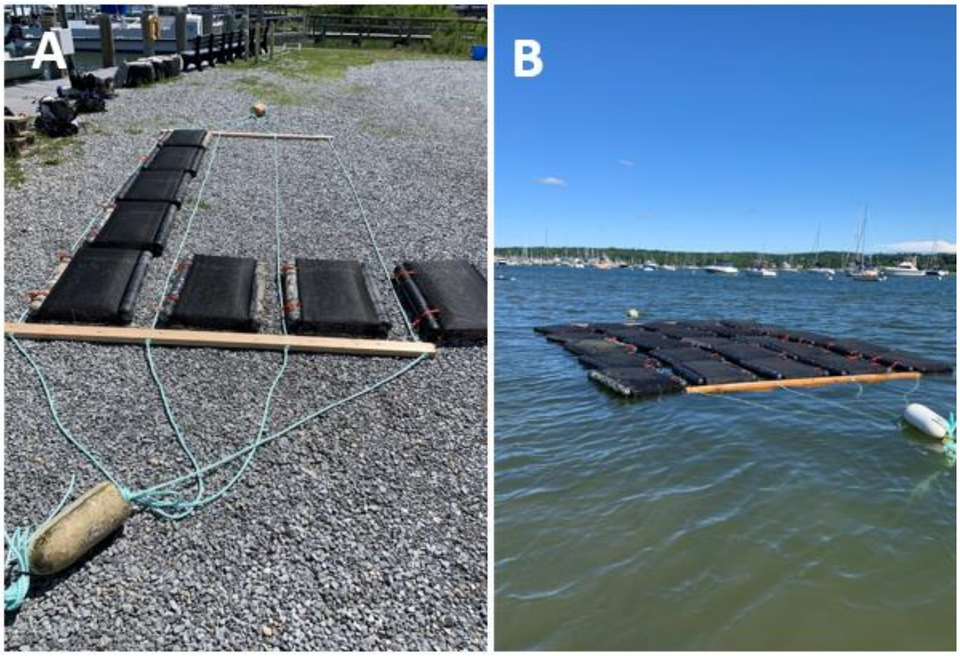


Figure 2c.2. Photos of the 25-bag array used to cultivate *Gracilaria* sp. and *Ulva* sp. in Northport Harbor and Mount Sinai Harbor, including **(A)** a photo on land showing the construction of the array including the spreader bars and parallel ropes for attaching bags, and **(B)** a photo of the full 25-bag array installed in Mount Sinai Harbor.

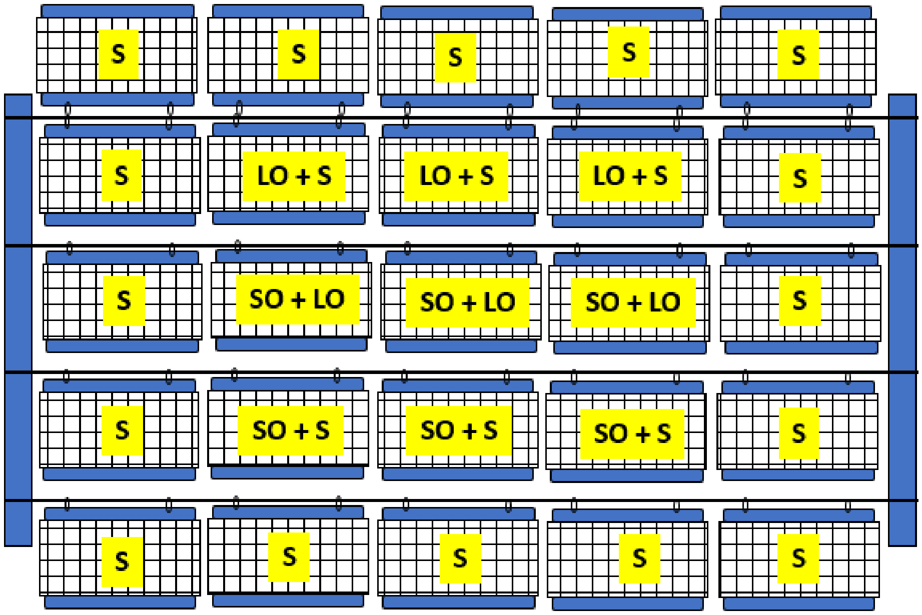


Figure 2c.3. Diagram of the 25-bag array used to cultivate *Gracilaria* sp. and *Ulva* sp. in Northport Harbor and Mount Sinai Harbor, with labels indicating the contents of each bag. Labels are as follows: S = seaweed; LO = large oysters; SO = small oysters. The 16 perimeter bags contained seaweed only, The interior nine bags contained oysters, with three bags containing large oysters and seaweed, three bags containing small oysters and seaweed, and the center three bags containing oysters only (large + small).

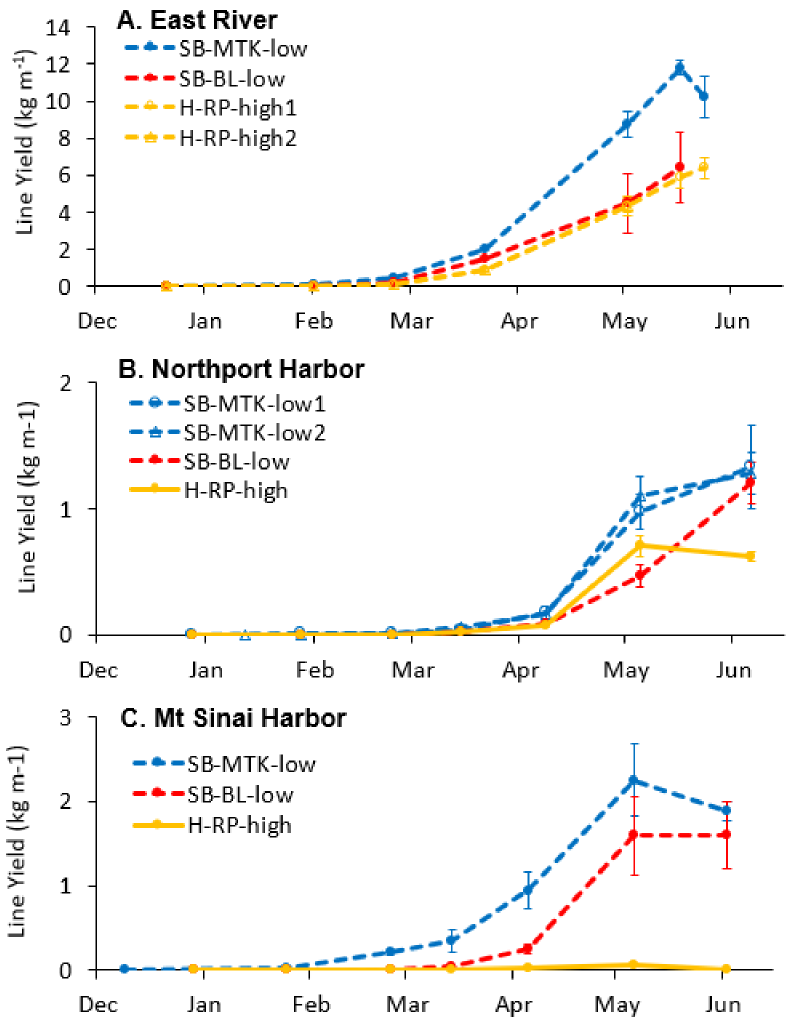


Figure 3a.1. Kelp growth along lines seeded with different seedstock at the three Long Island Sound study sites: (A) East River, (B) Northport Harbor, and (C) Mt. Sinai Harbor. Within each site, three batches of seedstock were deployed along separate lines, designated as follows: SB-MTK-low (blue), SB-BL-low (red), and H-RP-high (orange). The three-part designations indicate: 1) the hatchery where the seedstock was produced, either Stony Brook (SB) or Hart Lobster (H); 2) the location of the wild parental source, including Montauk (MTK), Black Ledge (BL), or Race Point, Fishers Island (RP); and 3) the spore setting density, described as either high (7,000 – 7,5000 spores ml^{-1}) or low (1,333-1,800 spores ml^{-1}). One to two lines of each of the three seedstock types were deployed at each site.

Figure 3a.2. Photos showing biofouling and blade deterioration of kelp at the end of the growing season, including **(A)** a field photo of kelp lines in Northport Harbor on 6/7/22 covered with the egg masses of eastern mud snails (*Ilyanassa obsoleta*), and **(B)** a lab photo of kelp blades collected from Mt. Sinai Harbor on 6/2/22 showing blade deterioration and loss of pigments, particularly at the blade tips..

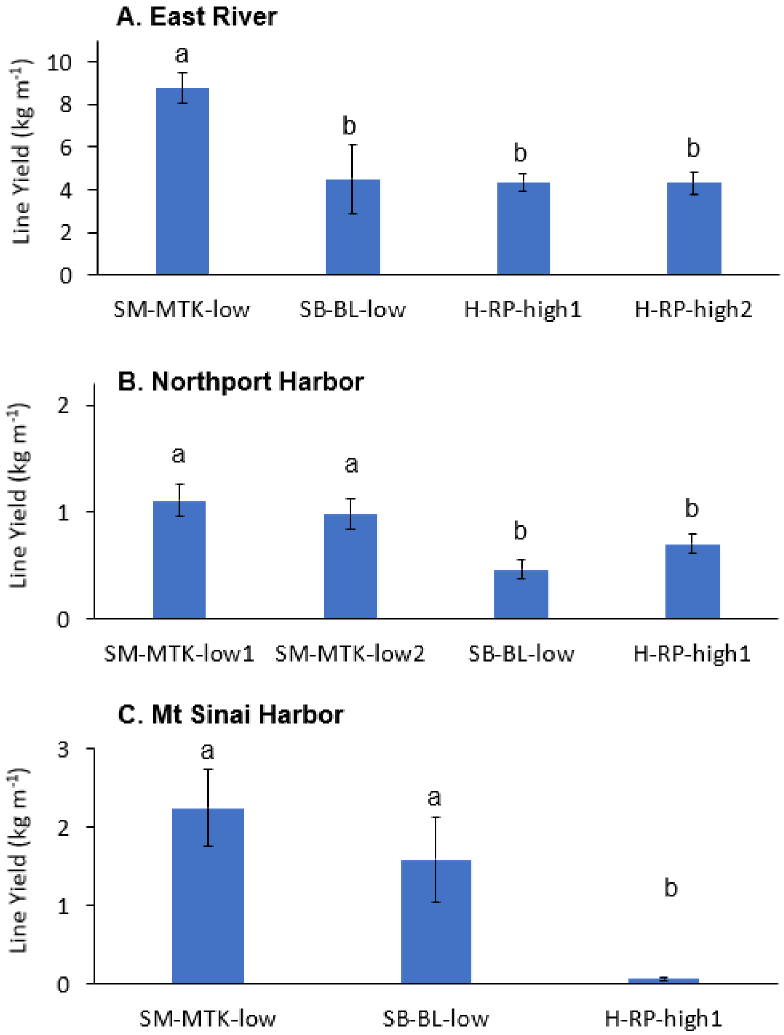


Figure 3a.3. Comparison of kelp line yields in early May among three seedstocks within each Long Island Sound study site, including: **(A)** East River (5/2/22), **(B)** Northport Harbor (5/5/22), and **(C)** Mt. Sinai Harbor (5/6/22). The three seedstocks include: SB-MTK-low (blue), SB-BL-low (red), and H-RP-high (orange). The three-part designation for each seedstock indicates: 1) the hatchery where the seedstock was produced, either Stony Brook (SB) or Hart Lobster (H); 2) the location of the wild parental source, including Montauk (MTK), Black Ledge (BL), or Race Point, Fishers Island (RP); and 3) the spore setting density, described as either high (7,000 – 7,5000 spores ml^{-1}) or low (1,333-1,800 spores ml^{-1}). One to two lines of each of the three seedstocks were deployed at each site. Letters above bars indicate significant differences. Error bars are standard errors.

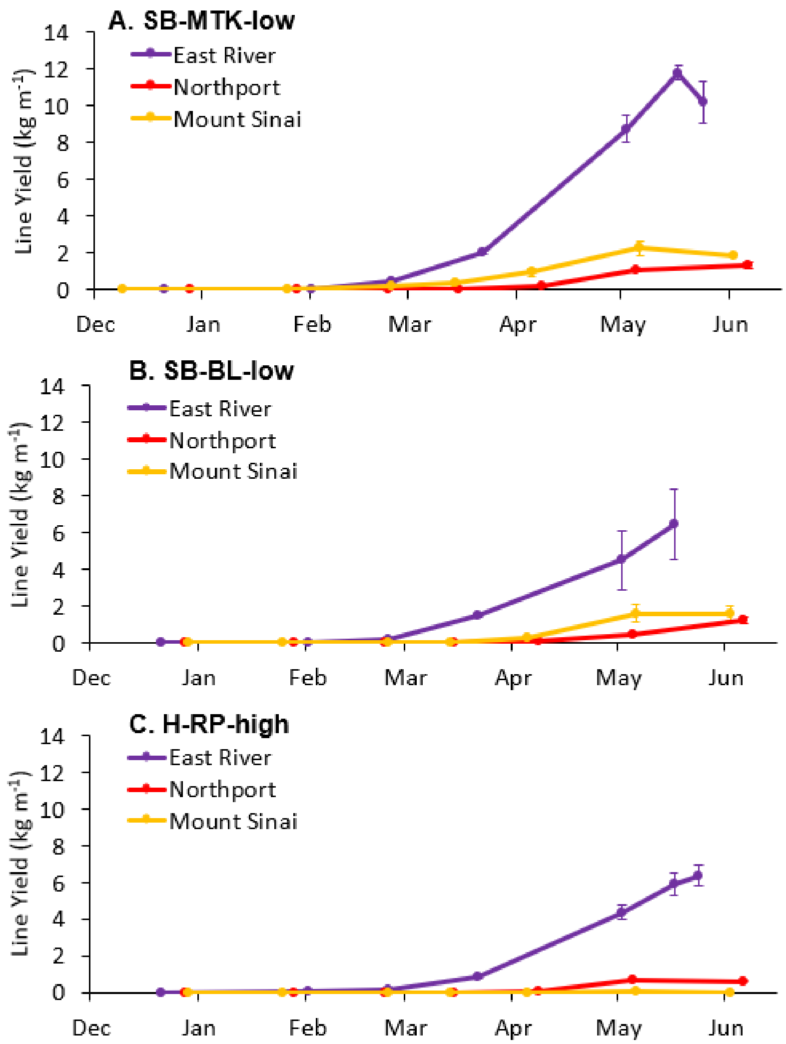


Figure 3a.4. Kelp growth at three Long Island Sound sites for each of three seedstocks, including: **(A)** SB-MTK-low, **(B)** SB-BL-low, and **(C)** H-RP-high. Each seedstock was deployed along separate lines at three LIS sites: East River (purple), Northport Harbor (red), Mount Sinai Harbor (orange). The three-part designations of each seedstock indicate: 1) the hatchery where the seedstock was produced, either Stony Brook (SB) or Hart Lobster (H); 2) the location of the wild parental source, including Montauk (MTK), Black Ledge (BL), or Race Point, Fishers Island (RP); and 3) the spore setting density, described as either high (7,000 – 7,5000 spores ml^{-1}) or low (1,333-1,800 spores ml^{-1}).

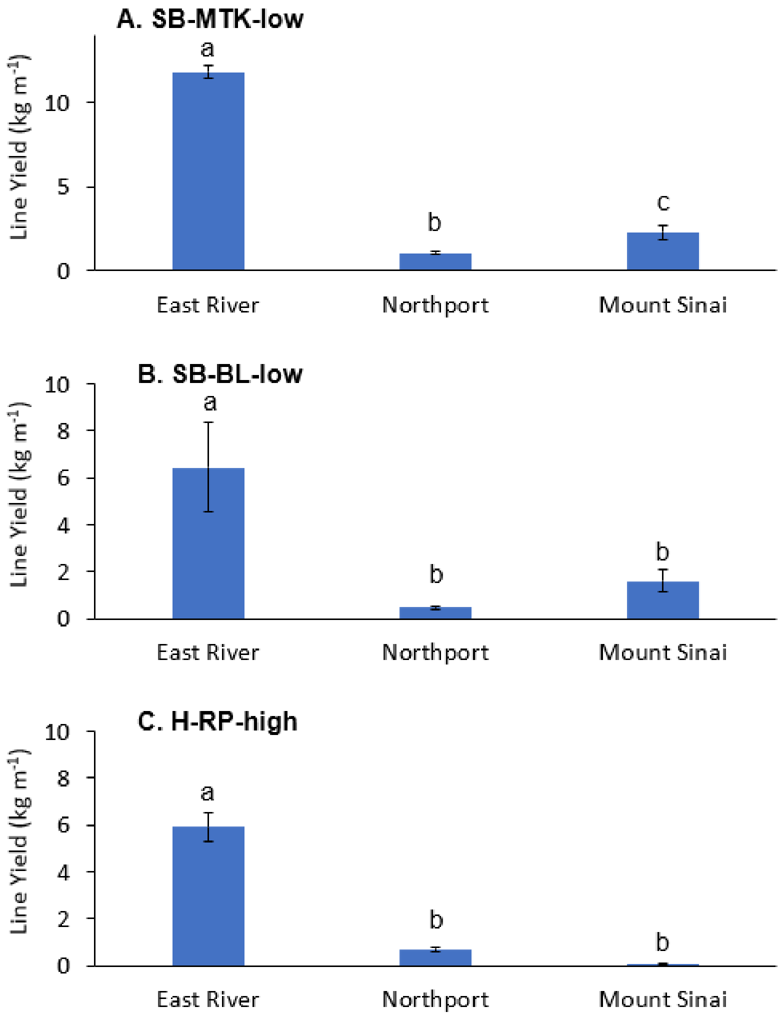


Figure 3a.5. Comparison of kelp line yields among three Long Island Sound sites for each of three seedstocks, including: **(A)** SB-MTK-low, **(B)** SB-BL-low, and **(C)** H-RP-high. Line yields are compared at optimal harvest times, which were considered to occur just prior to the onset of heavy biofouling and kelp deterioration when biomass yields were at or near maximum. Optimal harvest occurred on 5/17/22 at the East River site, 5/5/22 at Northport Harbor, and 5/6/22 at Mount Sinai Harbor. The three-part designations of each seedstock indicate: 1) the hatchery where the seedstock was produced, either Stony Brook (SB) or Hart Lobster (H); 2) the location of the wild parental source, including Montauk (MTK), Black Ledge (BL), or Race Point, Fishers Island (RP); and 3) the spore setting density, described as either high (7,000 – 7,5000 spores ml^{-1}) or low (1,333-1,800 spores ml^{-1}). Letters above bars indicate significant differences. Error bars are standard errors.

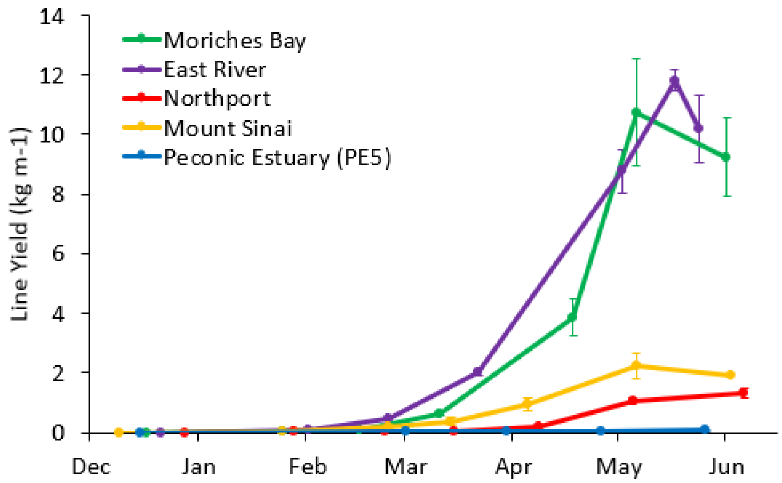


Figure 3a.6. Kelp growth in coastal water bodies surrounding Long Island during the 2022 kelp season (December 2021 to May/June 2022). Line yields are expressed as kilograms of kelp (wet weight) per meter of longline on which the kelp was cultivated. For figure clarity, growth in the Peconic Estuary is shown for only one site, PE5 (Southold Bay), where the highest growth was found. For all sites, line yields are for seedstock SB-MTK-low, which produced the highest yields at all sites. Error bars are standard errors.

Figure 3a.7. Photos comparing kelp size and color at the end of the 2022 growing among locations around Long Island, including (A) Moriches Bay, (B) the Eat River, (C) Northport Harbor, (D) Mt. Sinai Harbor, and (E) the Peconic Estuary (PE5).

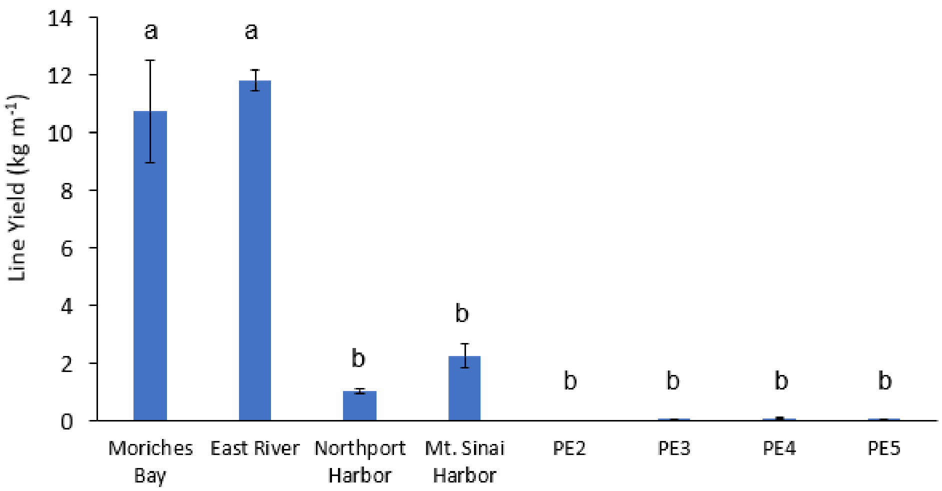


Figure 3a.8. Comparison of line yields at peak biomass among eight sites in coastal water bodies surrounding Long Island, NY, including one site in the South Shore estuary (Moriches bay), three sites in the Long Island Sound (East River, Northport Harbor, and Mt. Sinai Harbor), and four sites in the central Peconic Estuary (PE2 to PE5). Letters above bars indicate significant differences. Error bars are standard errors.

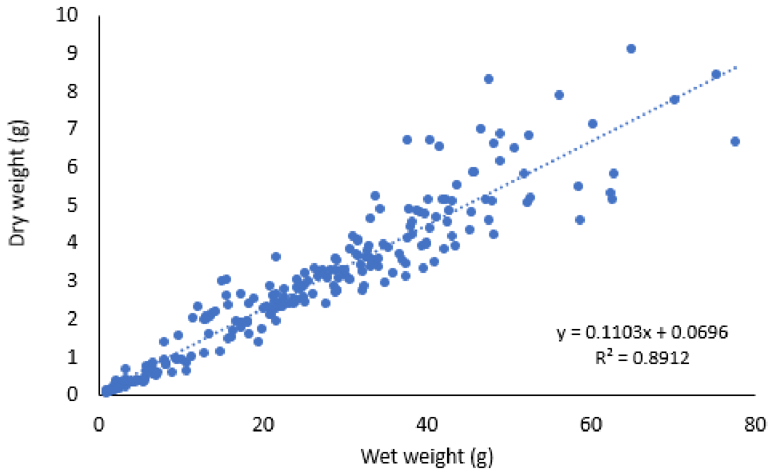


Figure 3a.9. Relationship between wet and dry weight for 227 kelp samples dried in 2022. Wet weights ranged between 1.1 to 77.6 grams. On average, dry weights were 11.2% of wet weights.

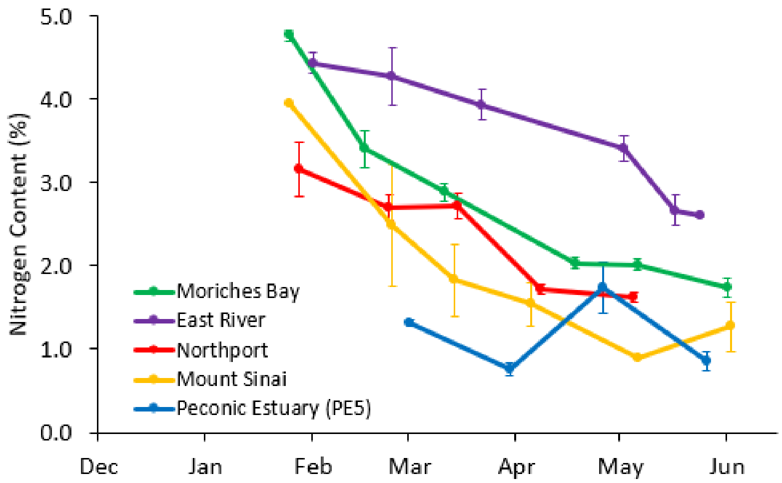


Figure 3a.10. Nitrogen content of kelp tissue over the course of the growing season at five locations around Long Island, including: 1) Moriches Bay (green), East River (purple), Northport Harbor (red), Mt. Sinai Harbor (orange), and the Peconic Estuary (blue). The nitrogen content is expressed as a percentage of the tissue biomass (dry weight). Error bars are standard errors.

Figure 3a.11. Comparison of the nitrogen content of kelp tissue at peak line biomass among the study sites. The nitrogen content is expressed as a percentage of the tissue biomass (dry weight). Letters above bars indicate significant differences. Error bars are standard errors.

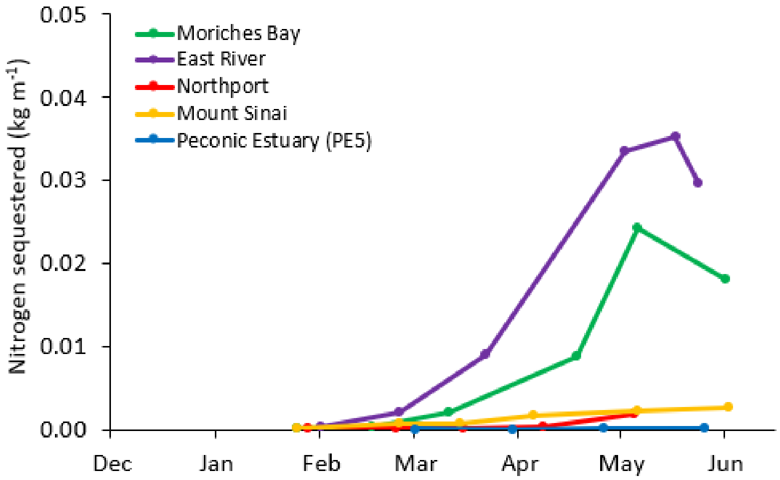


Figure 3a.12. Nitrogen sequestered per meter of kelp line over the course of the kelp growing season in five coastal water bodies around Long Island, NY, including 1) Moriches Bay (green), 2) East River (purple), Northport Harbor (red), Mt. Sinai Harbor (orange), and the Peconic Estuary (blue). The Peconic Estuary is represented by site PE5, where kelp line yields were highest among the four Peconic Estuary sites.

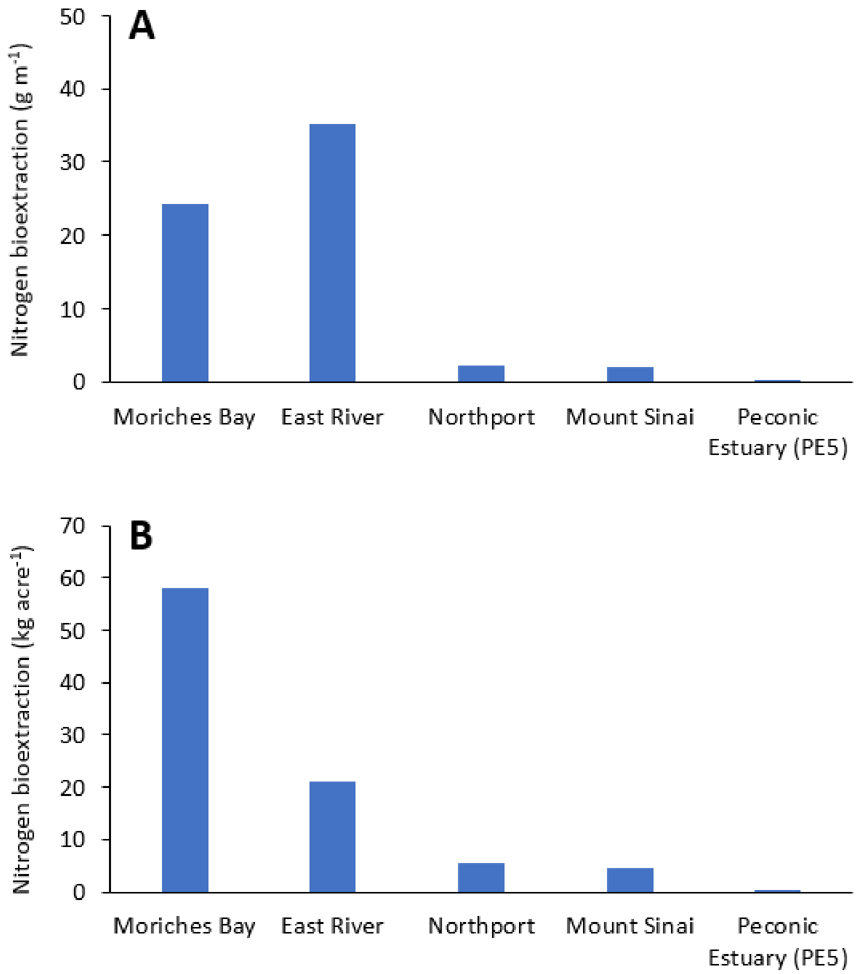


Figure 3a.13. Estimated nitrogen extracted at harvest **(A)** per meter of kelp line, and **(B)** per acre, in five coastal water bodies around Long Island, NY, including 1) Moriches Bay, 2) East River, 3) Northport Harbor, 4) Mt. Sinai Harbor, and the 5) Peconic Estuary. The nitrogen extracted per acre was extrapolated from the line yields, assuming that an acre could support 2,400 linear meters of kelp line in shallow waters (Moriches Bay, Mt. Sinai Harbor, Northport Harbor) and 600 linear meters of kelp line in deep waters (East River, Peconic Estuary).

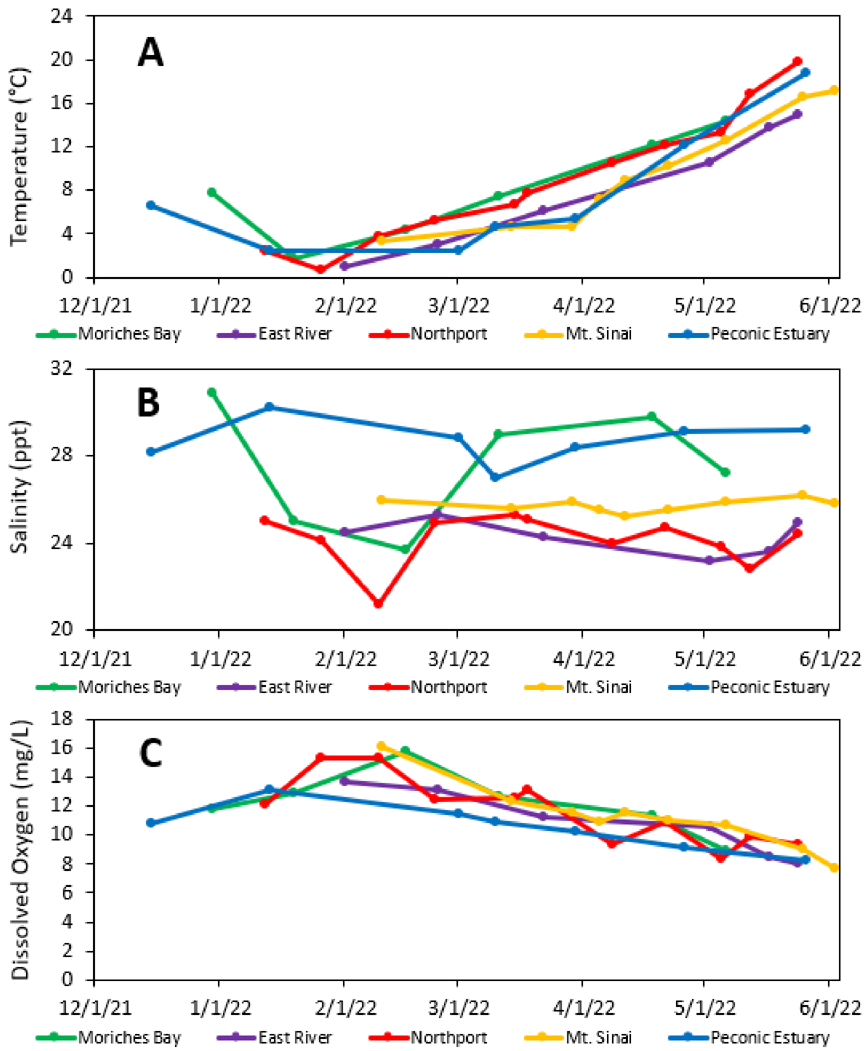


Figure 3a14. Comparison of **(A)** temperature, **(B)** salinity, and **(C)** dissolved oxygen among coastal water bodies around Long Island where kelp was grown during the 2022 season, from mid-December to early June. Discrete measurements were taken with a handheld YSI multiparameter instrument.

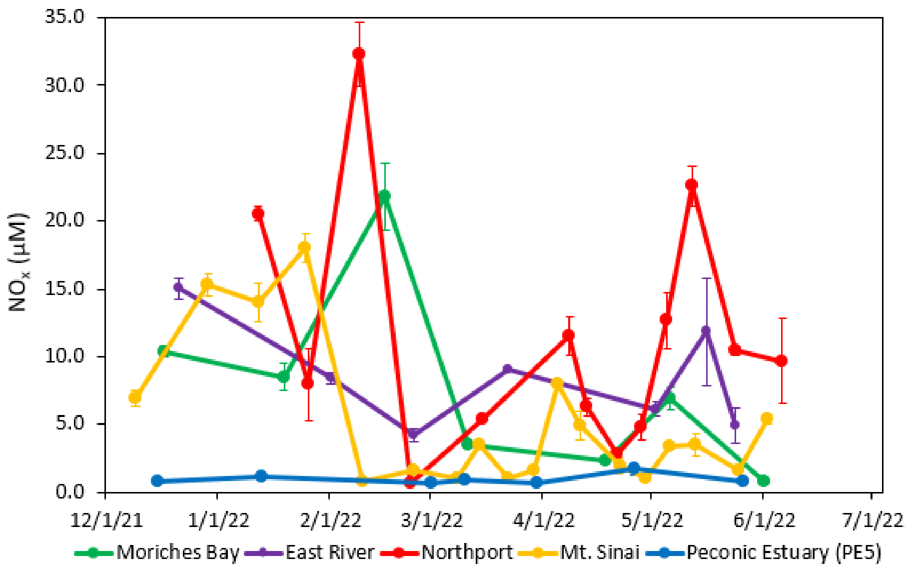


Figure 3a.15. Comparison of dissolved nitrate/nitrite (NO_x) concentrations among coastal water bodies around Long Island where kelp was grown during the 2022 season, from mid-December to early June. Error bars are standard deviation.

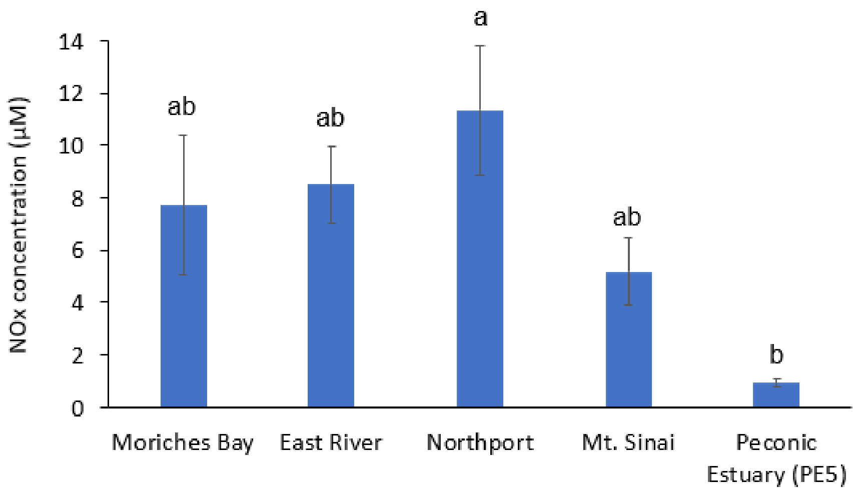


Figure 3a.16. Comparison of average NO_x concentrations during the kelp growing season among the study sites. Letters above bars indicate significant differences. Error bars are standard errors.

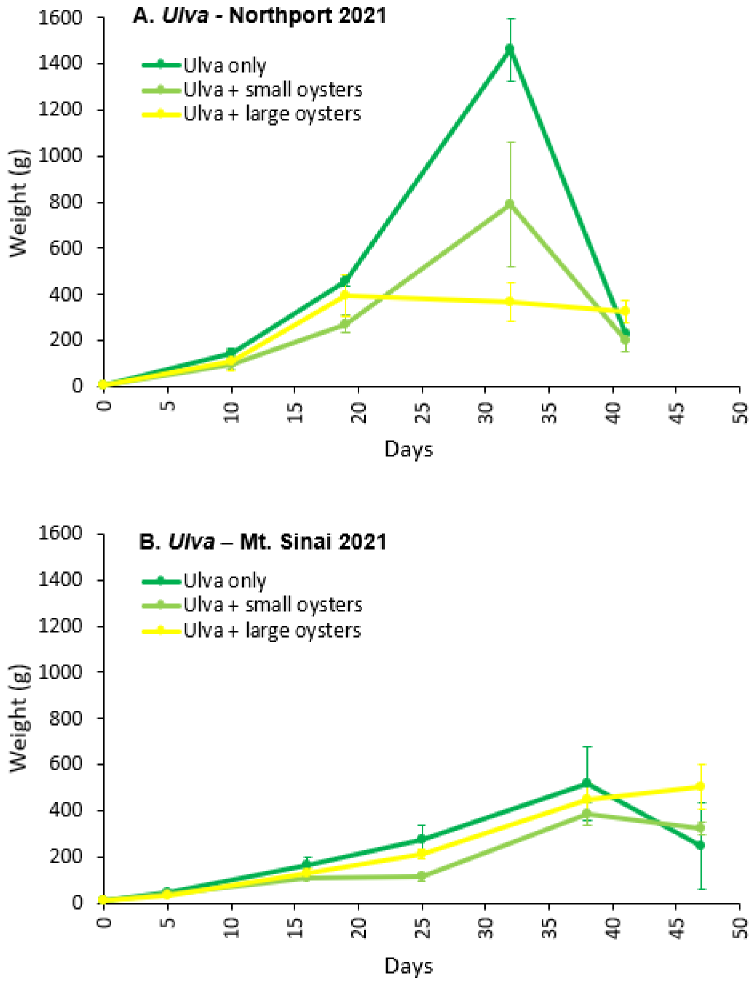


Figure 3b.1. *Ulva* growth over time in treatments with and without oysters during the 2021 cultivation experiments in **(A)** Northport Harbor and **(B)** Mt. Sinai Harbor.

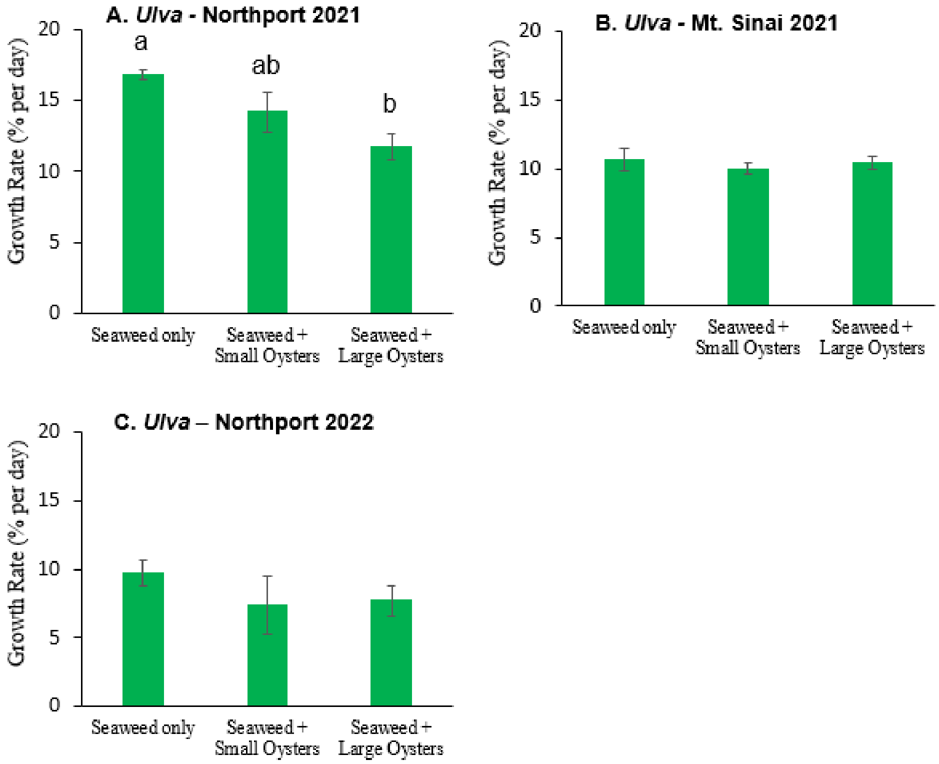


Figure 3b.2. Comparison of *Ulva* growth rates between treatments with and without oyster, in each three cultivation experiments conducted in **(A)** Northport Harbor 2021, **(B)** Mt. Sinai Harbor 2021, and **(C)** Northport Harbor 2022. Growth rates are expressed in percentage growth per day and were calculated over the optimal cultivation period (from t0 to peak biomass). Significant differences between treatments was only found in one experiment (Northport 2021). Letters above bars indicate significant differences.

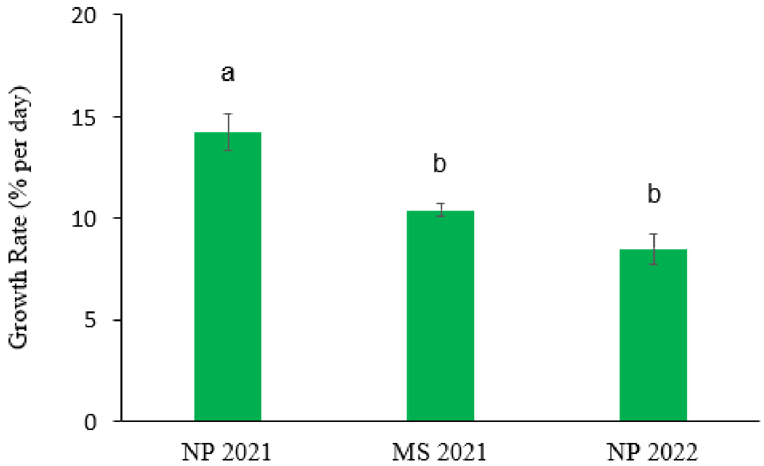


Figure 3b.3. Comparison of *Ulva* growth rates among three cultivation experiments: Northport Harbor 2021, Mt. Sinai Harbor 2021, and Northport Harbor 2022. Growth rates are expressed in percentage growth per day and were calculated over the optimal cultivation period (from t0 to peak biomass). Letters above bars indicate significant differences.

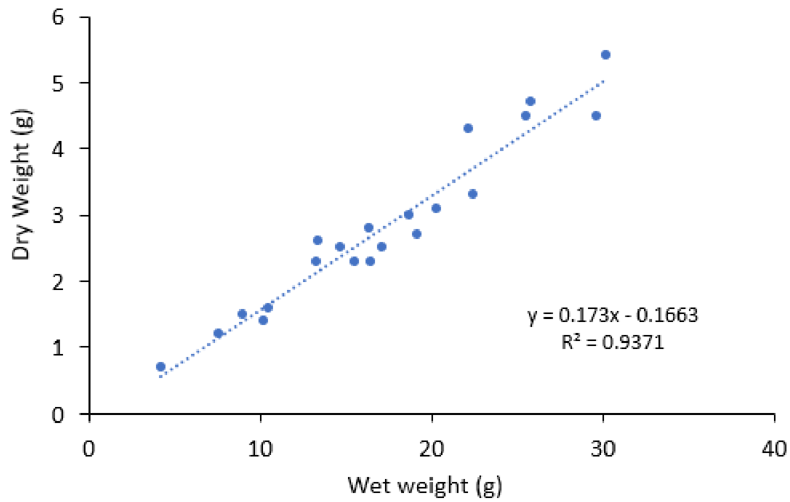


Figure 3b.4. Relationship between wet and dry weight for 21 *Ulva* samples dried. Wet weights ranged between 4.2 to 30.2 grams. On average, dry weights were 16.2% of wet weights.

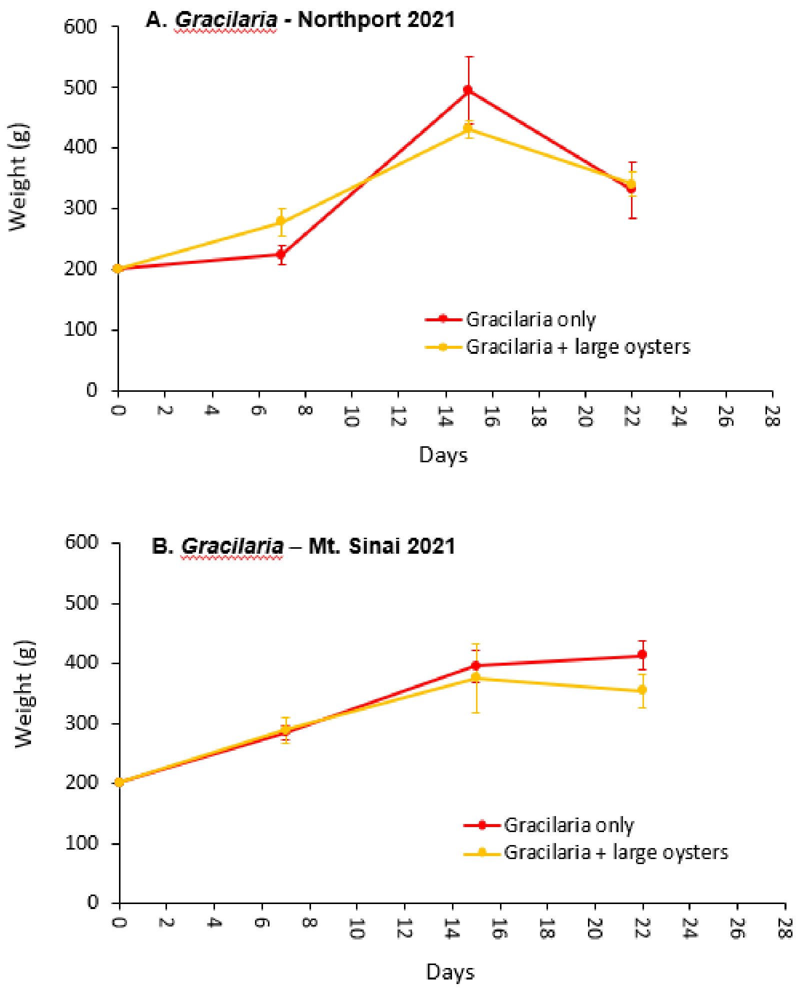


Figure 3c.1. *Gracilaria* growth over time in treatments with and without oysters during the 2021 cultivation experiments in **(A)** Northport Harbor and **(B)** Mount Sinai Harbor.

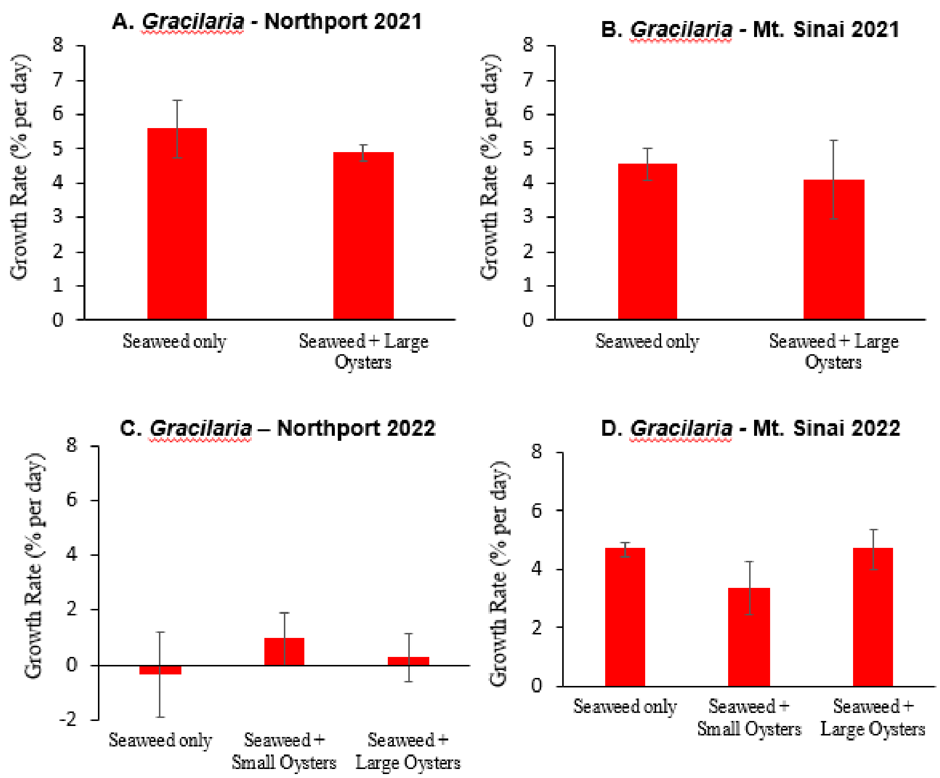


Figure 3c.2. Comparison of *Gracilaria* growth rates between treatments with and without oyster, in each four cultivation experiments conducted in **(A)** Northport Harbor 2021, **(B)** Mount Sinai Harbor 2021, (**C)** Northport Harbor 2022, and **(D)** Mount Sinai Harbor 2022. Growth rates are expressed in percentage growth per day and were calculated over the optimal cultivation period (from t0 to peak biomass). No significant differences between treatments were found within any of the four experiments.

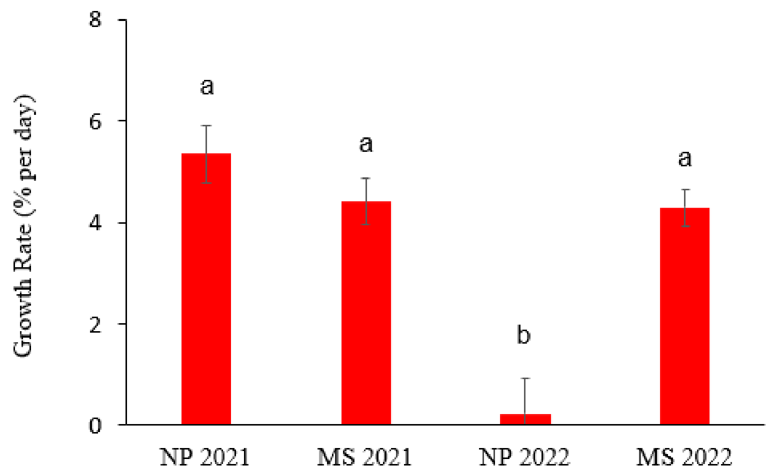


Figure 3c.3. Comparison of *Gracilaria* growth rates among four cultivation experiments: Northport Harbor 2021, Mt. Sinai Harbor 2021, Northport Harbor 2022, Mount Sinai Harbor 2022. Growth rates are expressed in percentage growth per day and were calculated over the optimal cultivation period (from t0 to peak biomass). Letters above bars indicate significant differences.

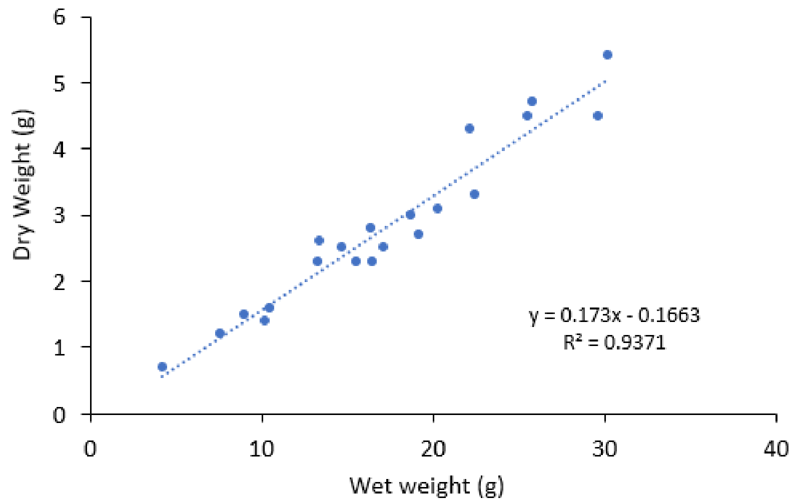


Figure 3c.4. Relationship between wet and dry weight for 29 *Gracilaria* samples dried. Wet weights ranged between 4.7 to 14.9 grams. On average, dry weights were 12.8% of wet weights.

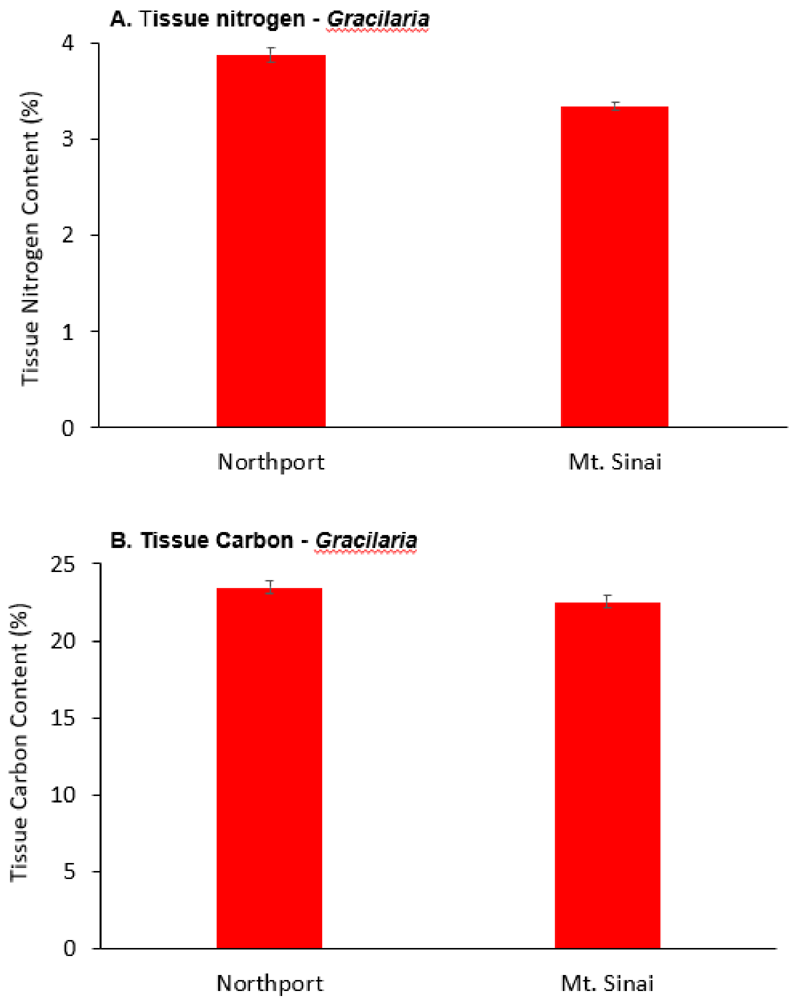


Figure 3c.5. Tissue **(A)** nitrogen content and **(B)** carbon content of *Gracilaria* cultivated in Northport Harbor and Mount Sinai Harbor. Nitrogen content was significantly higher in *Gracilaria* from Northport Harbor, but carbon content did not differ between harbors. Error bars are standard errors.

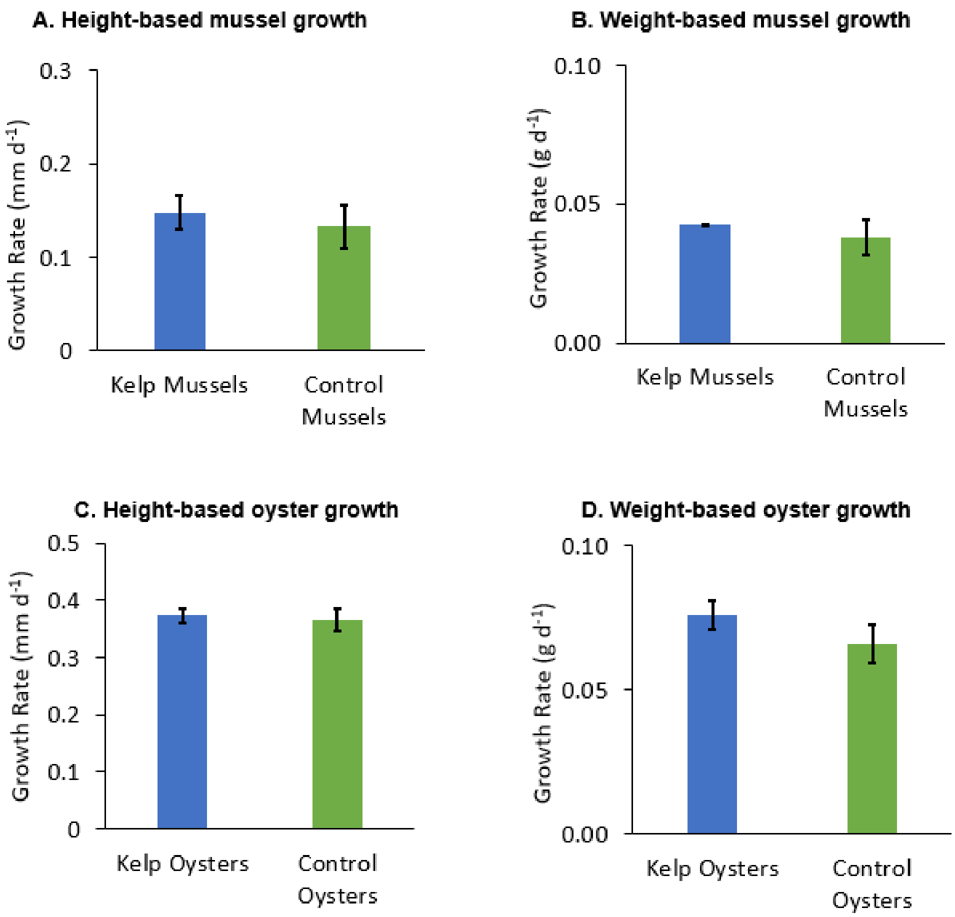
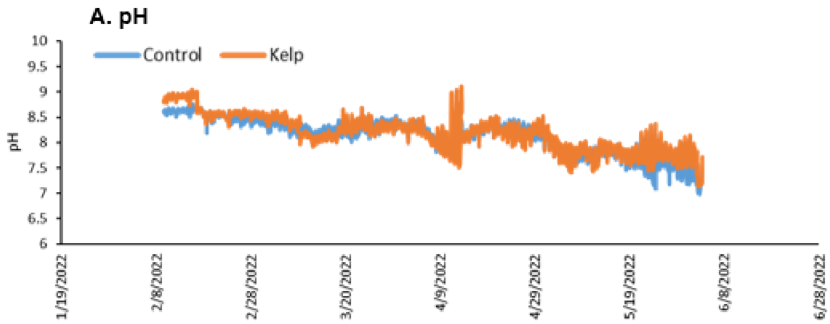


Figure 3d.1. Comparisons of blue mussel and oyster growth inside and outside of kelp lines in Northport Harbor, including **(A)** Height-based growth rate (mm d^{-1}) of blue mussels, **(B)** weight-based growth rate of blue mussels (g d^{-1}), **(C)** height-based growth rate of oysters (mm d^{-1}), and **(D)** weight-based growth rate of blue oysters (g d^{-1}). Initial timepoint to final timepoint was 59 days for blue mussels, and 34 days for oysters. Error bars indicate standard error. Asterisks indicate significant differences.



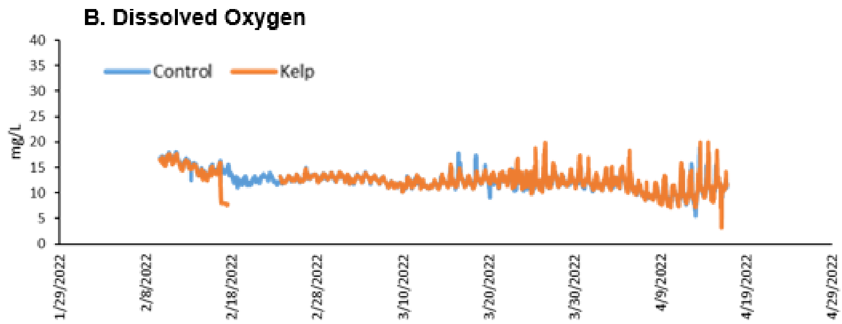


Figure 3d.2. Comparisons of (A) pH and (B) dissolved oxygen inside and outside of kelp lines in Northport Harbor during the 2022 kelp season.

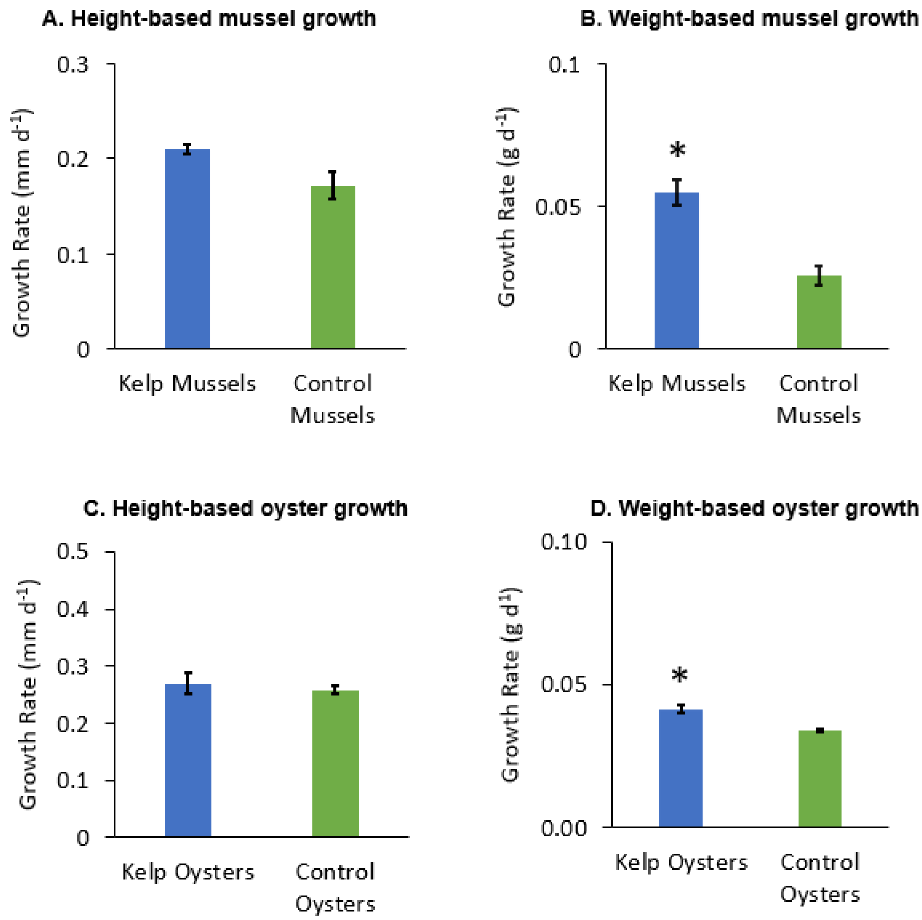


Figure 3d.3. Comparisons of blue mussel and oyster growth inside and outside of kelp lines in Mount Sinai Harbor Harbor, including **(A)** Height-based growth rate (mm d^{-1}) of blue mussels, **(B)** weight-based growth rate of blue mussels (g d^{-1}), **(C)** height-based growth rate of oysters (mm d^{-1}), and **(D)** weight-based growth rate of blue oysters (g d^{-1}). Initial timepoint to final timepoint was 55 days for blue mussels, and 31 days for oysters. Error bars indicate standard error. Asterisks indicate significant difference from control.

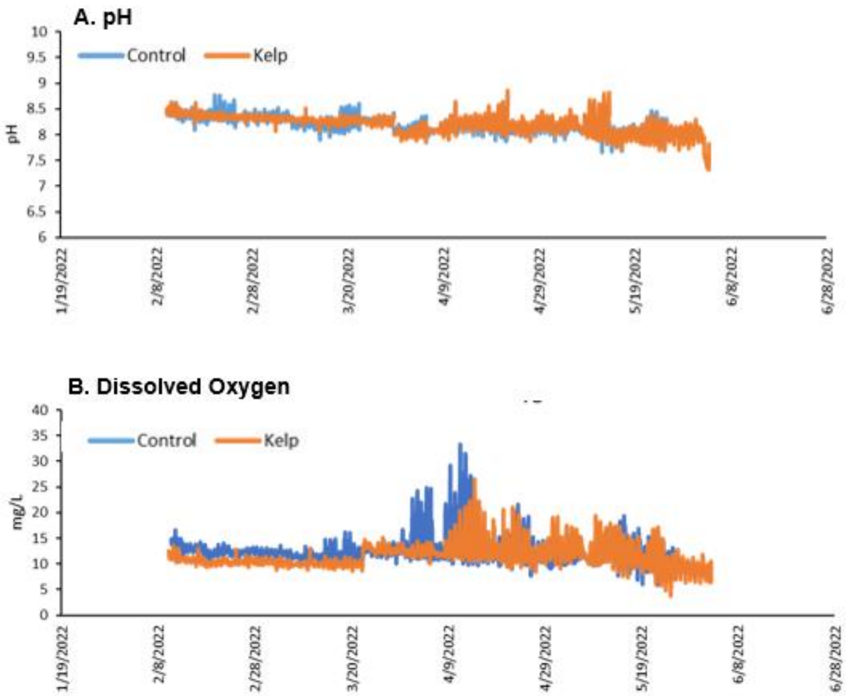


Figure 3d.4. Comparisons of (A) pH and (B) dissolved oxygen inside and outside of kelp lines in Mount Sinai Harbor during the 2022 kelp season.

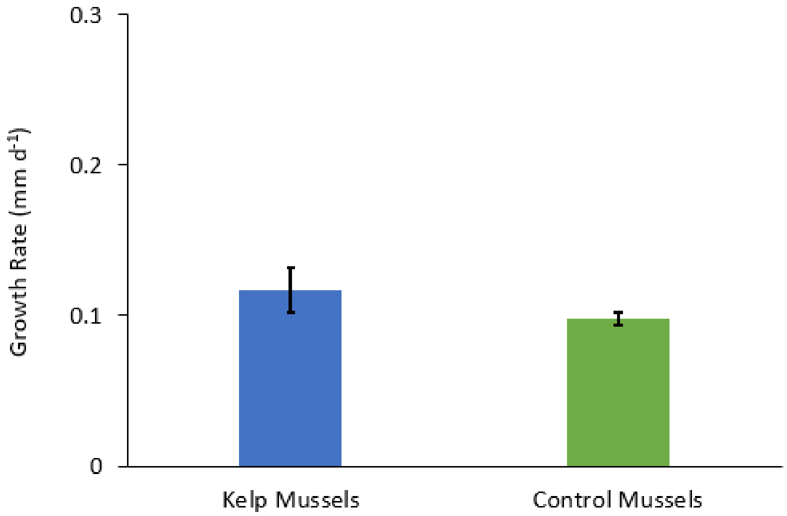


Figure 3d.5. Comparison of height-based growth (mm d-1) for blue mussels cultivated on vertical lines that were either suspended from kelp lines or deployed away from kelp lines at the Thimble Island Ocean Farm. Initial timepoint to final timepoint was 77 days. Error bars indicate standard error.

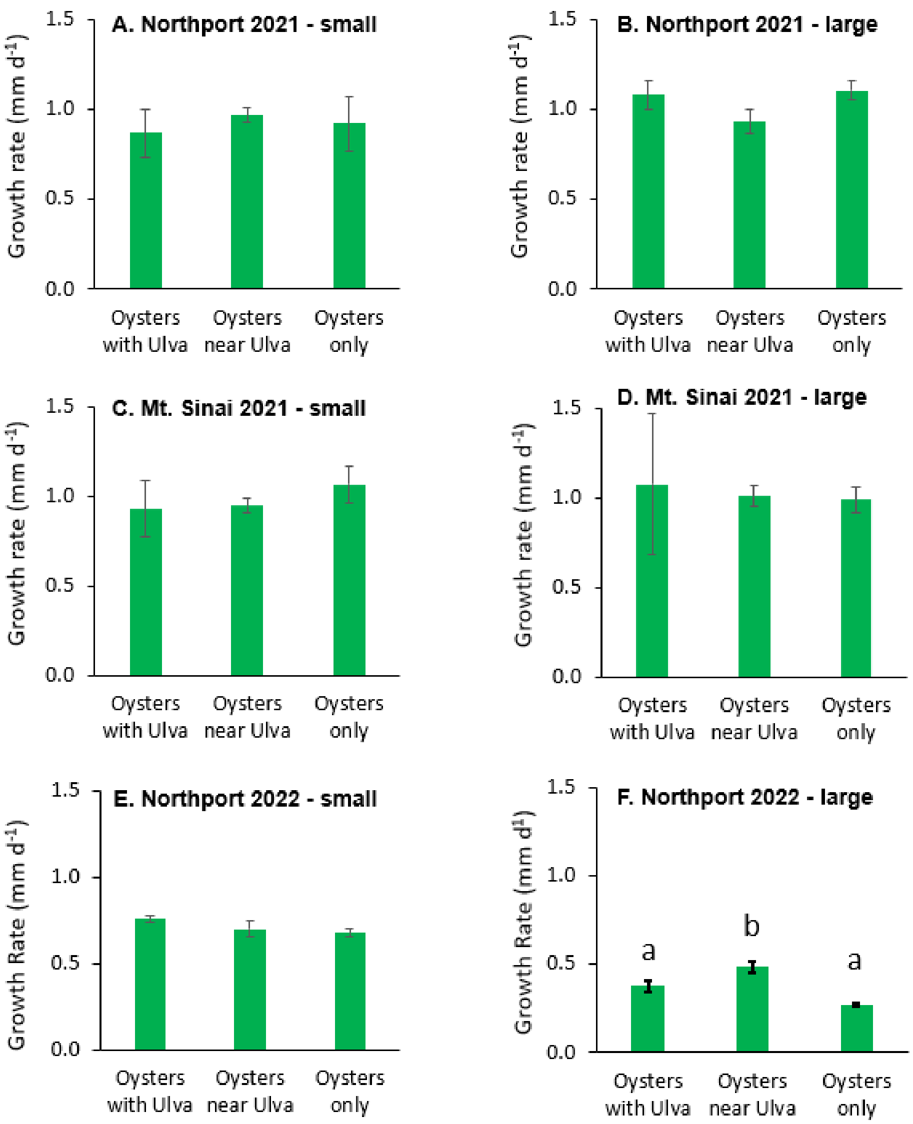


Figure 3d.6. Comparisons of height-based growth rates of small oysters (left panels) and large oysters (right panels) among three *Ulva* treatments in three cultivation experiments, including Northport Harbor 2021 (A, B), Mount Sinai Harbor 2021 (C, D), and Northport Harbor 2022 (E, F). The three Ulva treatments include oysters grown with Ulva (i.e., in the same grow-out bag with Ulva), oysters grown near Ulva (i.e., in separate bags surrounded by bags of Ulva), and in control oysters away from Ulva. Letters indicate significant differences. Error bars are standard errors.

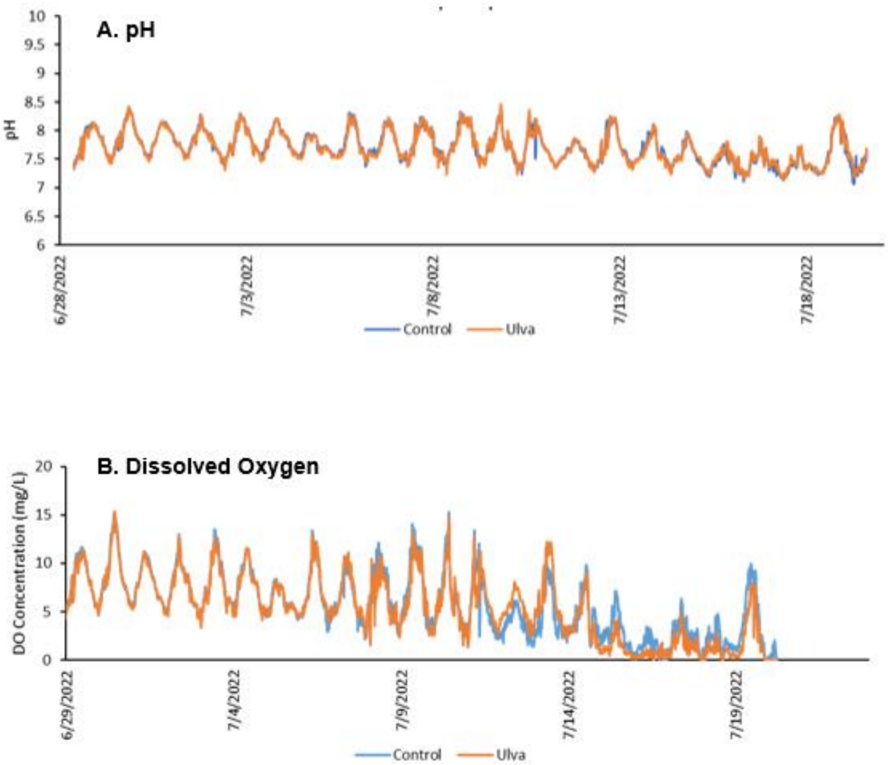


Figure 3d.7. Comparisons of (A) pH and (B) dissolved oxygen inside and outside of Ulva cultivation areas in Northport Harbor during the 2022 kelp season.

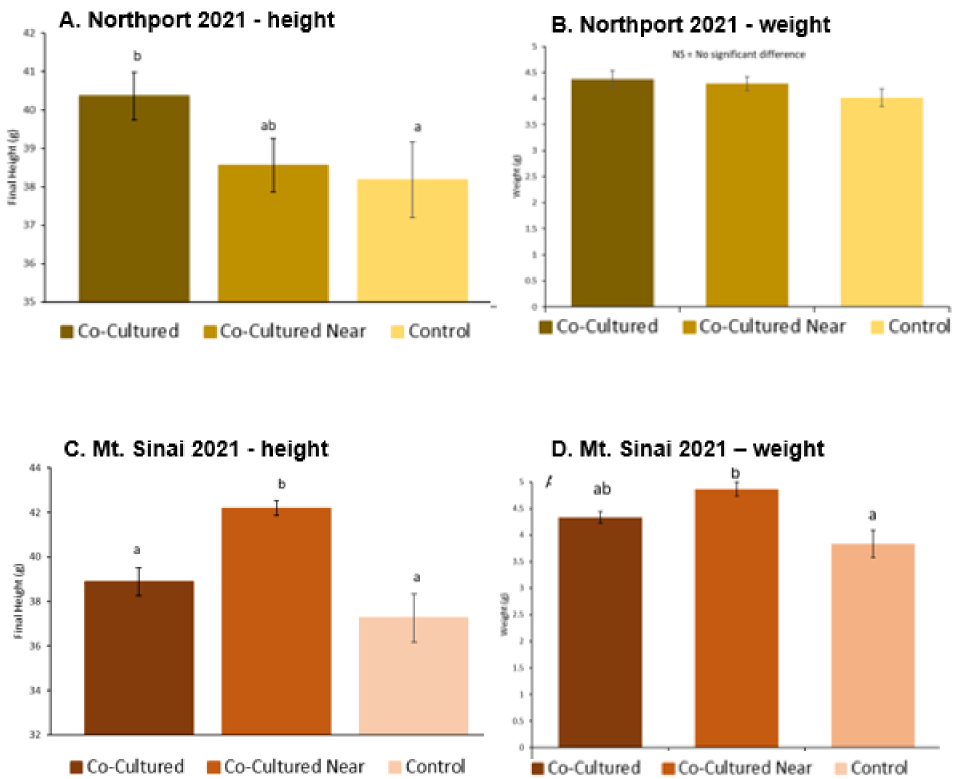


Figure 3d.8. Comparisons of height-based growth rates (left panels) and weight-based growth rates of oyster seed among three *Gracilaria* treatments in cultivation experiments in Northport Harbor 2021 (A, B) and Mount Sinai Harbor 2021 (C, D), The three *Gracilaria* treatments include oysters grown with *Gracilaria* (i.e., in the same grow-out bag with *Gracilaria*), oysters grown near *Gracilaria* (i.e., in separate bags surrounded by bags of *Gracilaria*), and in control oysters away from Ulva *Gracilaria*. Letters indicate significant differences. Error bars are standard errors.

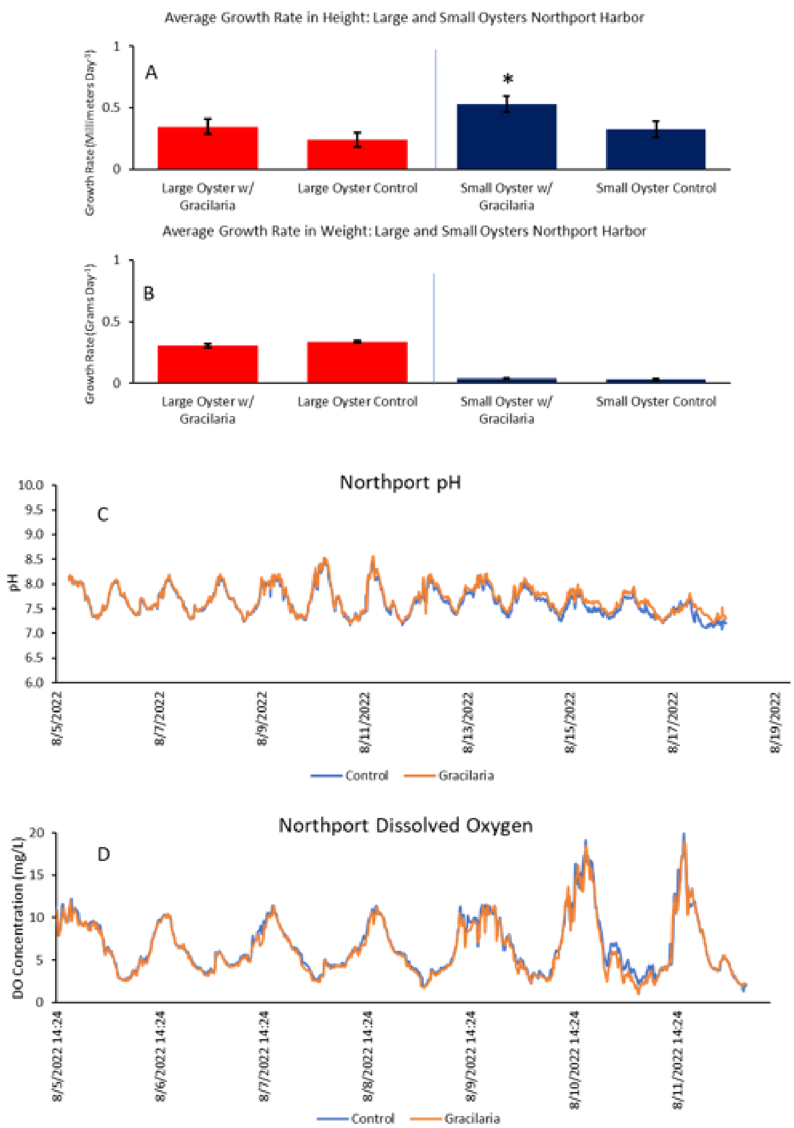


Figure 3d.9. Comparisons of (A) height-based oyster growth rates (B) weight-based oyster growth rates, (C) seawater pH, and (D) seawater dissolved oxygen in areas where oysters are co-cultivated with *Gracilaria* and control areas where oysters are grown without *Gracilaria*, in the Northport Harbor experiment in 2022. Initial timepoint to final timepoint was 13 days. Error bars indicate standard error. Asterisks indicate significant differences from control group.

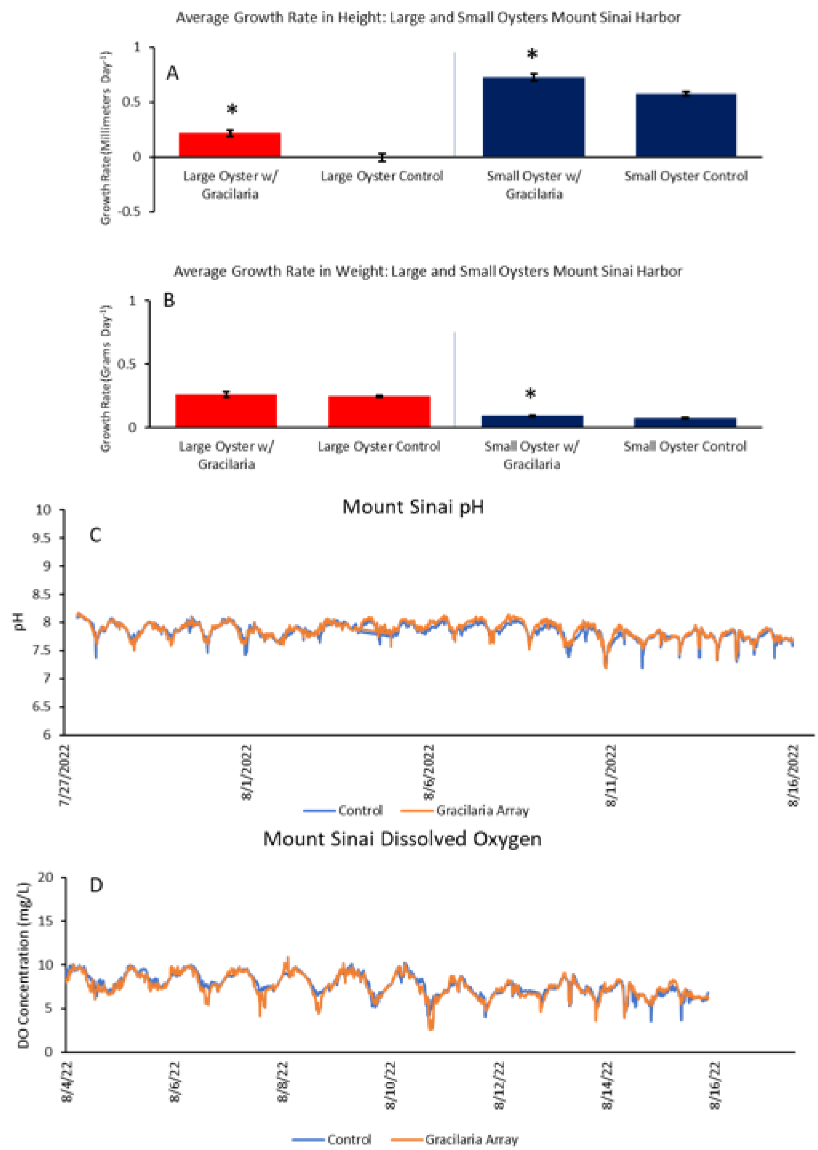


Figure 3d.10. Comparisons of (A) height-based oyster growth rates (B) weight-based oyster growth rates, (C) seawater pH, and (D) seawater dissolved oxygen in areas where oysters are co-cultivated with *Gracilaria* and control areas where oysters are grown without *Gracilaria*, in the Mount Sinai Harbor experiment in 2022. Initial timepoint to final timepoint was 20 days. Error bars indicate standard error. Asterisks indicate significant differences from control group.

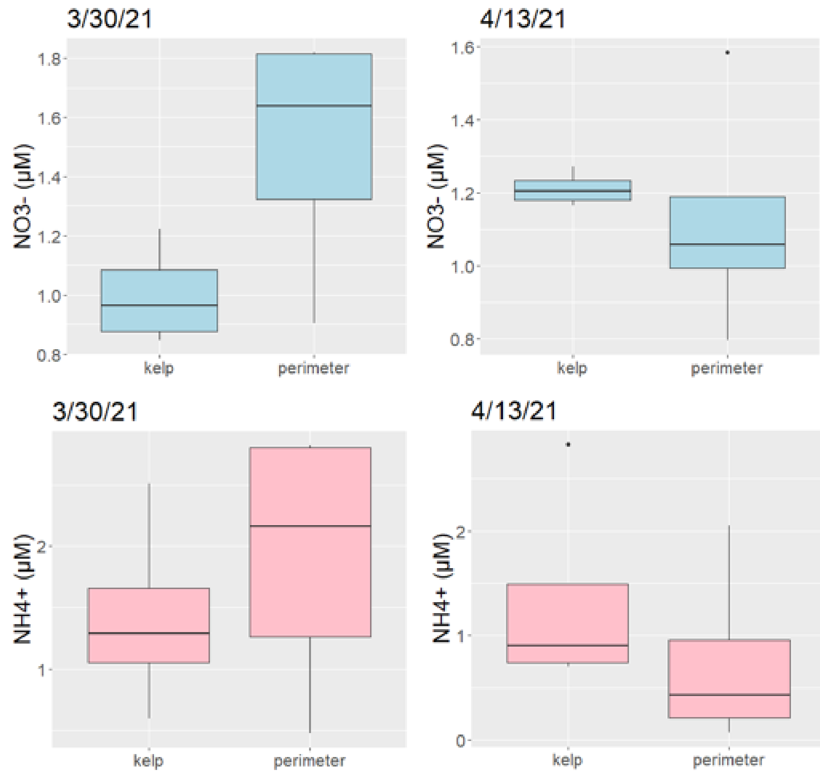


Figure 3e.1. Nutrient data from the 3/30 and 4/13 cruises. There were no significant nutrient differences between the kelp farm area and the perimeter.

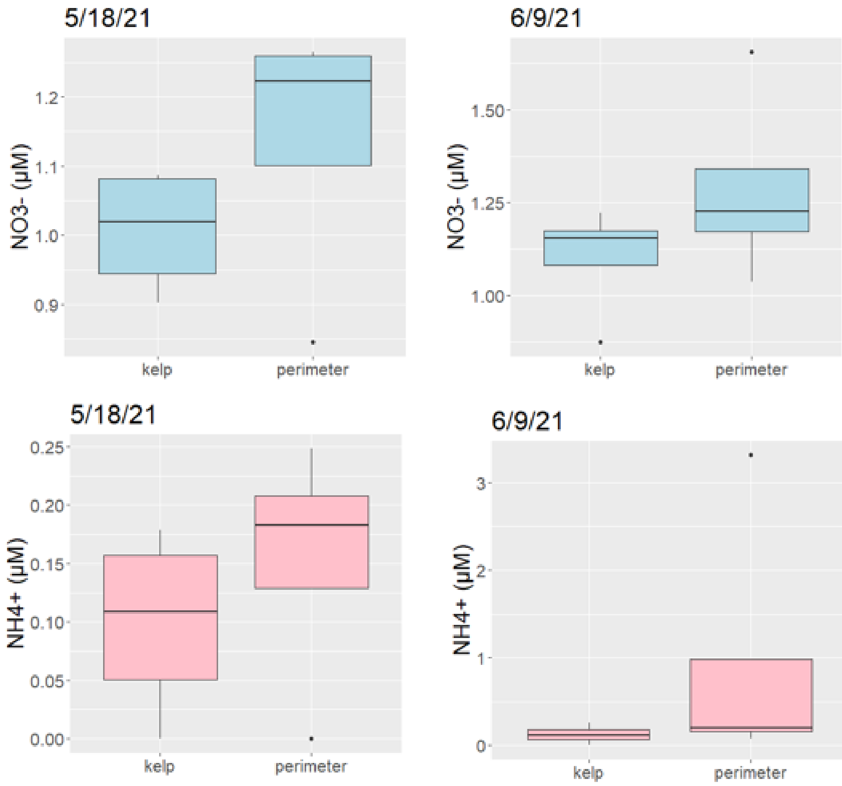


Figure 3e.2. Nutrient data from the 5/18 and 6/9 cruises. There were no significant nutrient differences between the kelp farm area and the perimeter.

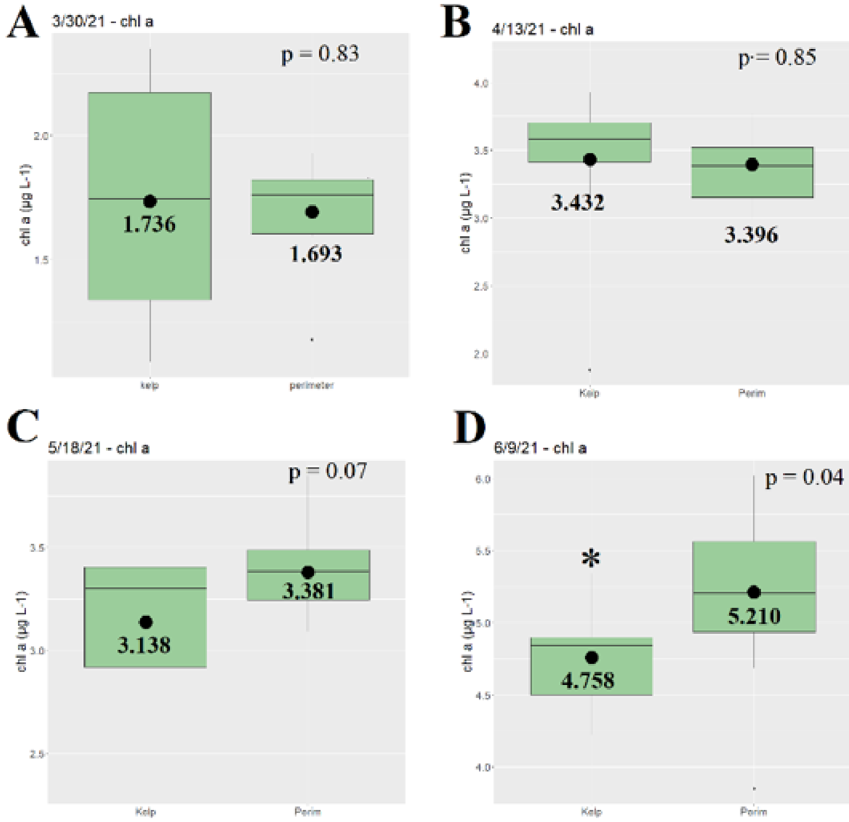


Figure 3e.3. Chlorophyll a data from all cruises. In the warmer months tested, chlorophyll was lower in the kelp farm than in the perimeter, significantly so on 6/9. Large dots on boxplots indicate mean value, with mean value shown in text bolded near dots. Asterisk indicates statistically significant difference between the within-kelp field and perimeter for that parameter.

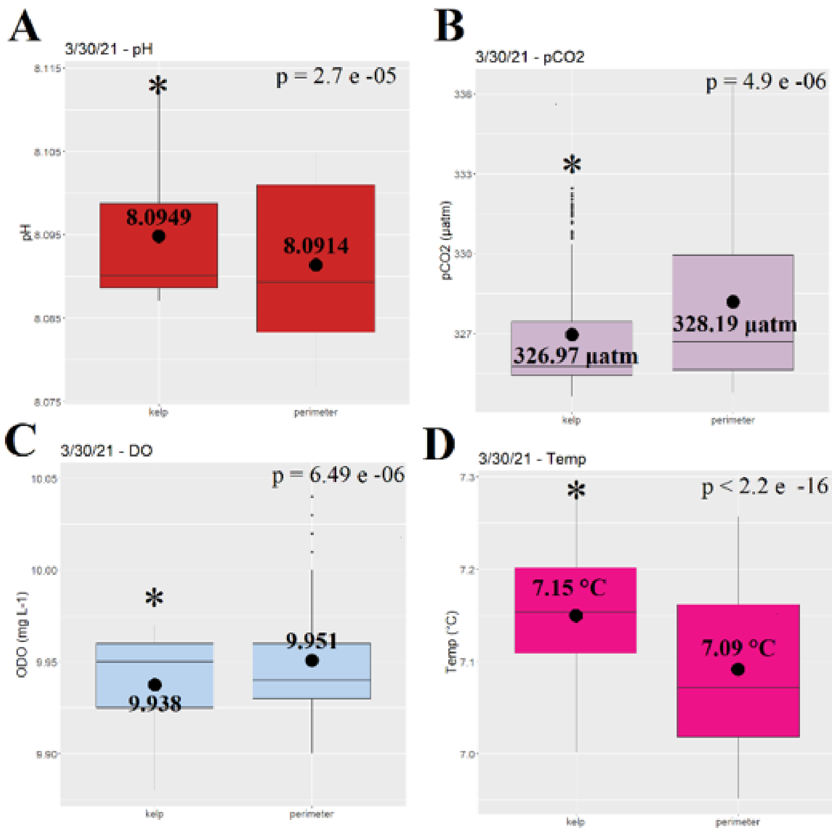


Figure 3e.4. Combined flow-through data from the 3/30/21 cruise, showing pH (A), pCO2 (B), dissolved oxygen (C), and temperature (D). Large dots on boxplots indicate mean value, with mean value shown in text bolded near dots. Asterisk indicates statistically significant difference between the within-kelp field and perimeter for that parameter.

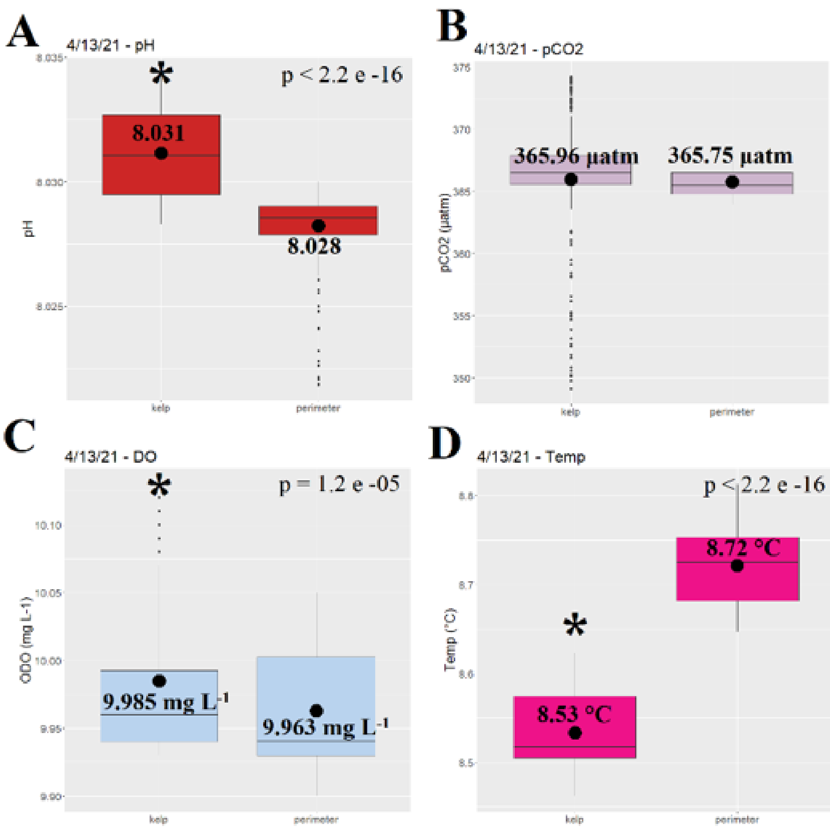


Figure 3e.5. Combined flow-through data from the 4/13/21 cruise, showing pH (A), pCO2 (B), dissolved oxygen (C), and temperature (D). Large dots on boxplots indicate mean value, with mean value shown in text bolded near dots. Asterisk indicates statistically significant difference between the within-kelp field and perimeter for that parameter.

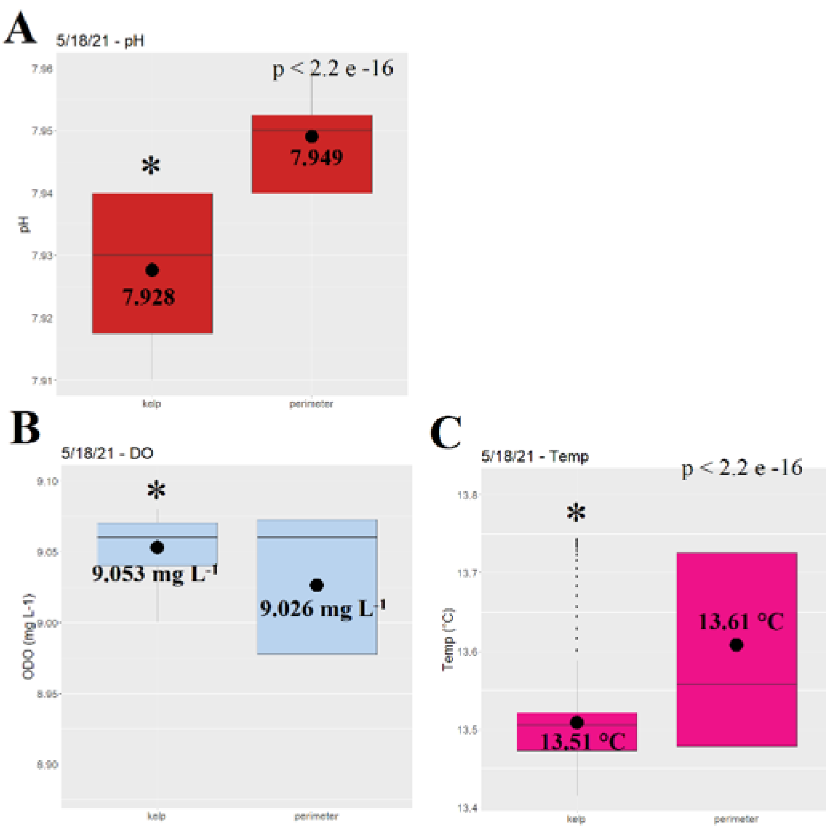


Figure 3e.6. Combined flow-through data from the 5/18/21 cruise, showing pH (A), dissolved oxygen (B), and temperature (C). pCO2 data was unavailable for this cruise. Large dots on boxplots indicate mean value, with mean value shown in text bolded near dots. Asterisk indicates statistically significant difference between the within-kelp field and perimeter for that parameter.

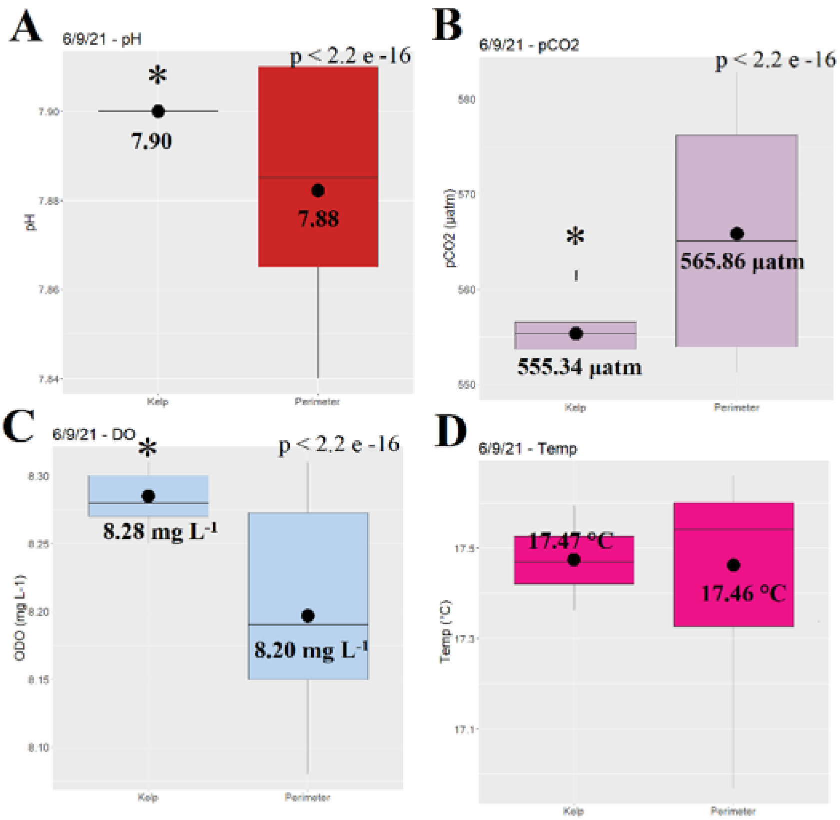


Figure 3e.7. Combined flow-through data from the 6/9/21 cruise, showing pH (A), pCO2 (B), dissolved oxygen (C), and temperature (D). SeaFET sensor was malfunctioning for this cruise, so EXO pH data is displayed instead. Large dots on boxplots indicate mean value, with mean value shown in text bolded near dots. Asterisk indicates statistically significant difference between the within-kelp field and perimeter for that parameter.

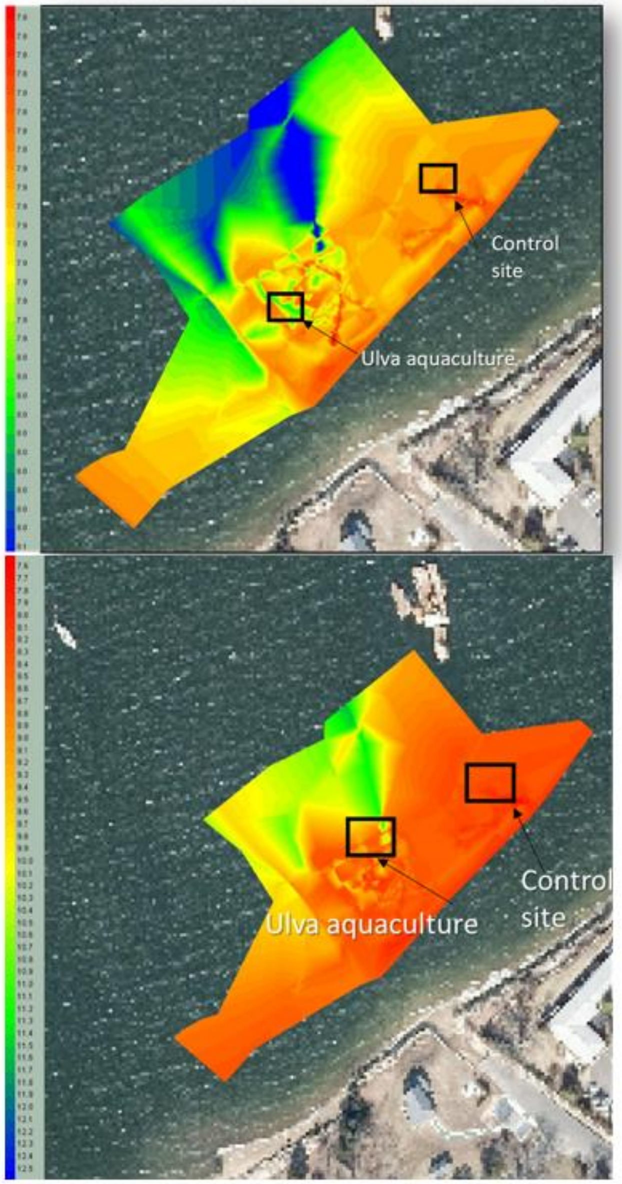


Fig 3e8. Surface pH (above) and DO (below) levels mapped around a 25-bag Ulva array deployed in Northport Harbor as depicted in Figure 2C2 by a Hycat autonomous surface vehicle equipped with a YSI EXO3 sonde.

Site	Estuary	MLW Depth	Date(s) seeded	Cultivation Method
East River	Long Island Sound	30 ft	12/21/22	Suspended lines
Northport Harbor	Long Island Sound	3 ft	12/28/22	Staked lines
Mt. Sinai Harbor	Long Island Sound	3 ft	12/9/22	Staked lines
Moriches Bay	South Shore Estuary	2 ft	12/17/22	Staked lines
PE2	Peconic Estuary	20 ft	12/15/21	Suspended lines
PE3	Peconic Estuary	20 ft	12/14/21	Suspended lines
PE4	Peconic Estuary	23 ft	1/13/22	Suspended lines
PE5	Peconic Estuary.	15 ft	12/15/21	Suspended lines

Table 2b.1. Summary of study sites where sugar kelp was cultivated during the 2022 kelp growing season, including estuary, water depth at MLW, line seeding date, and cultivation method.

Hatchery	Sorus Source	Spore Density (ml^{-1})	Treatment ID
Stony Brook	Black Ledge, 11/4/21	1,800	SB-BL-low
Stony Brook	Montauk, 11/3/21	1,333	SB-MTK-low
Hart	Race point, 10/24/21	7,500	H-RP-high

Table 2b.2. Summary of the three seedstocks used in the 2022 kelp cultivation studies, including hatchery, source of parental reproductive tissue (sorus tissue), and spore setting density.

Seaweed Species	Site	Start date	End Date	Duration (days)	Small oyster Initial shell height (mm)	Large oyster Initial shell height (mm)	Starting seaweed biomass (g)
Ulva	Mt. Sinai	6/26/21	8/12/21	47	10.9 ± 0.6	24.5 ± 0.9	10
Ulva	Northport	7/2/21	8/12/21	41	10.9 ± 0.7	22.6 ± 0.6	10
Ulva	Northport	6/24/22	7/20/22	26	11.8 ± 0.6	36.0 ± 1.0	20
Gracilaria	Mt. Sinai	9/14/21	10/6/21	22	n.a.	24.9 ± 2.4	200 or 1,500
Gracilaria	Mt. Sinai	7/27/22	8/16/22	20	13.5 ± 0.5	45.0 ± 1.1	20
Gracilaria	Northport	9/14/21	10/7/21	23	n.a.	26.0 ± 1.6	200 or 1,500
Gracilaria	Northport	8/5/22	8/18/22	13	12.8 ± 0.5	50.6 ± 1.5	20

Table 2c.1. Summary of the summer seaweed-bivalve co-cultivation experiments conducted in Northport and Mount Sinai Harbors in summer and fall of 2021 and 2022

Site	Wet weight line yield (kg m-1)	Dry weight line yield (kg m-1)	Tissue N (% of dry mass)	Nitrogen bioextraction (g m-1)	Kelp line per acre (m)	Nitrogen bioextraction (kg acre-1)
Moriches Bay	10.75	1.20	2.01	24.21	2,400	58.11
East River	11.82	1.32	2.67	35.27	600	21.16
Northport Harbor	1.04	0.12	1.62	1.90	2,400	4.56
Mt. Sinai Harbor	2.25	0.25	0.90	2.27	2,400	5.46
Peconic Estuary	0.06	0.01	0.85	0.06	600	0.04

Table 3a.1. Summary of peak harvest yields and nitrogen bioextraction of kelp aquaculture at each study site.

Site	Wet weight bag yield (g bag^{-1})	Dry weight bag yield (g bag^{-1})	Tissue N (%)	Nitrogen extraction (g bag^{-1})	Bags per acre	Harvests per year	Ulva wet biomass yields (kg $acre^{-1}$ $year^{-1}$)	Nitrogen extraction (kg $acre^{-1}$ $year^{-1}$)
Northport 2021	810.8	131.3	2.25	2.96	1,600	4	5,189	18.91
Mt. Sinai 2021	299.1	48.4	2.25	1.09	1,600	4	1,914	6.98
Northport 2022	175.9	28.5	2.25	0.64	1,600	4	1,126	4.10

Table 3b.1. Estimated nitrogen bioextraction achieved through *Ulva* aquaculture at each study site. The estimates of bag yields were obtained using the range of Ulva growth rates found in the three field experiments, and assuming that grow-out bags are inoculated with 20g of Ulva tissue and harvested after four weeks (the optimal cultivation period found in this study). Extrapolation from yields per bag to yields per acre were made assuming that a one-acre (63.6 m x 63.6 m) farm has the capacity to hold 1,600 floating bags, configured in 32 parallel rows spaced 2 meters apart, with 50 bags in each row. This assumption is based on actual floating bag densities used on shallow-water oyster farms on Long Island. It was also estimated that the growing season for *Ulva* is about 16 weeks, from mid-May to mid-September, allowing for 4 harvests per year. Tissue nitrogen content was measured from *Ulva* that was wild-collected from Shinnecock Bay, the parental source for tissue used to inoculate experimental grow-out bags in 2021 and 2022.

Site	Wet weight bag yield (g bag^{-1})	Dry weight bag yield (g bag^{-1})	Tissue N (%)	Nitrogen extraction (g bag^{-1})	Bags per acre	Harvests per year	*Gracilaria* wet biomass yield (kg $acre^{-1}$ $year^{-1}$)	Nitrogen extraction (kg $acre^{-1}$ $year^{-1}$)
Northport 2021	31.1	4.0	3.87	0.154	1,600	6	298.7	1.48
Mt. Sinai 2021	23.5	3.0	3.34	0.100	1,600	6	225.2	0.96
Northport 2022	0.8	0.1	3.87	0.004	1,600	6	7.7	0.04
Mt. Sinai 2022	22.6	2.9	3.34	0.097	1,600	6	217.2	0.93

Table 3c.1. Estimated nitrogen bioextraction achieved through *Gracilaria* aquaculture at each study site. The estimates of bag yields were obtained using the range of *Gracilaria* growth rates found in the three field experiments, and assuming that grow-out bags are inoculated with 20g of *Gracilaria* tissue and harvested after 18 days (the optimal cultivation period found in this study). Extrapolation from yields per bag to yields per acre were made assuming that a one-acre (63.6 m x 63.6 m) farm has the capacity to hold 1,600 floating bags, configured in 32 parallel rows spaced 2 meters apart, with 50 bags in each row. This assumption is based on actual floating bag densities used on shallow-water oyster farms on Long Island. It was also estimated that the growing season for *Gracilaria* is about 16 weeks, from mid-June to mid-October, allowing for 6 harvests per year. Tissue nitrogen content was measured in *Gracilaria* harvested at the end of cultivation experiments in Northport and Mount Sinai Harbors in 2021 (the same values were used for the 2022 calculations of nitrogen bioextraction).

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C2. Scientific Abstract:

Bivalves and seaweeds are well-known for their capacity to assimilation significant amounts of nitrogen, a process known as bioextration. This project grew multiple species of cold-water (*Saccharina latissima*) and warm-water (*Gracilaria tikvahiae*; *Ulva* spp.) seaweeds in multiple locations and at multiple scales in Long Island Sound to quantify net nitrogen (N) removal rates along with changes in water quality. This project also co-cultured bivalves with seaweeds to assess co-effects of these organisms when grown together and separated. In addition to growing bivalves and seaweeds at three sites across LIS (East River, Northport Harbor, Mount Sinai), sugar kelp (*S. latissima*) growth outcomes were compared to deployments in Moriches Bay and the Peconic Estuary using identical methods and source of kelp tissue. A novel staked line approach was pioneered for this project for the deployment of seaweeds in shallow waters specifically within Moriches Bay, Northport Harbor, and Mount Sinai Harbor. This approach provided growth and bioextraction yields that were equal to, or greater than, traditional seaweed deployment methods. Among the three seaweeds examined, kelp displayed the largest nitrogen bioextraction potential with 5 – 58 kg of N potentially removed per acre, followed by *Ulva* (4 – 19 kg N per acre), and *Gracilaria* (1 – 1.5 kg N per acre). Among the sites examined for kelp, Moriches Bay provided the greatest N bioextraction, followed by the East River, Northport Harbor, Mount Sinai Harbor, and finally the Peconic Estuary which had minor yield compared to the other sites. These patterns largely paralleled ambient concentrations of nitrate concentrations and suggests high N environments are ideal for maximizing bioextraction of N by kelp. For summer seaweeds, yields within Northport Harbor and Mount Sinai Harbor were similar when considering both field seasons of this project. Regarding, shellfish, in all locations and with all seaweeds, there were experiments during which the co-culture of bivalves with seaweeds led to significantly faster bivalve growth rates compared to the growth of bivalves in close proximity but without seaweeds ($p<0.05$). In many cases, increased bivalve growth was accompanied by higher levels of DO and pH, suggesting bivalve growth stimulation was caused by improvements in water quality caused by the seaweeds. In contrast, bivalves had minimal impact on seaweed growth. Water quality mapping at the Thimble Islands revealed the ability of dense deployments of kelp to increase DO and pH while decreasing levels of pCO_2 and chlorophyll *a*. Surface water mapping of large-scale *Ulva* deployments in Northport Harbor demonstrated the ability of this seaweed to regionally raise levels of DO and pH during the day. Collectively, this project demonstrated the great potential for seaweeds to bioextract N, increase DO, increase pH, and accelerate the growth of blue mussels and Eastern oysters.

C3. Problems Encountered:

Some restrictions associated with the COVID-19 pandemic slowed some progress on this project.

C4. New Research Directions:

This project expanded our research into red branching macroalgae like *Gracilaria* and helped lay the groundwork for our current NYSG project exploring this alga.

C5. Interactions:

We have monthly meetings with NYSDEC and the LISS Bioextraction Coordinator about this project and other Bioextraction initiatives in NY and on LIS.

C6. Presentations and Publications:

Publications:

CS Young, MH Doall, **CJ Gobler.** 2021. Dual benefit of ocean acidification for the laminarialean kelp Saccharina latissima: enhanced growth and reduced herbivory
Marine Ecology Progress Series 664, 87-102

Young, CS, **CJ Gobler.** 2021. Coastal ocean acidification and nitrogen loading facilitate invasions of the non-indigenous red macroalga, *Dasysiphonia japonica*. Biological Invasions, 1-25

Sylvers, LH, **CJ Gobler**. 2021. Mitigation of harmful algal blooms caused by *Alexandrium catenella* and reduction in saxitoxin accumulation in bivalves using cultivable seaweeds.
Harmful Algae 105, 102056

C Benitt, CS Young, LH Sylvers, CJ Gobler Inhibition of harmful algal blooms caused by Aureococcus anophagefferens (Pelagophyceae) using native (Gracilaria tikvahiae) and invasive (Dasysiphonia japonica) red seaweeds. Journal of Applied Phycology, 1-19

Presentations:

Gobler, C.J. 2023. Quantifying the ability of seaweed aquaculture in Long Island Sound to remove nitrogen, combat ocean acidification, improve water quality, and benefit bivalves. Presentation to the LISS STAC, June 2023.

D. Accomplishments: Complete the following sub-sections:

D1. Impacts & Effects:

Seaweeds are an emerging aquaculture industry in NY, CT, and LIS. While there are economic benefits to growing seaweeds as food, fertilizer, feed, or as an additive, this project has demonstrated the ability of seaweeds to improve water quality and, in turn, increase the growth rates of mussels and oysters. This outcome makes the aquaculture of seaweeds a significant benefit to shellfish farmers who may have only a single crop and whose crop may be threatened by poor water quality. This project should, therefore, lead to a more wide-spread adoption of seaweed aquaculture among shellfish farmers. We have grown seaweeds on 10 oyster farms during the past five years and several of those farms are now cultivating kelp as well.

D2. Scholar(s) & Student(s) Status:

Ian Dwyer, graduates spring 2025

Ann Marie Famularo, graduates spring 2025

Ben Kramer, graduated spring 2023

Rebecca Rogers, dropped out

Bradley McGuire, MS Student, graduated fall 2023; working at IAEA in Austria

Laine Sylvers, NYSG Scholar PhD Student, completion 2024

D3. Volunteers:

Lucas Chen, Research Technician

Andrew Lundstrom, Research Technician

Cameron Provost, Research Technician

Margot Eckstein, BS Student

Laine Sylvers, PhD Student

Stephen Tomasetti, PhD Student

D4. Patents:

None

D5. Leveraged Funding: List and describe any funding or grants (e.g. from NSF, etc.) you received with the help of the work or results from this project. Provide funding amount, funding source, project title, and project dates.

E. Stakeholder Summary:

Bivalves and seaweeds are well-known for their capacity to assimilation significant amounts of nitrogen, a process known as bioextration. This project grew multiple species of cold-water (*Saccharina latissima*) and warm-water (*Gracilaria tikvahiae*; *Ulva* spp.) seaweeds in multiple locations and at multiple scales in Long Island Sound to quantify net nitrogen (N) removal rates along with changes in water quality. This project also co-cultured bivalves with seaweeds to assess co-effects of these organisms when grown together and separated. In addition to growing bivalves and seaweeds at three sites across LIS (East River, Northport Harbor, Mount Sinai), seaweed growth outcomes were also compared to deployments in Moriches Bay and the Peconic Estuary. A new staked line approach was pioneered for this project for the deployment of seaweeds in shallow waters specifically within Moriches Bay, Northport Harbor, and Mount Sinai Harbor. This approach provided growth and bioextraction yields that were as good as traditional seaweed deployment methods. Among the three seaweeds examined, kelp displayed the largest nitrogen bioextraction potential, followed by *Ulva* and *Gracilaria*. Among the sites examined for kelp, Moriches Bay provided the greatest N bioextraction, followed by the East River, Northport Harbor, Mount Sinai Harbor, and finally the Peconic Estuary which had very little yield. For summer seaweeds, yields within Northport Harbor and Mount Sinai Harbor were similar when considering both field seasons of this project. Regarding, shellfish, in all locations and with all seaweeds, co-culture of bivalves with seaweeds led to significantly faster bivalve growth rates compared to the growth of bivalves in close proximity without seaweeds. In many cases, increased bivalve growth was accompanied by higher levels of DO and pH, suggesting bivalve growth stimulation was caused by improvements in water quality caused by the seaweeds. In contrast, bivalves had minimal impact on seaweed growth. Water quality mapping at the Thimble Islands revealed the ability of dense deployments of kelp to increase DO and pH while decreasing levels of pCO_2 and chlorophyll *a* concentrations. Surface water mapping of large-scale *Ulva* arrays in Northport Harbor demonstrated the ability of this seaweed to regionally raise levels of DO and pH. Collectively, this project demonstrated the great potential for seaweeds to bioextract N, increase DO, increase pH, and accelerate the growth of blue mussels and Eastern oysters.

F. Pictorial: See also figures

SBU Gobler Laboratory researchers inspecting a staked line rigging with sugar kelp at Mount Sinai Harbor.

An experimental modular dockside kelp rig being deployed in Britannia Marina, Northport by SBU Gobler Laboratory researchers.

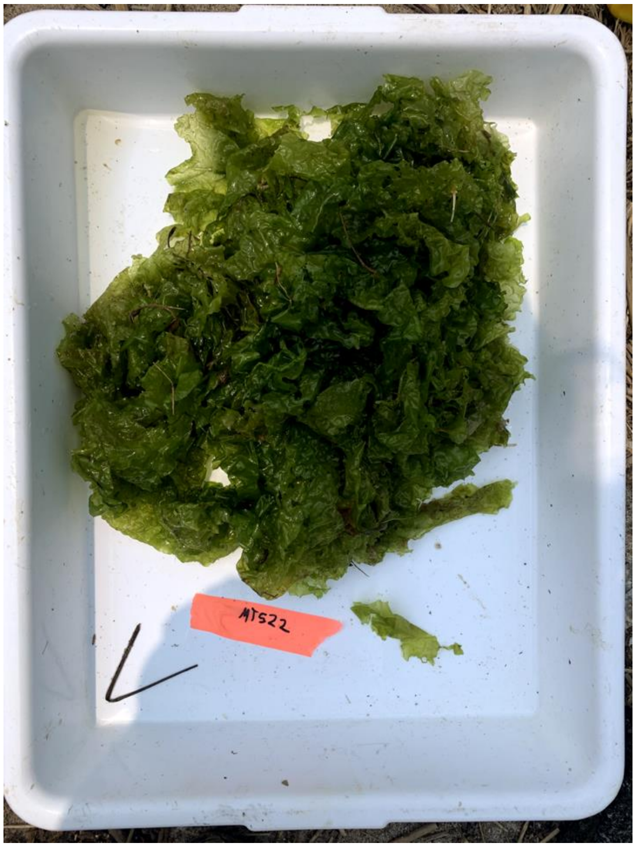
Sensors monitoring conductivity, pH, and DO continuously in Northport Harbor.

Floating array of bags containing oysters and seaweeds at Mount Sinai Harbor.

Deployment of oyster and seaweed bag array at Northport Harbor

Oyster and seaweed bags being inspected prior to reattachment to the array in Mount Sinai Harbor.

Oysters organized on grid paper for photographic height growth measurements at Mount Sinai Harbor.



Ulva grown in the experimental array at Mount Sinai Harbor.