Project Period	01/03/2011 - 07/01/2012
Award Amount	$123,999.00
Matching Contributions	$61,771.00
Project Location Description (from Proposal)	The mouth of the Bronx River Estuary, East River, Bronx, Bronx County, NY and off the coast of the Town of Fairfield,Fairfield County,CT.
Project Summary (from Proposal)	Pilot the use of seaweed to "bio" extract pollution and then measure reductions in pollution in New York at the mouth of the Bronx River and Connecticut off the coast of Fairfield.
Summary of Accomplishments	• Nutrient extraction for bioremediation was tested using the native red alga, Gracilaria tikvahiae at two sites: one off Fairfield, CT (LIS); and the other at the mouth of the Bronx River estuary (BRE). Gracilaria at the BRE site grew 11.8% and 10.0% d-1 at 0.5m and 1.0m deep, respectively, in Aug. Growth rates at the LIS site were 5.9% and 6.0% d-1 at the same depths. • Estimates of nitrogen removal rates by Gracilaria from the LIS site averaged 2.6 kg N ha-1 mon-1 from Aug.-Oct., and 5.5 kg N ha-1 mon-1 in Sept. and Oct. at the BRE site. During Aug. at the BRE site, nitrogen was removed at 10.3 kg N ha-1 mon-1. • Nutrients were rapidly assimilated and used to fuel the growth of new Gracilaria tissue at the BRE site, while nutrients appeared to limit the growth of Gracilaria at the LIS site during July and Aug. • Students and staff at the Bridgeport Regional Aquaculture Science and Technology Center, Rocking the Boat and Purchase College assisted at our seed stock nursery and at the farm sites. We have presented some of our results at scientific meetings and forums relevant for Long Island Sound. Our work was highlighted in E-The Environmental Magazine, CT-Mirror, CT Public TV program, 'All Things Connecticut,' The Day (also the Associated Press), NBC Universal WVIT news, and Aqua Kids.
Lessons Learned	• Gracilaria aquaculture can be a useful technique for nutrient bioremediation in urbanized coastal waters of the US. Since N removal varied with site and season, seaweed bioremediation might be best applied at nutrient hot spots in LIS and other New York estuaries trying to reduce nutrients from nonpoint and point sources. • While epiphytes were not noticeable on Gracilaria bundles or the long lines, fouling fauna can be a major problem during the summer months. Tunicates (e..g. Molgula) and hydroids (Turbularia) attached and grew on Gracilaria bundles and may have inhibited the growth of Gracilaria. A high density of tunicates was observed on the deeper water lines (1 m) in LIS. Therefore, adjustment of cultivation depth (<0.5 m) may be one way to control these and related epizoans. • The Gracilaria farm system also provides a good habitat for many small animals. A total of eighteen species of associated fauna were identified at both field sites. We would encourage conservation organizations to also look at food webs that are developing in and around the nutrient bioextraction systems. • Another major lesson was that the native species, Gracilaria tikvahiae, is being rapidly replaced by its non-native cogeneror, G. vermiculophylla! This was confirmed via molecular fingerprinting and extreme caution needs to be exercised in obtaining seedstock of the

native Gracilaria species.

Conservation Activities	See Narrative - Not Required
Progress Measures	Other Activity Metric
Value at Grant Completion	Not Required

Final Programmatic Report Narrative

Instructions: Save this document on your computer and complete the narrative in the format provided. The final narrative should not exceed ten (10) pages; do not delete the text provided below. Once complete, upload this document into the on-line final programmatic report task as instructed.

1. Summary of Accomplishments

In four to five sentences, provide a brief summary of the project's key accomplishments and outcomes that were observed or measured.

- A nutrient bioextraction using *Gracilaria tikvahiae* was tested at two sites: Long Island Sound (LIS) off Fairfield, CT, and at the mouth of the Bronx River estuary (BRE) in Bronx, NY. *Gracilaria* at the mouth of the Bronx River estuary (BRE) grew very well with growth rates of 11.8% and 10.0% d^{-1}at 0.5m and 1.0m deep, respectively, in August, while the growth rates at the LIS site were 5.9% and 6.0% d^{-1} at 0.5m and 1.0m depths, respectively, during the same period.
- *Gracilaria* removed nitrogen from the LIS site at 0.7, 3.2 and 3.9 kg N ha^{-1} mon^{-1} during the months of August, September and October, respectively; and 5.9 and 5.1 kg N ha^{-1} mon^{-1} in September and October, respectively, at the BRE site. During the month of August at the BRE site, the nitrogen removal was estimated at 10.3 kg N ha^{-1} mon^{-1}.
- The rates of growth and N removal of *Gracilaria* thalli suggested that nutrients were being rapidly assimilated and used to fuel the growth of new *Gracilaria* tissue grown at the BRE site, while the availability of nutrients appear to limit the growth of *Gracilaria* at the LIS site during July and August.
- We worked with students and staff at the Bridgeport Regional Aquaculture Science and Technology Center over 500 student hours), Rocking the Boat and Purchase College. The students and staff at BRASTEC have assisted in culturing *Gracilaria* at our BRASTEC seed stock nursery and at the LIS farm site. We have presented some of our results at scientific meetings and forums relevant for Long Island Sound. Our work has been highlighted in E-The Environmental Magazine (Oct, 2011), CT-Mirror (Dec. 6, 2011), Connecticut Public Television program, 'All Things Connecticut,' The Day (Mar. 11, 2012 also picked up by the Associated Press), NBC Universal WVIT news (June 6, 2012), and a syndicated TV show called Aqua Kids (June 25, 2012).

2. Project Activities & Outcomes

Activities

- Describe and quantify (using the approved metrics referenced in your grant agreement) the primary activities conducted during this grant.
- Briefly explain discrepancies between the activities conducted during the grant and the activities agreed upon in your grant agreement.

Outcomes

- Describe and quantify progress towards achieving the project outcomes described in your grant agreement. (Quantify using the approved metrics referenced in your grant agreement or by using more relevant metrics not included in the application.)
- Briefly explain discrepancies between what actually happened compared to what was anticipated to happen.
- Provide any further information (such as unexpected outcomes) important for understanding project activities and outcome results.

Task 1 – Isolation and establishment of laboratory cultures of *Gracilaria tikvahiae*

Figure 1. Strain selection. Left: Excising healthy tips and agar drag for cleaning; Right: Carpospore release from a cystocarp formed on the surface of a female plant.

Objective 1: Isolate and maintain cultures of southern New England species of *Gracilaria* to be used as "seed stock" for the production of young plants in a modular culture system at the University of Connecticut (UCONN, Stamford) and at The Bridgeport Regional Aquaculture Science and Technology Center (BRASTEC).

In 2010, we collected the native *Gracilaria tikvahiae* throughout southern New England. The species identification was confirmed by DNA sequencing using the ribosomal small-subunit 18s RNA gene.

Figure 2. Clone cultures in small flasks at UConn (left) and in 1,000L and 4,000 L tanks at BRASTEC (middle and right).

At UConn, we established and propagated vegetative cultures of male and female gametophytes and tetrasporophytes, isolated from spores. We also established cultures initiated from field collected plants through vegetative propagation. In both cases, clean plants in good condition were collected from the field and cleaned by gently wiping with sterile cotton balls. Vegetative branches were dragged through seaweed agar to remove epiphytes (Fig. 1). For vegetative cloning, the excised vegetative tips were transferred to von Stosch's enriched seawater (VSE) in 50 mm sterile Petri dishes and maintained at 15-20°C, 10 µmol m^{-2} s^{-1} light and day neutral (12:12, L:D) conditions (Ott, 1965). Tetrasporic and cystocarpic branches were transferred to sterile culture dishes containing VSE and were maintained at 20°C, 10 µmol m^{-2} s^{-1} light and day neutral conditions until spores have been released and begin to germinate. Individual germlings were transferred to VSE in 50 mm sterile culture dishes and maintained at 15-20°C, 10 µmol m^{-2} s^{-1} light and day neutral conditions. Over 10 strains of the native *Gracilaria* species have been isolated and maintained at the UConn and BRASTEC laboratories (Fig. 2).

Task 2 – Mass culture studies of *Gracilaria tikvahiae* in laboratory
Objective 2: Determine the optimum conditions to produce asexually propagated thalli of *Gracilaria tikvahiae* in mass culture units;
2-1. Development of new culture media for *Gracilaria* cultivation.

Table 1. Nutrient composition in each media

	VSE	CF1	CF2
Nitrogen	500 µM	500 µM (41% NH_3 and 59% NO_3)	500 µM (15% NH_3 and 85% Urea)
Phosphorus	30 µM	39 µM	34 µM
Iron	1 µM	0.6 µM	0.8 µM
Manganese	10 µM	0.3 µM	0.3 µM
EDTA	10 µM	not specified by maker	not specified by maker
Vitamins	Yes	-	-

The commonly used nutrient medium for red algal cultivation is the von Stosch Seawater enrichment (VSE) media. VSE contains several important nutrients including nitrate, phosphate, iron, manganese, EDTA and vitamins. However, VSE may not be applicable for the commercial scale seaweed cultivation for nutrient bioextraction due to its high material and preparation costs. We evaluated two potential culture media for *Gracilaria* cultivation using commercially available fertilizers (Table 1). Since vitamins are included in VSE, five media conditions were utilized, 1) VSE, 2) commercial fertilizer 1(CF1), 3) CF1 with vitamins (CF1V), 4) commercial fertilizer 2 (CF2) and 5) CF2 with vitamins (CF2V). Total nitrogen and phosphorus concentrations in each medium were adjusted as same those in VSE (500 µM and 30-39 µM, respectively). However, the nitrogen sources were different in each media (VSE, 100% NO_3, CF1, 41% NH_3 and 59% NO_3 and CF2, 15% NH_3 and 85% Urea). *Gracilaria* was cultivated at 100 µmol m^{-2} s^{-1}, 20°C and 12:12 L:D of photoperiod. The stocking density was 2 g L^{-1}. At one-week intervals for three weeks, all of the biomass in each flask was weighed (fresh weight; FW) and samples were taken for tissue analyses. For the analysis of tissue total N content, samples were dried at 60 °C before being ground. The powder was analyzed using a Perkin Elmer 2400 series II CHNS/O elemental analyzer.

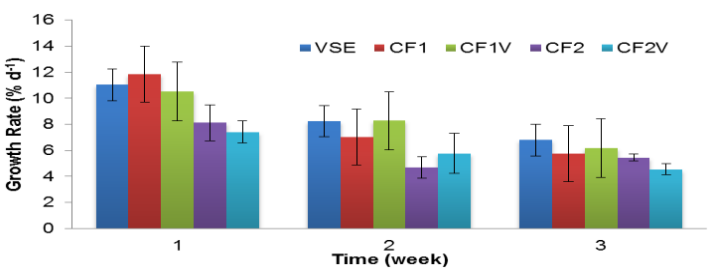


Figure 3. Growth rates of *Gracilaria* grown at different media. VSE: von Stosch enrichment, CF1: commercial fertilizer 1, 3) CF1V: CF1 with vitamins, 4) CF2: commercial fertilizer 2 and 5) CF2V: CF2 with vitamins.

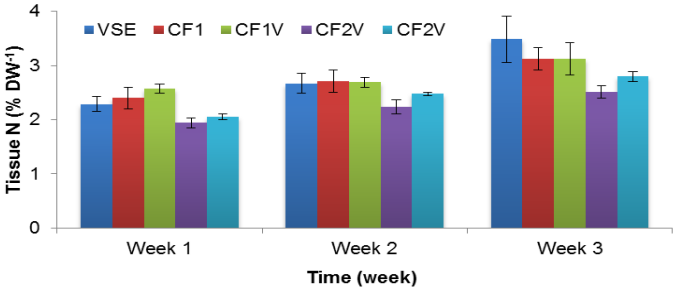


Figure 4. Tissue nitrogen contents of *Gracilaria* grown at different media. VSE: von Stosch enrichment, CF1: commercial fertilizer 1, 3) CF1V: CF1 with vitamins, 4) CF2: commercial fertilizer 2 and

The *Gracilaria* cultivated in the medium with CF1 fertilizer grew as well as the plant in VSE. The growth rates of *Gracilaria* in VSE, CF1 and CF1V were 6.8-11.0%, 5.8-11.8% and 6.2-10.5% d^{-1}, respectively. The growth rates using CF2 (5.5-8.1% d^{-1}) and CF2V (4.5-7.4% d^{-1}) were lower than those in other conditions (Fig. 3). Tissue nitrogen contents were also higher at VSE, CF1 and CF1V than at CF2 and CF2V (Fig. 4). Vitamins did not show any significant effect on the growth of this species, at least over the 7 days growth between culture medium changes (Fig. 3, 4). When the price of each media was compared (Table 2), CF1 proved to be the least expensive ($0.01 per m^3 of medium) than VSE ($1.62). Although the costs for vitamins were removed from VSE, the cost for CF1 media is still 2% of the cost for VSE without vitamins. This result suggests a potential uses of commercially available fertilizer in a seaweed nursery system.

Table 2. Price comparisons of different culture media

	Price per m^3 of culture medium
VSE	$1.623
CF1	$0.010
CF1V	$1.114
CF2	$0.012
CF2V	$1.116

2-2. Effects of temperature, light and photoperiod on the growth of *Gracilaria tikvahiae*

Apical segments of *Gracilaria* were cultivated in a matrix of photosynthetically active radiation (PAR), photoperiods and temperature to determine the optimum conditions for asexual propagation of *Gracilaria*. The combinations of temperature and PAR levels were generated using a light x temperature gradient table. The temperatures tested ranged from 14°C to 26°C in 3°C increments, and the PAR was regulated at 50, 100 and 200 µmol m^{-2} s^{-1}, respectively. Photoperiod was 12:12 L:D. For the combination of photoperiod and temperature, apical segments of *Gracilaria* were cultivated in different incubators with different temperature (15, 20 and 25 ˚C) and photoperiod regimes (12:12 and 16:8 L:D), simulating the spring-fall growing seasons. PAR was maintained at 100 µmol m^{-2} s^{-1}. For both experiments, approximately 1 cm of *Gracilaria* apical segments were placed in each petri dish containing VSE medium. All petri dishes were photographed weekly with a PixeLINK digital camera (Ottawa, Ontario, Canada), under a dissection microscope. The growth of apical segments was determined weekly by measuring the length of apices using the

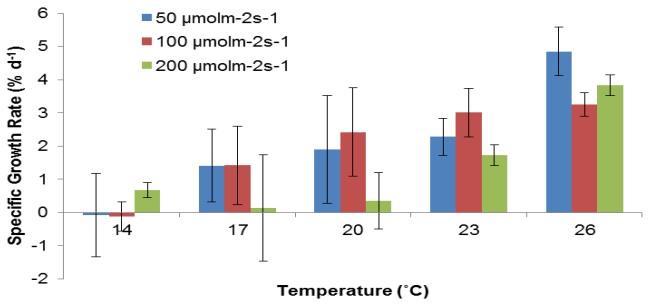


Figure 5. Growth rates of *Gracilaria* grown at different temperature and PAR conditions.

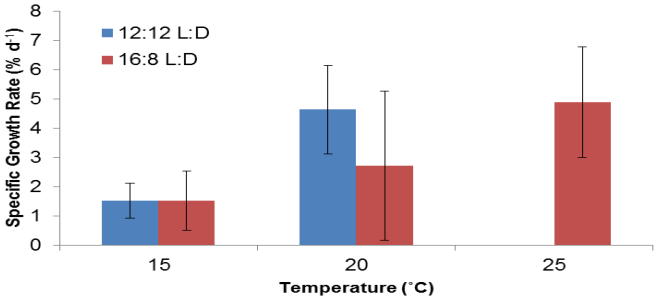


Figure 6. Growth rates of *Gracilaria* grown at different temperature and photoperiod conditions.

PixeLINK® µScope Microscopy Software (Ottawa, Ontario, Canada).

The growth rate was significantly affected by temperature at both experiments (Fig. 5 and 6). For example, the growth rates at 26 ˚C were 4.9, 3.3 and 3.8 % d^{-1} at 50, 100 and 200 µmol m^{-2} s^{-1}, respectively, and decreased as temperature decreased. At 14 ˚C, *Gracilaria* did not or barely grow at all PAR conditions (Fig. 5). Total photon flux did not show any significant pattern in terms of growth. *Gracilaria* grew equally well at neutral and long day conditions at 15 and 20 ˚C and at 100 µmol m^{-2} s^{-1} (Fig. 6).

2-3. Effects of a hypo-osmotic and temperature stress on the growth of *Gracilaria*

Recent studies have demonstrated that *Gracilaria vermiculophylla*, a non-native macroalga, is the dominant species in many local embayments throughout southern New England and Long Island Sound (Sears, 2002; van Patten, 2006; Nettleton et al., *In Press*.) and is replacing the local species *G. tikvahiae* (Yarish, unpubl.). Current molecular studies on the taxonomy of several *Gracilaria* species (including *G. tikvahiae* and *G. vermiculophylla*) populations throughout the world are revealing the wide distribution of *Gracilaria vermiculophylla*, a non-indigenous species from the Eastern Pacific (Saunders, 2009; Kim et al. 2010; www.algaebase.org).

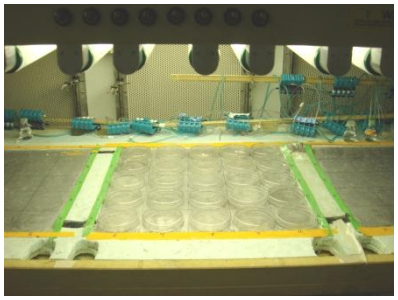


Figure 7. Apical segments of *Gracilaria* growing in petri dishes.

Salinity may drop drastically due to heavy run-off. *Gracilaria* in LIS grows mainly in the subtidal zone and therefore rarely is exposed to hyper-osmotic conditions, but it often experiences hypo-osmotic stress due to river water input. *Gracilaria* in estuaries, therefore, is likely to be better adapted to hypo- than to hyper-osmotic stresses. Resistance to low salinity and rapid recovery from the hypo-osmotic stress are also important pre-adaptations for successful invasion into brackish water. The salinity regime in areas of LIS inhabited by *Gracilaria* is generally 15 – 30 psu. However, *Gracilaria* is often found at much lower salinity conditions during heavy, fresh water depositing rainfall (e.g. < 5 psu in Holly Pond, Stamford, CT). In Holly Pond, a non-indigenous species *G. vermiculophylla*, appears to have replaced a native species *G. tikvahiae* (Nettleton et al. unpubl.). Therefore, our hypothesis is that the non-indigenous *G. vermiculophylla* may be more tolerant to hypo-osmotic conditions and may recover more quickly from these stresses than the native *G. tikvahiae*.

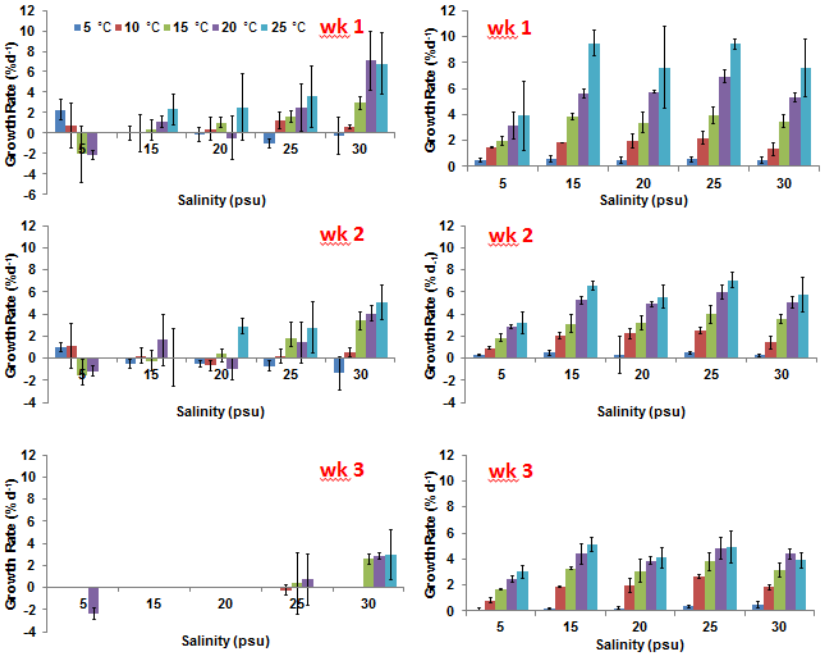


Figure 8. Growth rates of *Gracilaria* grown at different salinity and temperature conditions.

Two local strains of *Gracilaria* were used in this study. The native *G. tikvahiae* (G-RI-ST_1) was originally collected on April 18, 2010 from Potters Pond, Rhode Island (41º23'45.36" N - 71º32'12.52" W) and the ubiquitous occurring non-indigenous *G. vermiculophylla* (G-CT_2-ST_1), which was collected on April 23, 2010 in Holly Pond, Cove Island Park, Stamford, CT (41º02'57.03" N -73º29'56.55" W). Both strains were isolated and were cultured in von Stosch's enriched (VSE) seawater at 20 ºC, 28-30 ppt of salinity, 80-100 µmol$m^{-2}s^{-1}$ of PAR and 12:12 L:D photoperiod. Approximately one centimeter long apical segments from each *Gracilaria* species were cultivated in small petri dishes over three weeks at five different salinities, 5, 15, 20, 25, and 30psu and at five different temperatures, 5, 10, 15, 20 and 25 ºC (Fig. 8). The length of apical segments was measured weekly to estimate growth rates. All other conditions maintained at the optimum conditions for *Gracilaria* growth (i.e., VSE culture media, 100 µmol$m^{-2}s^{-1}$ PAR and 12:12 L:D photoperiod).

Both salinity and temperature affected the growth of both *Gracilaria* species. The native *G. tikvahiae* did not grow or even had negative growth rates at suboptimal conditions (<20 psu and <20 ºC). However, the non-native *Gracilaria vermiculophylla* grew equally well across the salinity range of 15-30 psu. The highest growth rates of *G. tikvahiae* were found at 30 psu and 20 and 25 ºC (6.8 - 7.1% d^{-1}). The highest growth rates of *G. vermiculophylla* were 7.6-9.5% d^{-1}at 25 ºC and 15-30 psu. The native *G. tikvahiae* did not survive over three weeks of culture at the suboptimal condition (<10 ºC and <20 psu) while the non-native *G. vermiculophylla* grew continuously at all conditions tested (Fig. 8). This result suggests that tolerance to the environmental stresses, especially hypo-osmotic stress, may role as a key factor determining the growth and survival of the invasive *Gracilaria* species in embayments and estuaries in Long Island Sound and adjacent estuaries.

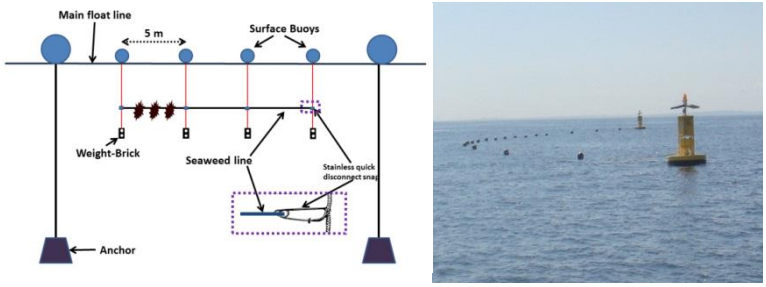


Figure 9. *Gracilaria* long line at the LIS seaweed farm site (left: representative side-view, right: *Gracilaria* long line.

Task 3 – Cultivation of *Gracilaria* at the demonstration sites

Objective 3: Produce biomass of *Gracilaria tikvahiae* in long-line culture units at two near-shore sites in Long Island Sound (Fairfield, CT) and at the mouth of the Bronx River Estuary, at two depths and two stocking densities.

3-1. Seaweed farm design

For each site, two long lines (1 at each depth) were

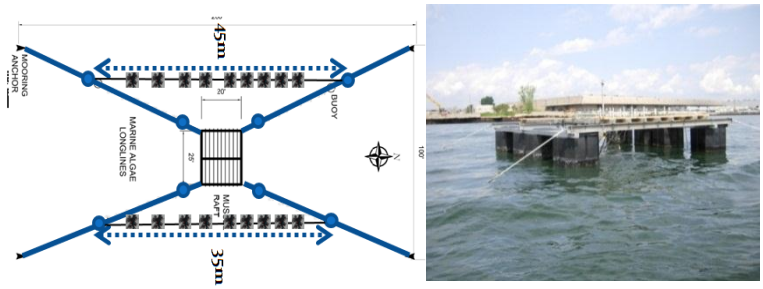


Figure 10. Left: Design of *Gracilaria* long line at the BRE farm site. Right: 6 x 7.5 m mussel raft with 30 x 7.5 m long pegged ropes and weight cases. Seaweed long lines were approximately 35 and 45 m long.

installed at each site. Due to the nature and constraints of the anchoring buoys, long lines approximately 100 and 130 m at the LIS site and 35 and 45 m at the BRE site were installed. Each long line was sub-divided into marked 5 m (LIS; Fig. 9) or 4 m units (BRE; Fig. 10) between which alternated the two stocking densities. These units were measured periodically for wet biomass to assess their growth at two depths and at two stocking densities (each unit of the long line can be unclipped and removed from the water to facilitate harvest or sampling at the designated interval; Fig. 9).

3-2. Salinity, water temperature and light penetration

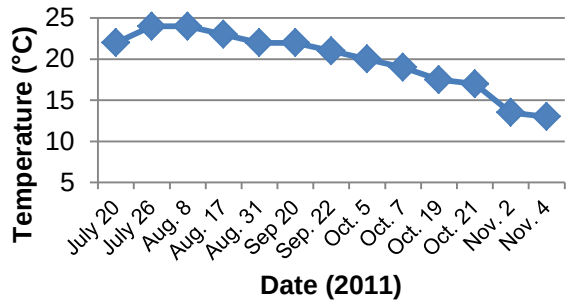


Figure 11. Average water temperature at the LIS and BRE farm sites.

Salinity at the LIS site during the growing season was ranged from 26 to 30 psu. The salinity at the BRE site was slightly lower and ranged from 20-25 psu. Light penetration did not differ between sites during the growing season. At the LIS site, the light penetration was 81.2% (±9.2%) at 0.5 m and 53.2% (±14.4%) at 1.0 m deep, during midday on cloudless days. At the BRE site, it was 80.5% % (±10.0%) at 0.5 m and 48.2% (±4.9%) at 1.0 m deep during mid day on cloudless days. The water temperature was also similar at both sites and at both culture depths. The water temperature from July to September was 22-24 °C and started to drop below 20 °C in October and reached below 13 °C in early November (Fig. 11).

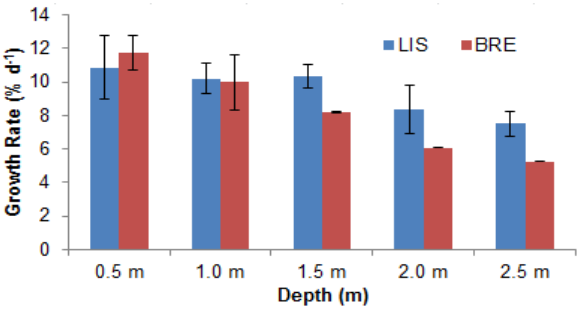


Figure 12. Growth rates of *Gracilaria* grown at different depths. LIS: growth in July 2011; BRE: growth in Aug 2011.

3-3. Determination of the appropriate depths for *Gracilaria* nutrient bioextraction system at the LIS and BRE farm sites.

Prior to the deployment of *Gracilaria* long lines, vertical lines with *Gracilaria* bundles at every 0.5 m down to 2.5 m depth were placed at each site. At the LIS site, the growth rates of *Gracilaria* grown at 0.5, 1.0 and 1.5 m were similar, 10.2-10.8 % d^{-1} and decreased at the depth of 2.0 m or deeper (Fig. 12). At the BRE site, the growth rates were higher at 0.5 and 1.0 m than other depth conditions (Fig. 12). Based on these results, the appropriate culture depths were determined to be 0.5 and 1.0 m at both farm sites.

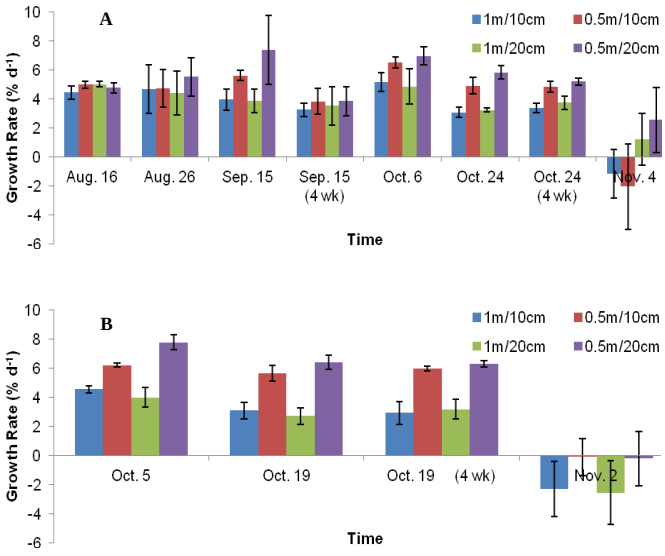


Figure 13. Growth rates of *Gracilaria* grown at different depths (0.5 and 1.0 m), stocking densities (10 and 20 cm) and different harvesting period (2 or 4 wk). Samples collected after two weeks unless indicated as four-week harvest. A) LIS and B) BRE sites.

3-4. Growth of *Gracilaria* at different depths, stocking densities, seasons and harvesting periods at the LIS and BRE farm sites.

Twenty gram bundles of *G. tikvahiae* thalli were inserted into nylon rope line for every condition tested, for a total of 100 m at the LIS and 65 m at the BRE sites. At the LIS site, the growth rates at the first four week harvest period in August were similar at all culture conditions, 4.4-5.5% d^{-1}. However, the growth rates from September and October were greater at the shallower depth. There was no significant effect of stocking density at either depth. However, growth rate dropped significantly in all density and depth combinations in November when the temperature was below 15 °C, indicating the critical temperature effect on the growth of *Gracilaria* (Fig. 13A and 14).

Gracilaria grown at the BRE site also showed higher growth rates at 0.5 m deep while stocking density did not show any significant effect. This result suggests that 10 cm of stocking density between bundles is enough to provide sufficient space

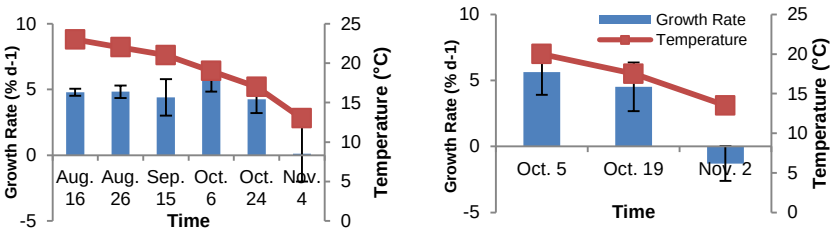


Figure 14. Growth rates of *Gracilaria* and temperature changes over time at LIS (left) and BRE (right).

for *Gracilaria* to grow over a four week period during the months of September and October. Similar to the LIS site, *Gracilaria* did not grow during the final harvest period (late October to early November; Fig. 13B and 14) due to the decrease below the critical growth temperature of 15 ˚C at each site.

Tissue nitrogen contents were 1.5-2.1% in *Gracilaria* from LIS while the tissue N contents were higher in *Gracilaria* from BRE site (4.4-4.6%) during the month of August. In addition, *Gracilaria* tissue harvested from the BRE site was almost black in color, while the tissue from the LIS site was light brown. These results indicate nutrient limitation in the summer months in LIS while nutrient were enrichment in the BRE site (Fig. 15).

Figure 15. *Gracilaria* grown at the LIS site (left) showed light brown in color and the plants from the BRE site showed black in color (right).

Task 4 – Evaluation of the nutrient bioextraction

Objective: Evaluate the nutrient removal capacity of the *Gracilaria tikvahiae* culture units during late spring through fall at each depth and stocking density for both sites. In addition, natural assemblages of the seaweed community on mooring lines of the mussel raft at each site and on long-lines will also be sampled for identification and quantification.

4-1. Carbon and nitrogen removal by *Gracilaria* at different depths, stocking densities, seasons and harvesting periods at the LIS and BRE farm sites

Tissue nitrogen (N) and carbon (C) contents were evaluated with *Gracilaria* samples collected from both farm sites. The material collected from the seaweed culture units were dried in an oven at 55ºC and later ground (Model MM200 Grinder, Retsch, Haan, Germany). The percentages of N and C in the tissue were determined using a CHN analyzer (Series II, CHNS/O 2400 Analyzer, Perkin Elmer Analytical Division of E.G. & G, Wellesley, MA, USA). Using the tissue N and C content combined with biomass data, the nitrogen and carbon removal was calculated using the following equation:

$$N\ removal = \frac{g\ FW\ produced}{m * d} * \frac{g\ DW}{g\ FW} * \frac{g\ N}{g\ DW} = \frac{g\ N\ removed}{m * d}$$

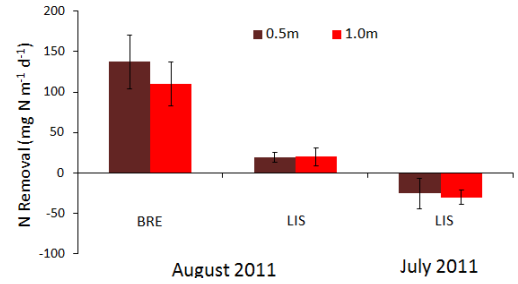


Figure 16. Estimated nitrogen removal by *Gracilaria* grown at different depths at the LIS and BRE farm sites.

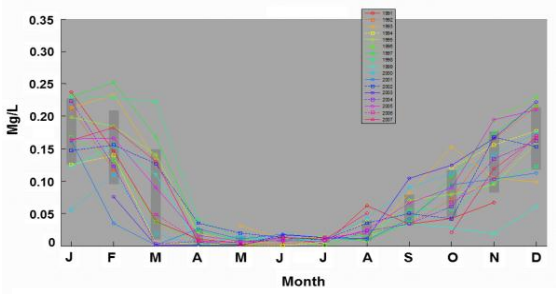


Figure 17. Annual cycle in the average Western Long Island Sound nitrite+nitrate by year (courtesy of J. O'Donnell).

The same calculation was used for C removal, substituting *C* for *N*. Prior to Tropical Storm Irene (Aug. 26-28, 2012), the growth rates at BRE site were 11.8% and 10% d^{-1} at 0.5 and 1.0 m deep, respectively (Fig. 12B). The estimates of nitrogen removal by *Gracilaria* during this period were approximately 14 and 11 mg N m^{-1} d^{-1} (Fig. 16). During the same period, the growth rates and nitrogen removal at the LIS site were 5.9% and 6.0% d^{-1} and 1.9 and 2.0 mg N m^{-1} d^{-1} at 0.5m and 1.0m deep, respectively. Interestingly, although the daily growth rates of *Gracilaria* at the LIS site in July were 10.8% and 10.2% at 0.5 m and 1.0 m deep, respectively (Fig. 12A), *Gracilaria* removed no nitrogen from the water (growth was supported by internal stores). This result indicates that nitrogen may be limiting in Long Island Sound for the growth of *Gracilaria* during the summer months when the nitrogen concentration in the water is the lowest (Fig. 17). For example, the nitrogen and phosphorus concentrations at the water column at the BRASTEC farm site during the month of July, 2011 was ranged 2.7-3.4 µmol L^{-1} and from 0.9-1.2 µmol L^{-1}, respectively (Fig. 18). The nitrogen and phosphorus concentrations at this site started to increase from late August and were as high as 8.4 and 4.7 µmol L^{-1}, respectively. The nutrient concentrations at the BRE site were significantly higher than those at the LIS site (37-55 µmol L^{-1} of nitrogen and 14-19 µmol L^{-1} of phosphorus, respectively, during the months of August through October, 2011).

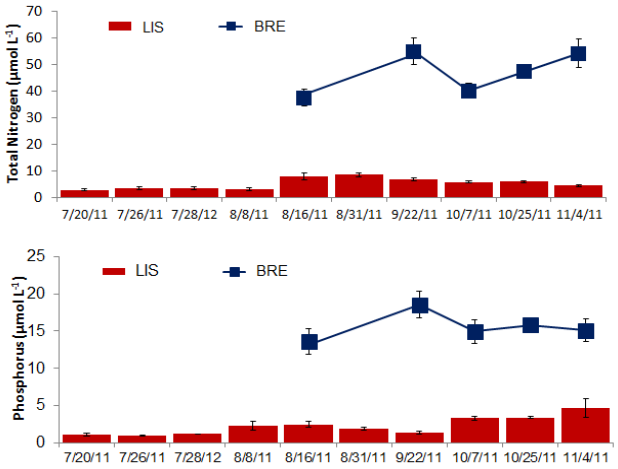


Figure 18. Total nitrogen (top) and phosphorus (bottom) concentration profiles during the *Gracilaria* growing season at the LIS and BRE farm sites. The water samples were analyzed by Dr. Shannon Meseck of the NMFS/NOAA Milford Laboratory (Milford, CT).

The rates of carbon and nitrogen removal were higher at the higher stocking density at both sites. *Gracilaria* grown at 0.5 m depth removed more carbon and nitrogen than the plants grown at 1.0 m (Fig. 19 and 20). For example, the carbon removal at the lower stocking density (20 cm) was only 29-77 % (LIS) and 37-62% (BRE) of those at the higher stocking density (10 cm), driven mostly by differences in yield. The nitrogen removal at the lower stocking density was only 41-64% (LIS) and 36-73% (BRE) of those at the higher stocking density with an exception at 0.5 m on Aug. 26, 2012 at LIS.

We have designed a *hypothetical* nutrient bioextraction 1 hectare *Gracilaria* farm system that assumes 4 m spacing between longlines. This spacing was determined to be the minimum spacing necessary for harvesting from a small work boat. Our *hypothetical* one hectare nutrient bioextraction farm system at the LIS site could remove 38.3 kg C ha^{-1} in August, 33.3 kg C ha^{-1} in September and 28.9 kg C ha^{-1} in October. At the BRE site, our *hypothetical* farm could remove approximately 33.7 and 36.9 kg C ha^{-1} during the months of September and October, respectively. For nitrogen, *Gracilaria* at the LIS site could remove 0.7, 3.2 and 3.9 kg N ha^{-1} during the months of August, September and October, respectively, while approximately, 5.9 and 5.1 kg N ha^{-1} could be removed at the BRE site in the months of September and October, respectively. During the month of August at the BRE site, the nitrogen removal was the highest, 10.3 kg N ha^{-1}.

4-2. Estimates of the contribution of nitrogen from nearby waste water treatment plants in *Gracilaria*.

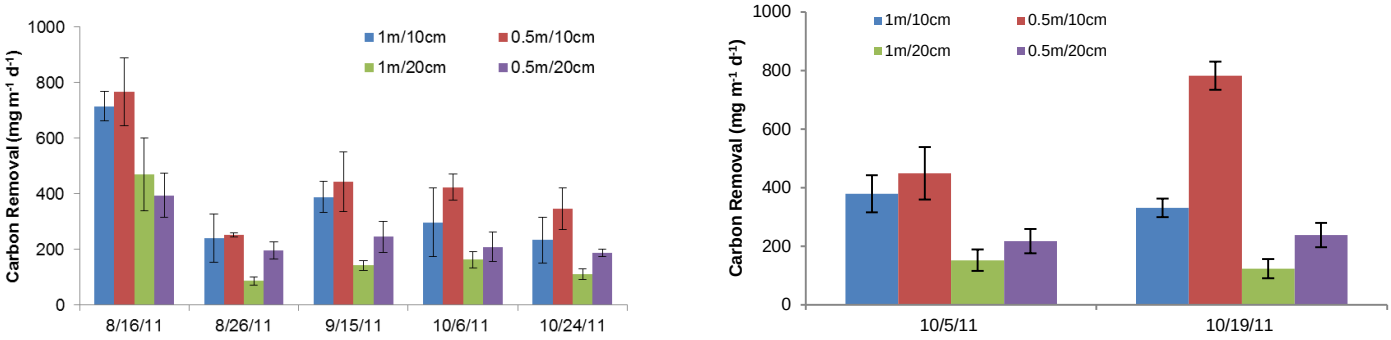


Figure 19. Net carbon removal during the *Gracilaria* growing season at the LIS (left) and BRE (right) farm sites.

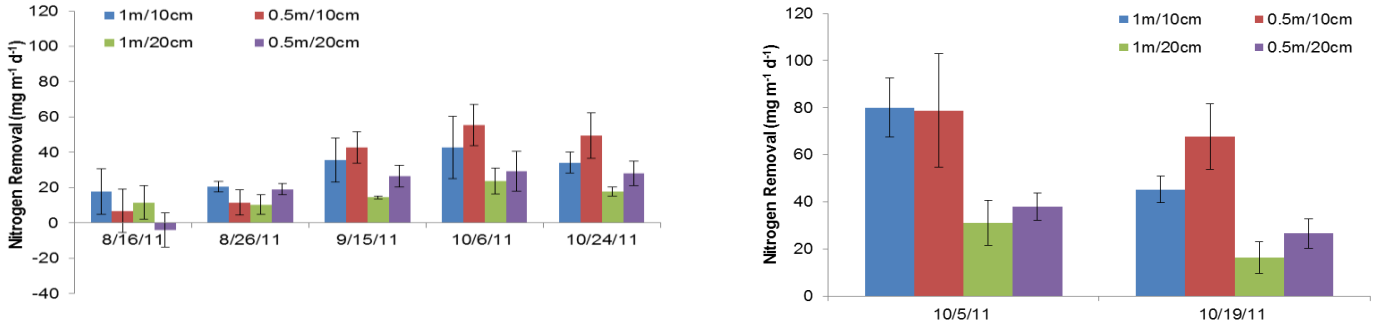


Figure 20. Net nitrogen removal during the *Gracilaria* growing season at the LIS (left) and BRE (right) farm sites.

The fate and uptake of dissolved inorganic nitrogen derived from sewage treatment plants (STPs) can be traced using the ratios of stable isotopes, $^{15}N/^{14}N$. While the untreated sewage carries a ^{15}N-depleted signature (e.g. nitrate- and ammonia-based fertilizers have $\delta^{15}N$ close to zero), treated sewage typically has an elevated $\delta^{15}N$ signature (> 20 ‰) relative to marine DIN $\delta^{15}N$ (4-6‰) (Heaton, 1986; Peterson and Fry, 1987; Owens, 1987; Savage, 2005). It is because natural microbial processes in the STPs are strongly discriminatory fractionation processes that selectively utilize ^{14}N over ^{15}N and produce a ^{15}N-enriched wastewater, therefore, elevated $\delta^{15}N$ signature (Heaton, 1986; Owens, 1987). This elevated $\delta^{15}N$ signature can be traced in primary producers including phytoplankton and seaweeds, and up to the food chain (Savage, 2005). The influence of sewage N could reach 24 km downstream but more significant within 10 km from the

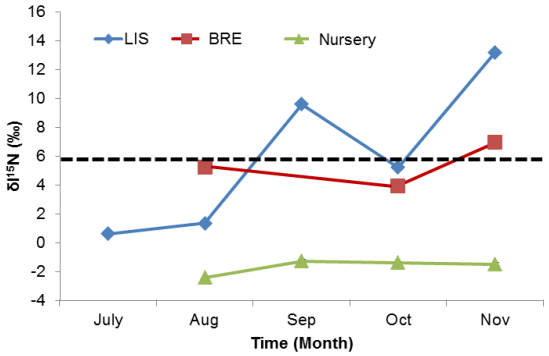


Figure 21. $\delta^{15}N$ signature in *Gracilaria* tissues grown at the LIS and BRE farm sites, and nursery tanks. Dotted line represents the highest $\delta^{15}N$ value in the marine dissolved inorganic N.

STPs. For instance, *Fucus vesiculosus* grown in Himmerfjarden embayment, Baltic Sea, assimilated proportionally more STD-derived N than other macroalgae growing further from the STD (Savage, 2005).

At the BRE site, a large sewage treatment plants is located < 100 m west of the longlines. At the LIS site, a sewage treatment outfall is located < 1 km east from the farm site. Therefore, at both farm sites, the impacts from the sewage treatments were expected. $\delta^{15}N$ values were measured in *Gracilaria* tissues collected from both farm sites. Tissues were dried at 55 ˚C and ground to a powder using a tissue grinder (Model MM200 Grinder, Retsch, Haan, Germany). The N stable isotope ratios in samples were then analyzed at the University of California Davis Stable Isotope Facility (Davis, CA). The $\delta^{15}N$ values in *Gracilaria* grown at the BRE site were similar to the $\delta^{15}N$ values in the marine dissolved inorganic N (4–6‰; Owens, 1987; Peterson and Fry, 1987), suggesting the primary N source at the BRE site is not the sewage treatment plant! The primary source of N could be from either resuspension from the sediments or from nonpoint sources from East river and Bronx river, but further studies are required to confirm this hypothesis. $\delta^{15}N$ values in *Gracilaria* at the LIS site in July (and Aug.) was -0.68 to 1.35 which is similar to the values in *Gracilaria* from the nursery tanks where *Gracilaria* grew before deployment. This $\delta^{15}N$ value confirmed that very little N (if not no N) was available in the western LIS during summer months. Interestingly, the $\delta^{15}N$ values at the LIS site in Aug. and Nov. were high, 9.59 and 13.19 ‰, respectively, suggesting a significant N contribution from the sewage treatment plants during this time period in the LIS site. *Gracilaria* grown in the nursery tanks showed very low values of $\delta^{15}N$ (-2.39 ~ -1.27 ‰) which was expected since N for the nursery system was supplied by a commercial ammonia and nitrate based fertilizer (Fig. 21).

Task 5 – Optimization of harvesting frequencies
Objective: Determine the optimum period of harvest of *Gracilaria tikvahiae* nutrient bioextraction culture units to maximize nutrient removal capacity of each culture unit.

To determine the optimum period of growth before harvest of the seaweed bundles, and to maximize the nutrient removal capacity of each culture unit, half of the units at each density was harvested every two weeks and half every four weeks. A harvest consists of untwisting the rope that holds bundles to the long line then pulling out the bundles, weighing and trimming the bundles until each returns to its original 20 g stocking weight, and then re-inserting them to the long line. In September at LIS at 0.5 m depth condition, the yield of *Gracilaria* harvested in two weeks were higher than those from 4 week harvest while there was no significant difference with plants grown at 1.0 m deep (Fig. 22). In terms of N removal, *Gracilaria* harvested in two weeks showed higher total N removed than those from four week harvest at both 0.5 and 1.0 m depth conditions. However, the harvest frequency had no significant effect on growth and N removal in October, when the water temperature started to decrease, at both sites except for N removal at 0.5m depth and the higher stocking density. This result suggests that harvesting *Gracilaria* every two weeks would provide higher N removal at the experimental sites.

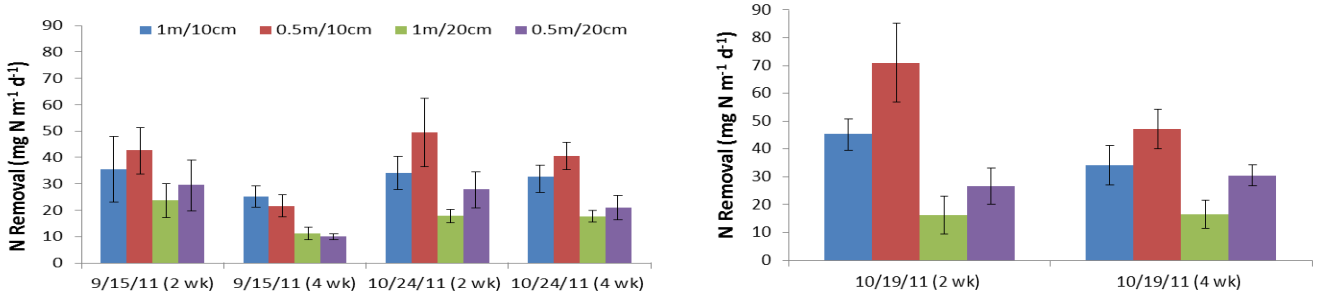


Figure 22. Estimated nitrogen removal by *Gracilaria* at different harvest periods (2 weeks vs. 4 weeks) at the LIS (left) and BRE (right) farm sites.

Task 6 – Outreach and results dissemination
Objective: Develop protocols/manual for implementation of the bioextraction technologies for *Gracilaria tikvahiae* and to present the results of the project in relevant and appropriate events (scientific and non-scientific meetings)

We have been closely working with students and staff at the Bridgeport Regional Aquaculture Science and Technology Center (BRASTEC) and Rocking The Boat (RTB). The students and staff at BRASTEC provided over 500 student hours in culturing *Gracilaria* in the nursery system at the two sites (Fig. 23). We have presented some of the project results in relevant and appropriate events, Northeast Algal Symposium (April 15-17, 2011 & April 20-22, 2012); Annual meeting of Phycological Society of America (July 12-16, 2011 & June 19-23, 2012); Sound Vision Press Conferences in LIS (Aug. 15, 2011 and Sep. 6, 2011); Keynote address at the Annual meeting of Sound Waters, Nov. 16, 2011; Citizens Advisory Committee of the LISS, Dec. 8, 2011; Aquaculture America 2012, Mar. 2, 2012; 32nd Milford Aquaculture Seminar (Mar. 12-14, 2012); and the Hudson River Foundation meeting held at RTB (May 16, 2012). Recently, our work was highlighted in various media including, E-The Environmental Magazine, The CT Mirror, Connecticut Public Television, The Day (and distributed nationally by the Associated Press to numerous other print media outlets), NBC Universal's WVIT news, the nationally syndicated AquaKids TV, etc. (See below for details).
Two papers are currently in preparation and will be submitted to peered review journals. We have also developed protocols/manual (http://digitalcommons.uconn.edu/cgi/siteview.cgi/wracklines/72) and companion DVD video (http://digitalcommons.uconn.edu/wracklines/71/) with instruction for the implementation and operation of the bioextraction technologies for *Gracilaria tikvahiae* with assistance of BRASTEC staff and students.
With support from School of Business of UConn, Prof. T. Dowding (UConn School of Business) and his team are currently developing optimization model to determine the value of both nutrient bioextraction and the commodities (including biofuels, agar, animal feed, fertilizer, etc.) derived from the harvested *Gracilaria* as a method to maximize potential multiple revenue streams while reducing any residual biomass waste to zero. We provided the output from our project to the Dowding team for their modeling efforts. Drs. Yarish and Kim have also assisted the Dowding team providing biological and economic information for *Gracilaria* farming. Their final presentation is scheduled on Aug. 21st, 2012. The N removal data from our projects will be freely available for eutrophication or nutrient bioextraction modeling efforts in LIS and other urbanized estuaries in the US through the LISS of the US EPA.

Figure 23. BRASTEC students assisting water tests in the *Gracilaria* nursery system (A). Rocking The Boat students assisting outplating of *Gracilaria* at the BRE farm site (B). Sound Vision Press Conferences (C) and UConn (Sena Gong) and Greenwich high school (Mary Cirino) students presenting posters at the Milford Laboratory Seminar (D).

3. Lessons Learned

Describe the key lessons learned from this project, such as the least and most effective conservation practices or notable aspects of the project's methods, monitoring, or results. How could other conservation organizations adapt their projects to build upon some of these key lessons about what worked best and what did not?

- One of the key lessons learned from this project is that the seaweed (*Gracilaria*) aquaculture can be a useful technique for nutrient bioextraction in the urbanized coastal waters including LIS and Bronx River estuary. *Gracilaria* can remove a significant amount of nitrogen (N) from the water column. Not surprisingly, N removal varies with site and season. Therefore, seaweed bioremediation might be best applied at nutrient hot spots in LIS and New York estuaries.
- Epiphytes were not noticeable on *Gracilaria* bundles or the long lines. Very few seaweed species including *Ulva* spp., *Neosiphonia* sp., etc. were observed on mooring lines of the mussel raft. However, the biomass was negligble (< 10g from all mooring lines and the mussel raft) throughout the growing season. Fouling fauna, however, can be a major problem for *Gracilaria* cultivation during the summer months. Especially, tunicates (*Molgula*) and hydroids (*Turbularia*) attached and grew on *Gracilaria* bundles and might have inhibited the growth of *Gracilaria*. These animals are *suspension feeders* removing organic nutrients from the water column. The biomass of tunicates was up to 60 gram per one meter of *Gracilaria* longline in September, 2011, but dropped when the water temperature dropped below 20˚C. Fouling fauna at the BRE site was not a significant issue during the months of September and October. It is probably because of the delay of deployments at BRE site. A high density of tunicates was observed at deeper water condition (1 m) while the density at a shallow depth (0,5m) was minimum at least for 4 weeks of growth period. Therefore, adjustment of cultivation depth (< 0.5 m) can be one way to control the tunicates and hydroids. The other way can be deploy fresh *Gracilaria* after four weeks of culture period.

- *Gracilaria* farm system also provides a good habitat for animals. A total of eighteen species in fourteen families of fouling organisms were identified at both sites (pooled data), including *Ampithoe vailida, Ampithoe longimana, Corophium insidiosum, Jassa falcate, Unciola irrorata, Leptocherius pinguis, Caprella penatis, Idotea balthica, Carcinus maenas, Hemigrapsus sanguineus, Semibalanus balanoides, Balanus improvises, Paleomonetes* sp., *Molgula manhattensis, Turbularia* sp. *Eubranchus exiguous, Phascolopsis gouldii* and *Nereis pelagica*. Most of these animals are known as microcarnivores.
- One major lesson learned was that our native species, *G. tikvahiae,* is being rapidly replaced by its non native cogeneror, G. *vermiculophylla*! While we were going out and trying to establish new cultures for this project and a related CT Sea Grant Project, we felt the need to develop the capacity to do molecular fingerprinting of our isolates. This effort gave us the information that our native species is under attack in the LIS ecosystem.

4. Dissemination

Briefly identify any dissemination of lessons learned or other project results to external audiences, such as the public or other conservation organizations.

Figure 24. CPTV documentary filming (left). NBC Universal, WVIT news anchor, Brad Drazen interviewing a BRASTEC student Kevin Kollar during NBC Universal, WVIT news story filming (right).

Our work was highlighted in a CPTV program, 'All Things Connecticut,' http://www.cptv2.org/allthingsct/episode/savin-rock-west-haven;
"The Seaweed and Shellfish Solution" by Renee Cho. September-October, 2011. E-The Environmental Magazine, Vol. 22 (5):12-14 (http://reneecho.files.wordpress.com/2011/02/pub-article.pdf or http://www.unbsj.ca/sase/biology/chopinlab/articles/files/Cho%202011%20E%20The%20Environmental%20Magazine.pdf;
http://www.ctmirror.org/story/14641/long-island-sound-legislation-stalled-washington-politics (Long Island Sound legislation stalled by Washington politics, The CT Mirror, Dec. 6, 2011, Jan E. Spiegel.); The Day (Mar. 11, 2012), http://www.theday.com/article/20120311/NWS01/303119895/ (Newspaper article in The New London Day was picked up by the Associated Press and repeated in many major newspapers throughout the US in the month of March); NBC Universal, WVIT news (June 6, 2012, http://www.nbcconnecticut.com/video/#!/on-air/as-seen-on/Brastec--A-Kelp-Farm/157580315); www.aquakids.tv (2011-24 Seaweeds - Week of June 25, 2012) (Fig. 24); WCBS 880-AM News (Aug. 22, 2012, Commercial Seaweed Farm Coming to the Long Island Sound, http://newyork.cbslocal.com/2012/08/21/commercial-seaweed-farm-coming-to-the-long-island-sound/); Stamford Advocate (UConn Finds Seaweed Could Be Cash Crop, Aug. 22, 2012, http://www.stamfordadvocate.com/local/article/UConn-finds-seaweed-could-be-cash-crop-3805535.php), this article was also in Connecticut Post, Danbury News Times, Hartford Courant (via McClatchy News Wires) and Greenwich Time; and Stamford Times (Aug. 22, 2012, UConn Professors: Seaweed Farming Is The Wave Of The Future, http://www.thehour.com/stamford_times/news/uconn-stamford-professors-seaweed-farming-is-the-wave-of-the/article_9c96b3b7-dcdf-5b69-9461-62fec52cbd63.html).

5. Project Documents

Include in your final programmatic report, via the Uploads section of this task, the following:

- 2-10 representative photos from the project. Photos need to have a minimum resolution of 300 dpi and must be accompanied with a legend or caption describing the file name and content of the photos;
- report publications, GIS data, brochures, videos, outreach tools, press releases, media coverage;
- any project deliverables per the terms of your grant agreement.

POSTING OF FINAL REPORT: *This report and attached project documents may be shared by the Foundation and any Funding Source for the Project via their respective websites. In the event that the Recipient intends to claim that its final report or project documents contains material that does not have to be posted on such websites because it is protected from disclosure by statutory or regulatory provisions, the Recipient shall clearly mark all such potentially protected materials as "PROTECTED" and provide an explanation and complete citation to the statutory or regulatory source for such protection.*

New England *Gracilaria* Culture Handbook

System Implementation & Operation
For Production of Young Plants

Project Title: Seaweed Aquaculture for Bioextraction of Nutrients from LIS and Bronx River Estuary (Project # 24266)**

Date: Oct. 12, 2012

Sarah Redmond, Jang K. Kim, Charles Yarish*
University of Connecticut
(*charles.yarish@uconn.edu, 203-251-8432)

**Long Island Sound Futures Fund (LISFF) and
The National Fish & Wildlife Foundation Award Notification (Project # 24266)

This project was funded by the Long Island Sound Futures Fund (National Fish and Wildlife Foundation), under project title “Bioextraction of Nutrients from Long Island Sound” (1/3/2011-4/2/2012) to Dr. Charles Yarish, University of Connecticut. Additional support was provided by the Connecticut Sea Grant College Program (Project Number R/A-38), under the title, “Development of Seaweed Culture System Technologies to Support Integrated Multi-trophic Aquaculture and Sea Vegetable Aquaculture in New England Coastal Waters” (2/1/2010-1/31/2013). The principal investigator wishes to thank his co-PIs including Dr. George P. Kraemer (Purchase College, Purchase College), Mr. John Curtis (Bridgeport Regional Aquaculture and Science Technology School in Bridgeport, CT and to his UConn seaweed research team including Sarah Redmond and Dr. Jang K. Kim. We would also like to thank the many the UConn and Purchase College undergraduate students, BRASTEC students and staff, and Rocking the Boat (Mr. Adam Green and Ms. Dawn Henning) for all their efforts logistical support for the Bronx River estuary and Long Island Sound sites.

Preface

The seaweeds are a diverse group of large marine macroalgae that are as important to the marine world as land plants are to our terrestrial world. Seaweeds were the precursors to land plants, and like land plants, they are critical primary producers, forming living links between the inorganic and the organic world, using photosynthesis to convert CO_2 and nutrients into living biomass. These primary producers support and encourage other marine life through the production of oxygen, contribution to marine food webs, and by providing structure and habitat for refuge, breeding, and nursery grounds for fish and invertebrates. Seaweeds are an important resource for humans. Coastal peoples have always utilized seaweeds for food, minerals, medicine, fertilizer and fodder, and these sea-plants continue to provide for an ever-increasing world population. A multi-billion dollar industry worldwide, seaweeds are consumed as nutritious sea vegetables, processed to extract valuable phycocolloids including agar, carrageenan, and alginate, and utilized in the agricultural industries for animal fodder or plant fertilizers.

Wild harvest still constitutes a significant portion of seaweed production, however, there is an ever increasing amount of seaweed production from aquaculture, principally in Asia (China, Korea and Japan) and South America (Chile). Seaweed aquaculture makes up about 24% of all aquaculture production (~19 million metric tons) with a value of ~US $5.65 billion (FAO, 2012). Most of the cultured harvest is the kelps, *Saccharina japonica* and *Undaria pinnatifida,* the red algal carrageenophytes species including *Kappaphycus and Eucheuma*, the edible red algal species known as nori (including *Porphyra* and *Pyropia* species) and the red algal agarophyte species known as *Gracilaria*. China is the world's top producer of cultured seaweeds, though other countries in Asia (Japan, Korea, and the Philippines) and in Europe (France, Ireland, Norway, Scotland, and Spain) also grow seaweed. In North America, seaweed industries are mainly small wild-harvest cottage operations located along the East and West Coasts of Canada and the United States, however, there is a newly developed sugar kelp industry in the Gulf of Maine (Maine and New Brunswick, Canada).

As populations expand, culture of seaweeds will be important to supplement the wild resource. Seaweeds can be cultivated like land plants, in the sea on suspended lines, rafts, or nets, or on land in tank-based culture systems. A sustainable, low- impact process, seaweed culture can provide much needed employment and independence to rural coastal areas. The development of a seaweed aquaculture industry can also encourage development of other aquacultured species higher up in the food chain, since seaweeds are bioextractive organisms, taking up excess nutrients generated by other species, such as fish or shrimp. The integrated culture of fed aquaculture (fish and shrimp) with extractive aquaculture (seaweeds and shellfish) has been called 'Integrated Multi-Trophic Aquaculture', or IMTA. The IMTA concept is an ecologically

based model that couples an inorganic bioextractive organism (seaweed) with an organic bioextractive organism (shellfish) to balance the intensive culture of fed organisms (finfish and shrimp), in order to produce a more sustainable, cleaner, and diversified aquaculture system (Neori *et al*., 2007). The development of new ecologically based, sustainable culture technologies will ensure future employment for coastal communities, healthier coastal ecosystems, and the protection of important wild populations.

There are many interesting seaweed species in the Northeast with great economic and environmental potential. There are economically important kelp species—*Alaria esculenta, Laminaria digitata, and Saccharina* latissima, which are all large brown algae. They are the largest of the North American seaweeds, some species exceeding 10 m in length. They are an excellent source of iodine and other trace minerals, as well as a source of alginate, a phycocolloid used in many different industries (Sahoo and Yarish, 2005). The native red seaweeds of interest in the Northeast include *Gracilaria tikvahiae, Chondrus crispus*, and *Porphyra/Pyropia* (hereafter referred to as *Porphyra*) species. *Gracilaria* species are cultivated at a large scale in some countries for food, as a feed for abalone, and for agar, an important phycocolloid in the food, medical, and microbiological industries. *Chondrus crispus* is cultured in land- based tank systems for sea vegetables and wild harvested for a variety of carrageenans, which are important in the food and consumer products industries as thickeners and stabilizers. *Porphyra* species are cultivated on nets in Asia and are pressed and dried into the valuable nori sheets that are an integral part of the Asian diet. In New England, the development of *Porphyra* cultivation in land-based recirculating IMTA systems has been a topic of particular interest (Yarish and Pereira, 2008; Pereira and Yarish, 2010). However, it will be the goal of this manual to introduce cultivation techniques for native red alga *Gracilaria* tikvahiae to the inquisitive aquaculturalists, as well as providing a resource to coastal managers. A video that compliments this Manual on Gracilaria cultivation may be found at http://digitalcommons.uconn.edu/wracklines/71/.

TABLE OF CONTENTS

Introduction

Worldwide demand for seafood has increased steadily over the years, while for the past 20 years the amount of seafood harvested from wild populations has remained constant (FAO, 2002, 2003, 2006, 2010, 2012; New 1999). The increased demand has been met entirely through marine aquaculture, which is a multi-billion dollar industry (FAO, 2010, 2012; Fig 1). The United States is a major consumer of marine aquaculture products, yet we grow only a small fraction of what we consume (FAO, 2010). A major obstacle to the growth of a U.S. aquaculture industry has been the need to find environmentally sustainable methods of farming fish and crustaceans. Two issues of particular concern are 1) nutrient loading of coastal waters from effluent generated by coastal and shore-based fish aquaculture operations, and 2) the depletion of wild fish stocks harvested for fishmeal used in marine finfish aquaculture diets.

One goal for the development of domestic sustainable aquaculture is to ensure that commercial aquaculture has minimal adverse effects on the environment. One way to achieve this goal is through development of improved methods of waste management for land-based and coastal/offshore aquaculture. Fish, through metabolic processes, excrete nitrogen, phosphorus and carbon dioxide (Beveridge 1987; Mugg, *et al.* 2000; Neori *et al.* 2004, 2007). Up to 49 kg nitrogen (N) and 7 kg phosphorus (P) can be released per ton of finfish produced per year (Chopin *et al.* 1999; Kautsky *et al.* 1996; Troell *et al.* 2003). In coastal waters, high levels of these nutrients can trigger harmful algal blooms and contribute to excessive growth of nuisance algae, which in turn have serious negative consequences on coastal ecosystems and the economy. These nutrients could instead be used to support the growth of economically important seaweeds (Neori *et al*. 2004; Chopin *et al.* 2008, Yarish & Pereira, 2008).

On a global basis, seaweed aquaculture represents 24% of world marine aquaculture production on a weight basis and $5.7 billion U.S. dollars on a monetary basis (FAO 2012). Nearly all seaweed aquaculture occurs in China, Korea and Japan. North America has very few seaweed aquaculture operations. The most notable in the Northeast is Acadian Seaplants, LLC, in Charlesville & Dartmouth, Nova Scotia, CA, producing the red seaweed *Chondrus crispus* for export to the Japanese food market (Craigie and Shacklock, 1995, Craigie

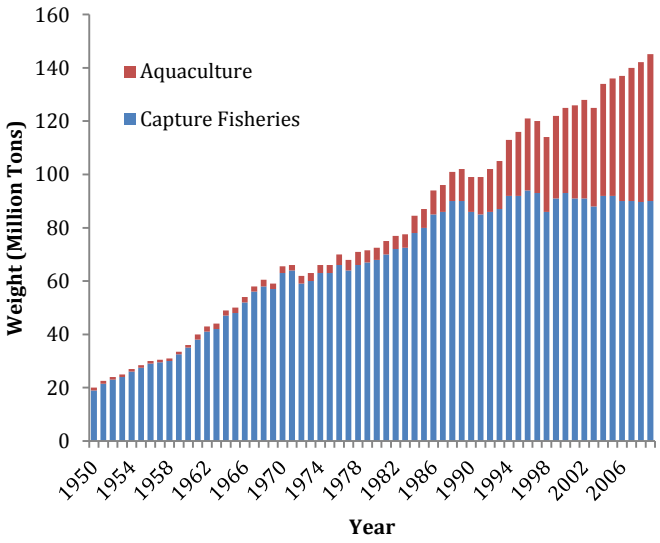


Figure 1. World Aquaculture Production and Capture Fisheries Data (FAO, 2012).

et al. 1999). The primary commercial use of seaweed is as human food, either as sea vegetables or functional foods (Cordero, 2006; Nisizawa, 1987, 2006; Smit, 2004; Teas, 2006). Seaweeds are also used as a source of colloids for the food and cosmetic industries, as an ingredient in pharmaceuticals and neutraceuticals, as a supplement in livestock feed and as a soil amendment in agriculture (Yarish and Pereira, 2008; Neori, 2008; Braden *et al.* 2007; Saker *et al.* 2003; Turner *et al*. 2002; Pereira and Yarish, 2010, Pereira *et al*., 2012; Holdt and Kraan, 2011; Craigie, 2011; Browdy *et al*. 2012). It has been used as a food source for abalone and urchin aquaculture and is currently being investigated as a replacement for fishmeal in finfish diets (Neori *et al.* 2004; Neori *et al.* 2007; Robertson-Anderson *et al.* 2008; Francis *et al.* 2008, Walker *et al.* 2009). In the 1980s, there was significant interest in seaweeds as a biomass source for methane production (Flowers and Bird, 1984; Gao and McKinley 1994), and there is current renewed interest in seaweed as a biofuel source for ethanol and methanol production (Horn *et al*. 2000; Matsui *et al.* 2006; Yokoyama *et al*. 2007). However, the potential of most seaweeds as a feedstock for biodiesel is low, as the lipid content generally tends to below 5% dry weight except for some species of *Porphyra*/*Pyropia*, *Codium* and *Hypnea* (Sanchez-Machado *et al.* 2004; Pereira and Yarish, 2010). There are a number of potential applications for habitat restoration (Carney *et al.* 2005), have been proposed as large-scale carbon sinks, as a method of removing heavy metals from marine environments (Davis *et al*, 2003), and even as a way to detoxify and remove TNT from seawater (Cruz-Uribe *et al.* 2007).

Seaweeds have been successfully incorporated into a number of demonstration and pilot-scale IMTA and nutrient bioextraction systems. A pilot-scale coastal IMTA project in New Brunswick, Canada uses kelp (*Saccharina latissima* and *Alaria esculenta*) and mussels (*Mytilus edulis*) as the extractive components in close proximity to salmon (*Salmo salar*) net pens (Chopin *et al.* 2008). In Portugal, Matos *et al.* (2006) demonstrated the effectiveness of three red seaweeds, *Palmaria palmata, Gracilaria bursa-pastoris* and *Chondrus crispus* in removing nutrients from the effluent of tank-based production of turbot (*Scophthalmus maximus*) and sea bass (*Dicentrarchus labrax*). In Israel, Neori (1996) and Neori *et al.* (1996, 1999, 2000, 2003, 2004, 2007, 2008) have developed small commercial scale IMTA systems incorporating gilthead seabream (*Sparus aurata*), the green seaweed *Ulva lactuca*, abalone and sea urchins. In South Africa, kelp (*Ecklonia maxima*) grown in the effluent of abalone aquaculture tanks was fed back to the abalone. Nutrient load in the effluent was significantly reduced and more of the exogenous nutrients were converted to abalone biomass (Bolton *et al.* 2006; Troell *et al*. 2006; Robertson-Andersson, 2008; Francis *et al.* 2008). The seaweed in these systems can be used for human food, as a protein source in finfish aquaculture diets, as a source of pharmaceuticals, phycocolloids and other biochemicals, or as a carbon source for biofuel production (Horn *et al.* 2000; Chopin and Sawhney, 2009; Chopin *et al.* 2008; Buschmann *et al.* 2008a; Kim *et al*., 2010; Pereira *et al*., 2012).

A trial of nutrient bioextraction technologies in Long Island Sound and the Bronx River estuary cultivating native strains of *Gracilaria tikvahiae* has been supported by the Long Island Sound

Futures Fund and the Connecticut Sea Grant College Program (Yarish *et al*., 2012a,b; Kim *et al*. 2012). To grow seaweed in a nutrient bioextraction (or IMTA systems), it is necessary to have a source of young plants. In Asia, native species have been isolated from wild populations; strains have been selected for desirable traits and are maintained as "seed" cultures. Seaweed farmers often belong to a cooperative and obtain "seed" nets or lines from a seaweed culture facility (*i.e*. a seaweed nursery). This manual describes how to set up a seaweed culture laboratory, defines the basic resource needs of these plants, and provides a culture system roadmap for the production of young "seed" plants of the native red alga, *Gracilaria tikvahiae*.

General Seaweed Culture System Components

System

In order to cultivate seaweed, a simple but well-equipped laboratory is essential. The three most important components of a culture system are seawater media (seawater and nutrients), temperature and light. This chapter offers a broad overview for laboratory system components that will be applicable to all types of seaweed culture. For more information on algal culturing techniques, the reader is urged to consult Andersen (2005).

Seawater

Successful seaweed culture requires a reliable supply of clean seawater. Natural seawater is preferred over artificial seawater, as natural seawater contains all of the essential mineral components (over 50 known elements) in the natural ratios, which may or may not be available in artificial seawater. Seawater salinities should be between 28-34 parts per thousand (ppt), and it is critical that it be clean and free of any organic or inorganic contaminants. To avoid terrestrial runoff or salinity variation, natural seawater collection should occur during periods of dry weather, preferably at high tide, and at depths of approximately 3 meters (10 feet) below the surface. Seawater can be collected by boat or from a shore facility with a well-placed intake pump. Water storage and transfer can be done in polyethylene tanks or 20-liter (5 gallon) carboys. All new containers need to be leached before use by filling with clean water (ideally distilled water, but clean seawater can be used) and allowed to sit for several days before rinsing for use. All containers and tanks should be well sealed to prevent contamination, and kept in a cool dark place to prevent growth of unwanted algae.

Seawater should initially be filtered through a coarse filter during collection from the sea, using a sand filter or a canister filter, then further fine-filtered from there. Initial rough mechanical filtration can be accomplished with sand filters (pool filters) or polyester bag filters with 20-35 micron pore sizes. Further filtering can be accomplished with common household water filters, down to 0.2 microns, by pumping water through a series of canister filters of decreasing filter size (10-, 5-, 1-µm). A 0.45-micron filter will separate out organics from dissolved inorganics, and filtering water down to 0.2 microns should eliminate almost all possible biological contaminants in the water, though organisms can get through if there are tears or holes in the filters. For this reason, further sterilization of the water and periodic inspection of filters may be necessary to eliminate any contaminants that could threaten the culture (Fig 2).

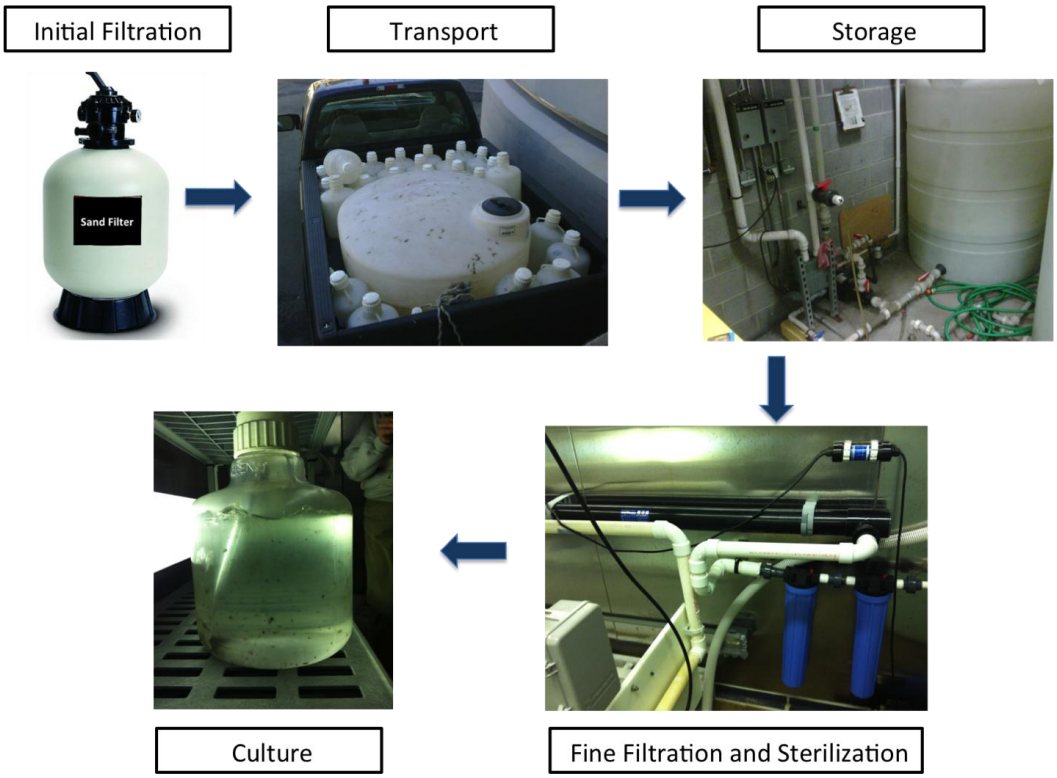


Figure 2 . Seawater system for culture. Initial filter, transport, storage, second filter, sterilization (UV, fine filter, or autoclave) culture.

Artificial seawater (AS) can be used, but caution is advised, for artificial mixes tend to have limited success compared to natural seawater. If it is necessary to use AS, a certain amount of experimentation may be required to determine the best source of mixed salts. They tend to be inconsistent, with great variability both between brands and within batches of the same brand. Artificial mixes may not have the same ratios of essential microelements present in natural seawater, which is why natural seawater is recommended for all culture work.

Sterilization

Once filtered, seawater should be sterilized, especially during culture initiation. While it is not necessary to have axenic ("pure") cultures to grow seaweed, the microscopic and juvenile phases are sensitive to contamination. There are a host of microorganisms that can feed on, compete with, or overgrow the macroalgal cultures, including other algae, fungi, bacteria, cyanobacteria and micro zooplankton (primarily protozoans). Protozoan grazers can especially be a problem for the microscopic stages of the kelp and other seaweeds, if present at high concentrations. The only way to completely ensure sterility of seawater is through heat treatment.

There are a few different methods available to sterilize seawater, and the best method will depend on the facilities available. The most effective method of sterilizing seawater is by autoclaving, which ensures sterility through a treatment that applies extreme heat and

pressure to seawater (Fig. 3). Autoclaves are heavy walled closed chambers that can be used to sterilize liquids, metals, glass, or autoclavable plastics. Seawater can be sterilized in polypropylene, polycarbonate, or Pyrex containers. Containers should only be filled ¾ of the way, with loosely applied caps, to allow for pressure changes within the container during the heating and cooling of the liquid. Aluminum foil covers can be applied around the loose caps during the sterilization process to minimize exposure to air currents. A liquid cycle (121°C; 1-2 PSI, 15-30 minutes depending upon the volume of liquid) is sufficient to kill all living organisms, including bacteria, viruses, and heat resistant spores. In order to ensure the entire volume of liquid reaches the required temperature for a sufficient amount of time, containers should be allowed to equilibrate to the temperature of the warmed-up autoclave (30min-1hour) before running the cycle, and the cycle time should be increased for larger volumes of liquid. After the cycle has run, the door of the autoclave should not be opened until the pressure inside is the same as the room to avoid boiling over of the liquid.

Figure 3. Autoclave for sterilization of seawater and glassware.

Autoclaving can result in the formation of precipitates, especially phosphates, which are a critical micronutrient for seaweed growth. This occurs because the high temperature and pressure applied drives CO_2 gas out of the seawater, resulting in an increase in pH. Precipitation increases with higher levels of nutrients, so to avoid this, seawater is sterilized first, then nutrient solutions are added after it has cooled. Seawater should be removed from the autoclave when it is safe to do so, and allowed to cool at room temperature before storing in a cool dark place. It is best to store all sterilized water at 5-10°C to minimize chances of recontamination. After the water has cooled, caps should be completely tightened to avoid any chance of contamination through air exposure. Autoclaved seawater should be allowed to sit for several days before using or aerated with regular air to restore CO_2 equilibrium and lower pH levels. If a traditional autoclave is not available, a large pressure cooker may be used to apply heat and pressure to sterilize smaller amounts of water in the same way.

Seawater can be sterilized by application of heat only, through the processes of pasteurization and tyndallization. Pasteurization is accomplished by heating seawater up to a boiling point, to 90-95°C for 30-60 minutes, and then cooling rapidly to less than 10°C. Tyndallization applies the process of pasteurization three consecutive times to the liquid, over the course of three days. This method takes longer to accomplish, but provides extra treatment to kill heat resistant spores that will not be effectively destroyed in the first pasteurization. This can be

accomplished with a stovetop or a microwave oven, making sure that the seawater is well covered to avoid contamination.

Another way to sterilize water by application of heat is through flash sterilization. This will kill most organisms in seawater, but may not kill heat resistant spores. This method heats water up to a high temperature (70-90°C) in a very short time by passing through a tube or plate heat exchanger. This may not kill all possible contaminants, but can be an effective way to treat larger amounts of seawater.

Filter sterilization of liquid uses very fine filtration to exclude all living organisms, effectively sterilizing water without the application of heat. This method is used for small volumes of seawater or heat sensitive liquids, such as nutrient media and vitamin solutions. Liquid is filtered through a 0.2-micron filter, which, in theory, should exclude all organisms, though imperfections or defects in the filter pore size can allow some through. For small volumes, a reusable or disposable filter assembly is set up on a side arm flask, and liquid is pulled through the filter using a vacuum pump. Fine filtering can also be a part of a multi canister filter system, where seawater is pumped through a series of decreasing filter sizes.

A filtering system coupled to a UV light system may ensure extra treatment of seawater. Enclosed high-energy UV bulbs can be installed as part of the water filtration process. Filtered seawater passing through a UV sterilizer will be exposed to UV radiation, destroying any remaining organisms that may have passed through the filter. UV radiation may not be 100% effective, but can be an additional treatment to incoming seawater or water that cannot be autoclaved or heat sterilized.

Seawater can be sterilized with a chlorine treatment, by adding liquid bleach (5% sodium hypochlorite) to sterilize, and sodium thiosulfate to neutralize after treatment. After water is filtered to remove organic matter, bleach is added at concentrations of 1-5 mL of bleach per liter of seawater. Water should be left to stand for several hours, avoiding exposure to direct sunlight. After treatment, the bleach needs to be sufficiently neutralized before using. To neutralize, a sodium thiosulfate solution ($Na_2S_2O_3$ $5H_2O$) is added at concentrations of 1 mL per 4 mL bleach added. The sodium thiosulfate solution is made by dissolving 250 grams of $Na_2S_2O_3$*$5H_2O$ in 1 liter of water (Kawachi and Noel, 2005). Neutralization of seawater should be verified with a chlorine test kit.

Table 1. Seawater sterilization options.

Seawater Sterilization Options			
Method	Temperature	Time	Notes
Autoclave	121°C	15-30 min entire volume	Containers filled ¾ Loosely applied covers
Pasteurization & Tyndallization	Heat 90-95°C, Cool 10°C	Heat 30-60 min, Rapidly cool	Repeat 3 times over 3 days
Filter Sterilization	n/a	n/a	Filter size 0.2-microns For heat sensitive liquids
UV Sterilization	n/a	Seconds of contact time	Most effective in recirculating water systems
Flash Sterilization	70-90°C	Rapid heating of seawater passing through heat plates	For large amounts of seawater
Chlorine Treatment	n/a	30 min – 24 hrs	Neutralize with sodium thiosulfate solution (1mL per 4mL bleach)

Nutrient Media

While natural seawater contains many of the necessary trace elements needed for seaweed culture, the quality and amount of nutrients can be variable and insufficient for culture work. Both natural and artificial seawater need to be enriched with a concentrated nutrient solution after the water has been sufficiently sterilized. There are several different types of media, but all provide a mixture of essential macronutrients (nitrogen, phosphorus, calcium, potassium, sodium, chloride, etc.) and trace elements (iron, manganese, zinc, molybdenum, copper, cobalt, zinc, etc.), metal chelators (EDTA), vitamins (B_{12}, thiamine, biotin), and hydrogen-ion and metal-ion buffers (TRIS, EDTA). Reagent laboratory-grade chemicals should be used to reduce the likelihood of contamination or impurities. Macronutrient, trace metal, and vitamin solutions are usually made up separately then added together to produce the concentrated stock solution. All solutions should be filter sterilized and refrigerated. Vitamin solutions can be frozen or refrigerated, but care should be taken to maintain sterility (Harrison & Berges, 2005).

Nutrient solutions are made up in large quantities beforehand, filter-sterilized into clean stock bottles, and refrigerated. It is useful to pre-proportion the nutrient solution into smaller bottles that are ready to be added to the volume of the culture tank to make seawater preparation easier. Preparation of enrichment solution requires a clean, sterilized table space, clean, sterilized glass and plastic ware, and sterile techniques. Only non-reactive metal instruments or glass should be used, as some latex and rubber can be toxic if leached into the media. In order

to filter sterilize media, self- assembly glass filter units with disposable filter papers (0.2 micron or µm) can be used. These units are placed into the mouth of a side-arm flask, and the liquid is pulled through the filter paper by suction underneath, created by a vacuum pump (Fig. 4). Instructions for preparation of this media can be found in Appendix A.

Figure 4. Vacuum pump assembly for filter sterilization (0.2 µm) of nutrient media (top). Filter assemblies can be disposable (left, bottom) or reusable (right, bottom).

Seawater media must be changed regularly during the culture period in order to provide sufficient nutrients to the growing plants. This is a simple operation that involves transferring plants from one culture vessel to a new one with fresh seawater media, taking care that the new culture water is at the same temperature as the old culture water to avoid stressing the seaweed. This should be done in a clean environment, using sterilized forceps and gloves, avoiding any chance of contamination through air currents or contact. Culture changes are usually done once per week or more in actively growing or dense cultures, and once every two to four weeks in cultures that are just being maintained and not actively growing.

Diatom Control

The most common and problematic contaminants in a seaweed culture are single celled microalgae called diatoms (Fig. 5). Diatoms are one of the most common types of phytoplankters, characterized by unique symmetric cell walls made of silica. Centric diatoms are radially symmetrical (round), while pennate diatoms are bilaterally symmetrical (shaped like a long thin football). Diatoms can exist as single cells, colonies, or filaments. They are able to rapidly proliferate through both asexual and sexual means, and tend to smother all available surfaces, including culture substrates and young blades. They are very likely to be introduced into cultures through natural seawater or on blades collected from the wild. Their small size and variable shapes allow them to pass though filtration systems and even UV sterilization. In order to control growth of diatoms in cultures of young seaweeds, a saturated solution of germanium dioxide is added to the culture media at 1-2 mL per liter of seawater. This compound inhibits silica deposition in diatoms (necessary for cell wall formation) and effectively eliminates diatoms in the culture (Lewin, 1966). Germanium dioxide should only be used when initiating cultures, and will not be a cost effective measure in large volumes of water.

Figure 5. Diatoms problematic in seaweed culture. Top: diatom embedded in kelp blade, second: pennate diatoms, third: pennate and centric diatoms, bottom: centric diatom. Scale bar = 10 microns.

Temperature

Water temperature control is a critical component in any seaweed nursery operation. Seaweeds have an optimal temperature range for growth, as well as a range of upper and lower survival temperatures (see Lüning, 1990 for a general introduction to environmental factors regulating seaweed growth and production). Cultures can either be placed inside a refrigerated space (cold rooms or incubators) or in individual tanks that may be cooled with an aquarium-type chiller (Fig. 6). If standard household refrigerators are available they can be converted into homemade incubators by installing fluorescent lights on the inside walls (make sure to install ballasts on the outside). Temperature alarm monitoring systems with automatic dialers can alert culturists in the event of a temperature failure (Omega Systems make a variety of these alarms monitoring devices).

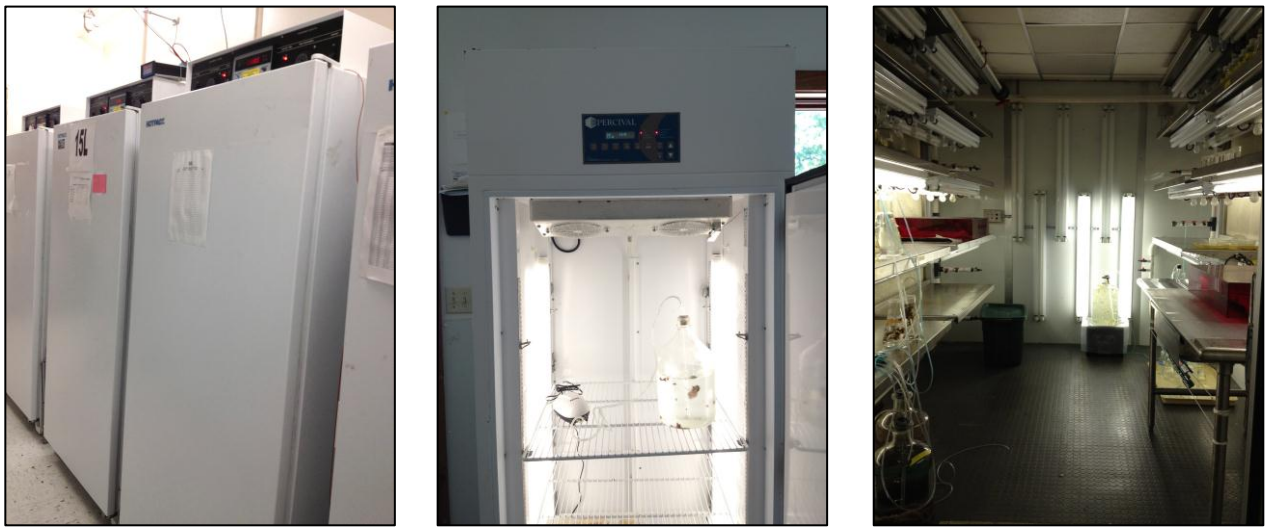


Figure 6. Environmental Culture Chambers, also known as incubators. These can be any size, and can be purchased commercially or can be homemade. Environmental chambers all have a means of temperature control, a light source with photoperiodic control, and aeration. Left: Hotpack brand Incubator, Middle: Percival Biological Incubator, Right: Room- sized environmental chamber.

Lighting

Seaweeds are photosynthetic organisms that incorporate inorganic materials into organic biomass using the energy of the sun. Seaweeds, like land plants, utilize photosynthetically active radiation (PAR), which is the portion of light available for photosynthesis. PAR comprises the same part of the light spectrum as visible light, and can be broken down into its colored components, from blue/violet (400nm, high energy) to red (700nm, low energy). The underwater light environment is highly variable and dynamic, because as light passes through water it is absorbed and scattered by water molecules and particles. The longer, lower energy red wavelengths are usually absorbed near the surface, while shorter, higher energy blue wavelengths tend to penetrate deeper into the water column. While all of the algal groups (green, red, and brown) contain chlorophyll *a* and carotenoids, they have different photosynthetic accessory pigments. Each type of pigment absorbs wavelengths in different parts of the spectrum, as shown below (Fig. 7), however, only certain wavelengths elicit a physiological response (often referred to as the action spectrum).

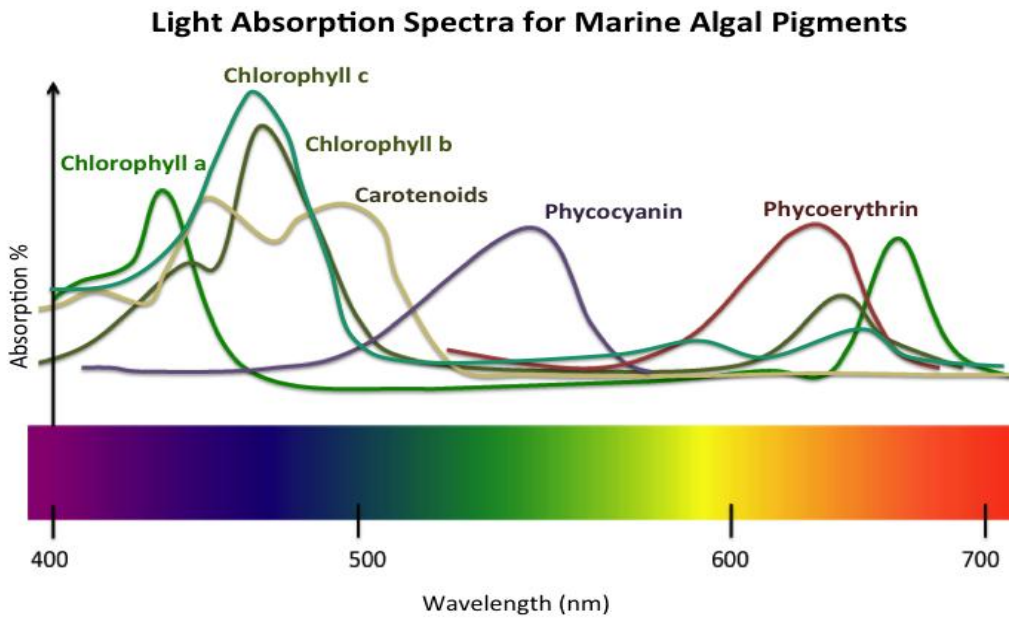


Figure 7. Light absorption spectra for photosynthetic pigments

For the laboratory, fluorescent light bulbs will provide sufficient light energy for the culture of plants. There are many different varieties of bulbs on the market, and the technology is ever evolving, so it is helpful to understand what type of light each bulb offers. Light quality and quantity are important when choosing a bulb. Higher output bulbs will provide higher light intensity or photon fluence levels. For this reason, the "high output" bulbs (or "HO") are used in order to ensure enough light for the cultures. The quality of light available from each type of bulb is determined by the difference in the inside coating of the glass. There are three main types of bulbs, differentiated by their color temperature. The color temperature is a value in Kelvins printed on the bulb. Daylight bulbs have the highest color temperature, at 5000K or above, and are made to simulate natural sunlight. Cool white bulbs have more light in the blue, with a color temperature around 4000K. Warm white bulbs emit light in the red portion of the spectrum, with a lower color temperature of 3000K or less. Cool white bulbs are best, because most of the photosynthetic pigments in algae are able to absorb light in the blue portion of the spectrum (Fig. 8).

Figure 8. Fluorescent bulb color temperatures

To provide artificial lighting in the laboratory or indoor culture facility, cool white high output T12 fluorescent bulbs with electronic ballasts are best. However, more energy efficient bulbs

including T8 and T5 technologies are now replacing T12 bulbs. One note of caution, the spectral composition of all the bulbs are not equivalent, hence our reticence to use T5 bulbs vs the older T12 bulbs. The spectral compositions of all these bulbs are readily available by the manufacturers including General Electric, Phillips and Sylvania. Light can be provided from above or from the sides of clear containers, and several independently controlled bulbs can be used to increase or decrease light levels. Window screening can also be used to control light intensity by placing layers between the light source and the cultures, each layer of 1mm mesh screen resulting in a 40-50% reduction in light.

It is important to know the intensity of light available to plants, as light requirements change over time. Usually, new cultures require lower light levels, while developing plants require more light. Very low light levels (1-10% of normal culture conditions) can be used to maintain cultures over a long period of time. Quantum light meters are used to measure the amount of light available for algal growth. Light meters measure photosynthetic photon flux density, given in units of micromoles of photons per meter squared per second (µmol m^{-2} s^{-1}). Very good handheld quantum meters (*e.g*. Apogee Instruments, LI-COR) can be found on the Internet for order, with a wide variation in the cost, ranging from $300 – $2000.

Lights should be connected to an interval timer to control photoperiod. Photoperiod is the alternating period of light and dark, which is very important for normal development and growth of seaweeds. A neutral photoperiod is commonly used, which is 12 hours light followed by 12 hours of darkness (12:12, L:D). To establish the photoperiod, all outside light sources (such as windows or other light fixtures) should be eliminated. A long day photoperiod consists of long days and short nights, usually 14-16 hours of light followed by 8-10 hours of darkness. A short day photoperiod consists of only 6-8 hours of light followed by 16-18 hours of dark. Algae reproduction and growth is often triggered by changes in photoperiod and/or temperature, so manipulating photoperiod is a useful tool in a culture center (see Lüning, 1990, for further information on photoperiod).

Laboratory

Basic laboratory equipment for successful algal culture will include a microscope, a thermometer, a refractometer, a light meter, and some simple laboratory glassware.

A compound microscope is essential to determine successful spore release, spore density, and development of microscopic stages (Fig. 9). A high-powered dissecting scope is also very useful for monitoring larger plants throughout the culture period, or for isolating spores. Microscopes should be kept covered with dust covers when not in use, and should be kept clean and dry. Microscope

Figure 9. Dissecting (left) and compound (right) microscope.

accessories include lens paper, Windex or alcohol, and glass slides and coverslips. A haemocytometer, specially etched for the counting of microscopic cells, is useful for spore solution density determination.

Some useful equipment to have in the lab will include a refractometer (Fig. 10), a light meter, a pH meter, a thermometer, and a magnetic stir plate. A refractometer is a simple handheld tool that measures salinity of seawater. A pH meter is required for culture media preparation, as well as for monitoring culture pH. pH meters need to be calibrated with buffer solutions before each use, rinsed before and after each use in deionized water, and stored in an electrode storage solution. A magnetic stir plate with stirrer bars is used for preparing nutrient media solutions (Fig. 11).

Figure 10. A Refractometer.

Glass and plastic ware for the lab will include volumetric flasks, graduated cylinders, beakers, flasks, and various culture dishes (Fig. 11). Borosilicate glass, Teflon, and polycarbonate are all common laboratory materials. Any material used in the lab should be non-reactive and non-toxic, and thoroughly cleaned before use. New glass or plastic ware needs to be pre-cleaned before use. This is done by washing, soaking in a dilute 10% hydrochloric acid solution, followed by a soaking in deionized water for at least 1-2 days. This step ensures the removal of any residue or chemical that could leach into the seawater or nutrient media. All lab ware should be carefully washed using laboratory detergent, rinsed in tap water, followed by a rinse in deionized water to remove any residual nutrients, or trace or heavy metals that can be present in tap water. All types of rubber, metal, or reactive materials should be avoided, and new tubing should be thoroughly rinsed before use. The size and type of glassware will depend on the needs of the grower. Glassware, brushes, and glassware detergents are available from laboratory supply companies. If buildup occurs on glassware with use, it can be soaked in a 10% hydrochloric acid bath for at least several hours, and then rinsed twice with deionized water. Acid baths should be prepared with proper protective equipment (goggles, gloves, and apron) and stored in a closed container to avoid evaporation. Any critical portion of the culture process should utilize only clean, rinsed, covered and sterile glassware to avoid any contamination. Washed, dried, and sterilized glassware should be stored in a clean cupboard, away from air currents.

Figure 11. Useful lab items (from top left to right): forceps, sterilized glassware, lab book, pH meter, stir plate, Bunsen burner, pipettes.

Glassware, tanks, and all laboratory equipment and working spaces should be clean and sterile to avoid contamination in cultures. Glassware and metal instruments can be sterilized by applying moist heat or dry heat. Moist heat is applied in an autoclave or pressure cooker, (1-2 bars of pressure at 121°C), making sure the steam penetrates the material, and caps (either aluminum foil or a plug) are applied loosely to containers. Dry heat can be used to sterilize glass or metal, by heating in an oven for 3-4 hours at 150°C. Aluminum foil is used to cap or cover equipment, ensuring that all openings are covered to maintain sterility upon removal from oven or autoclave.

Figure 12. Necessary laboratory equipment for successful cultivation: Left: sterile vacuum filter for making stock solutions, "Algal Culturing Techniques", various sized Erlenmeyer flasks, beaker, refractometer, thermometer, graduated cylinder, Petri dishes, multiwall plates, air pump, and Pasteur pipettes. Right: Custom filter (10-100 micron mesh), squirt bottle with sterilized seawater, and glass stir rod with rubber policeman scraper useful for

A spray bottle of bleach solution or ethyl alcohol solution is very useful to have in the lab, for it can be used to sterilize and clean working areas and other equipment (Fig. 12). A clean working station will ideally be set up in a laminar flow cabinet (Fig. 13), but if this is not available, a clean room with minimal air movement is best. To set up a clean working station, the working surface should be cleaned with a microbial disinfectant (*e.g.* Amphyl ® cleaner & disinfectant) or 70% ethyl alcohol solution before and after use. The ethyl alcohol solutions can be stored in small bottles or plastic containers. 95% solutions of the ethyl alcohol should be available to sterilize stainless steel tools (forceps, dissecting needles, blades). An open flame from an oil lamp or gas burner is also useful for sterilizing metal tools that are dipped in the ethyl alcohol solution. Parafilm®, a plastic wrap used in laboratories, is very useful for covering and sealing containers, minimizing contamination and reducing evaporation of cultures.

Figure 13. Two types of clean hoods. Left, a biological safety cabinet, suitable for working with cultures or hazardous materials. Right, a laminar flow cabinet, suitable for working with cultures and non-hazardous materials.

A record of all cultures in the lab, nursery, or culture center should be kept in the lab with the source, history, and details of all of the cultures. This will allow the grower to track cultures over time. All cultures, chemicals, and seawater media should be well labeled, and appropriate federal and state laws should be followed in the handling, storage, and disposal of all chemicals and cultures.

Long-Term Maintenance

It is a good idea to maintain stock cultures of all cultivars in the lab. Stock cultures should be kept at lowered temperatures and light levels to minimize growth and need for culture changes. For long-term maintenance, cold-temperate plants should be kept at 5-10°C, warm temperate plants at 15-20°C. Illumination is best kept at 1-10% of normal culture conditions, which is at or near compensation level (generally 2-7µmol photons m^{-2} s^{-1}).

Gracilaria

Gracilaria tikvahiae

Gracilaria tikvahiae is the only *Gracilaria* species native to New England (Fig. 14). The genus *Gracilaria*, in the Phylum Rhodophyta (a group of the red seaweeds), contains over 100 species found around the world, and many are wild harvested and cultivated for food, animal feed, and the phycocolloid called agar. They are warm water seaweeds, usually preferring temperatures in the 15-30°C range, making them a potential summer species for culture in New England. *Gracilaria* is an ideal candidate for aquaculture due to its warm-water growing season, ease of propagation, relatively high growth rates, high tolerance to a range of environmental conditions, and its existing and potential commercial value.

Figure 14. *Gracilaria tikvahiae in culture*

Biology

Gracilaria tikvahiae has a variable morphology, which depends on the strain and growing conditions. It is a bushy, branching seaweed, comprised of rounded branches which are irregularly or dichotomously branched from rounded, compressed, or flattened axes (Fig. 15). Blades are usually red, but can be brownish, green, or almost black depending on light and nutrient conditions. Blades arise from a flattened disc that is formed from a spore. Growth occurs by an apical meristem, located at the tip of each branch. Blades can reproduce through spores or by vegetative propagation, and can be found as either attached or free floating in coastal areas.

Figure 15. *Gracilaria tikvahiae*

Gracilaria is a species that has a wide range of tolerance for changing environmental conditions. It is common in estuaries or bays, often found in intertidal or shallow subtidal areas, less than 1 meter deep, either attached to rocks or free floating. It is often found in embayments, which are environments with reduced water flow and may be rich in ammonia and nitrate. They are a euryhaline species, which means they can tolerate a wide range of salinities, from about 10-40 ppt, though they grow best in the 25-33ppt range. They can survive temperature ranges from 0-35°C but have an optimal range of 20-28°C.

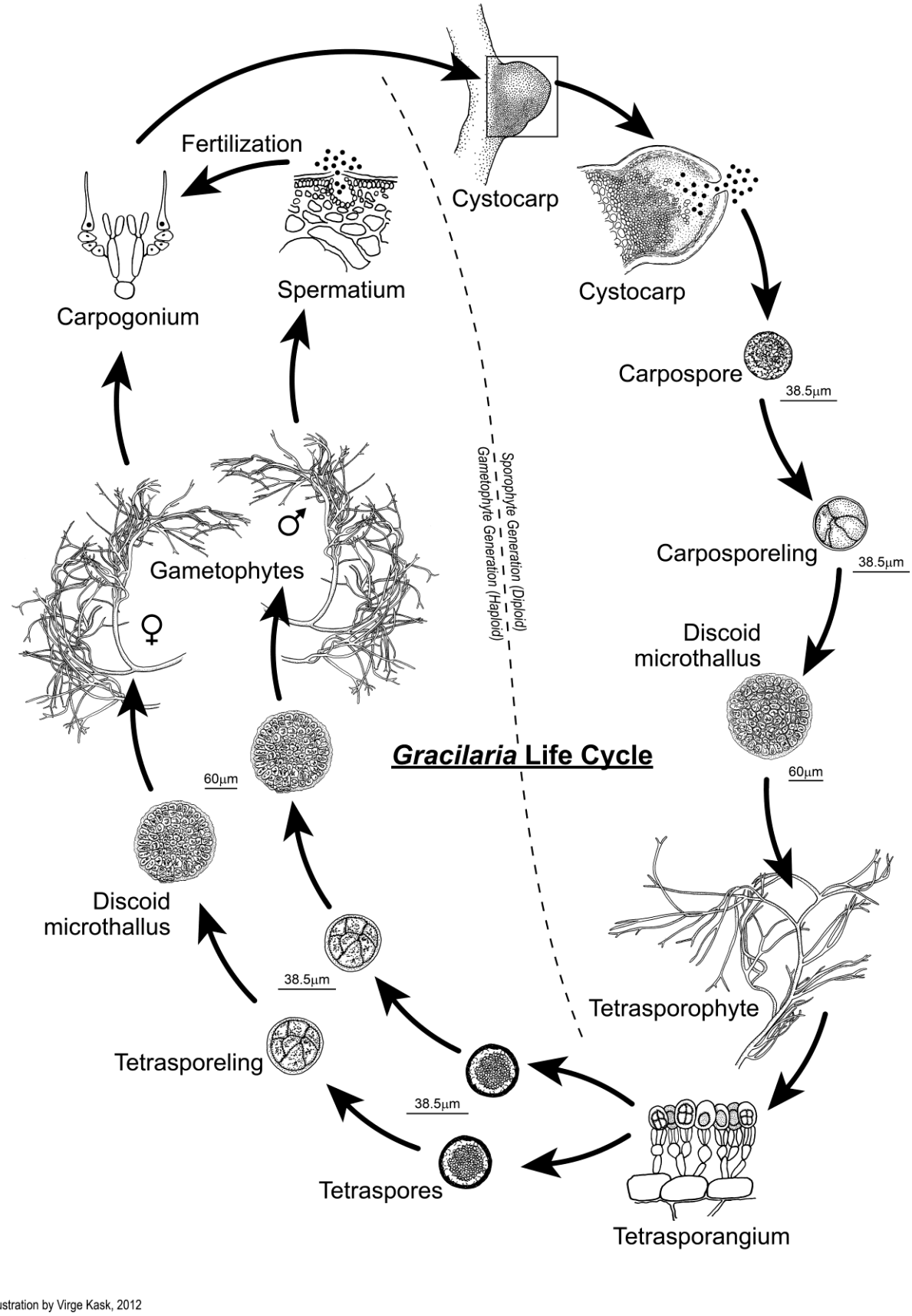


Figure 16. The *Gracilaria* life cycle.

Gracilaria has a three-stage life history that is similar to many other red seaweeds, often called a *Polysiphonia*-type life history (Fig. 16). It has an isomorphic ("same shape") alternation of generations. This indicates that two of its three life stages, the tetrasporophyte and the gametophyte stage, are morphologically identical. The life cycle has three different stages; a diploid tetrasporophyte stage, a haploid gametophyte phase, and a diploid carposporophyte phase that occurs on the female blades. These stages are distinguishable only by microscopic examination of the reproductive structures or presence of the third stage, which appear as bumps on the branches of the female blade. The mature diploid tetrasporophyte produces four haploid tetraspores within each tetrasporangium by undergoing meiosis. Tetrasporangia occur in the cortex (the outer edge of cells) of the thallus, and can be found anywhere on the blade. The tetraspores appear as red cross-shaped (cruciate) spots, and can be observed with a microscope or even a hand lens. When tetraspores are released from the blade, they drift passively in the water column until they settle and adhere to a substratum. The spore will begin to internally divide, then enlarge and develop a multicellular disc. The center of this disc will then develop a raised dome and the blade will develop from this initial growth. Each disc may produce many upright thalli, each of which may separate from the disc and continue to grow as a free-floating plant.

The plants that develop from tetraspores are either male or female haploid gametophytes, indistinguishable until maturity. Mature male gametophytes produce white spotted areas with spermatangia on their thallus, observable under a microscope. Female gametophytes become apparent when their eggs (carpogonia), produced within the cortex of the plant, are fertilized by spermatia (non-motile sperm), and new tissue is built up around the zygote. The new diploid, globular structure is the third phase of the life cycle, the carposporophyte, occurring on the haploid female thallus. The small bump is a cystocarp, and inside the cystocarp, the original zygote undergoes many cell divisions (mitosis), eventually producing many diploid spores, called carpospores. These non-motile, spherical spores are released into the water column and carried to a suitable substratum by water currents. Once settled on a substratum, the spores will adhere, divide and form a multicellular disc. These multicellular discs will initially produce a protuberance that develops into an upright thalli, which will develop into a tetrasporophytes, thus completing the life cycle. Each disc may also produce many upright thalli, each of which may separate from their disc and continue to grow as a free-floating plant.

Besides the relatively complicated three-phase sexual reproductive life cycle, *Gracilaria* is also able to reproduce asexually, through vegetative propagation. A single individual has the capacity to become hundreds or thousands of individuals, through continual fragmentation. Each fragment produced will grow and develop into an individual, and these blades can be further fragmented into several individuals, and so on. This is possible because the growth occurs in the apical meristem, which is growth from the tips of the branches. Each tip, then, has the capacity to grow and branch into its own blade. This allows for a much simpler means

of propagation, and also creates consistency in a culture environment. Vegetative propagation is the most common means of culture, as it is quicker, easier, and more efficient than starting from spores, and it allows for consistency, as all blades in a culture can be genetically identical, all having the same parent (sometimes referred to as a clone). This is very important if the blades are being grown for a specific characteristic, such as agar consistency, specific morphology, or favorable growth rates and biomass yields. However, the vegetative propagation of a blade does not have an attachment stage, so the culture method needs to be adapted to the local needs and situation of the grower.

Cultivation

There are several different methods used for the cultivation of *Gracilaria*, and the ideal system for any one grower may be a combination of two or several of these following options:

Culture Initiation

- Tip Isolation
- Spore Isolation

Biomass Production

Asexual (Vegetative) Propagation:

- Tank culture
- Suspended Rope Culture
- Bottom Culture

Sexual Propagation:

- Spore seeded substratum (the use of carpospores or tetraspores)

Culture Initiation

To initiate a culture, it is necessary to establish a unialgal culture (containing no other alga) by either spore or tip isolation. Clean, healthy, actively growing, and/or reproductive 'parent' fronds exhibiting desirable characteristics should be selected. Fronds (sometimes referred to a thallus) may be chosen from wild populations or from existing cultures.

When making a wild collection, fronds should be held and transported in an environment similar to (or cooler than) the one it was collected in to minimize stress. Clean plastic bags, plastic containers, or buckets can be used for collection and transport. *Gracilaria* should be transported in moist paper towels or gauze to avoid drying and exposure. Regardless of the culture initiation method used, the critical step in culturing *Gracilaria* (or any seaweed) is the cleaning process. Any wild collected frond will be carrying a host of microscopic organisms

(diatoms, protozoans, fungi, other micro-, macro-organisms, or cyanobacteria) that can potentially contaminate, inhibit or destroy cultures. An initial rinse of the seaweed in the field can remove any visible fouling organisms, followed by a more thorough cleansing process in the lab. Whenever possible, it is recommended to make voucher herbarium specimens for more rigorous genetic study.

A successful culture is established in the lab with a healthy, actively growing, unialgal isolate free of any contamination. One successful culture can provide all of the 'seedstock' necessary to "seed" an entire farm. It is important to maintain a 'seedstock' culture in the lab for back-up and preservation purposes. To increase biomass from one isolate, fronds are fragmented and given sufficient light, space, and nutrients to multiply in number and size, and the process is repeated until enough biomass is reached to either "seed" a larger farm system or for direct harvest.

Tip Isolation

The area of new and active growth is located at the tips of every branch on a *Gracilaria* frond, the apical tissue. To obtain new 'starter plants' to initiate a culture, tips are cut from the parent frond, cleaned thoroughly, and placed in favorable growing conditions. Each tip will then grow, elongate, and branch into a new frond. To prepare a tip for isolation, a clean working area should be set up in a clean room with a draft-free, clean working space and a flame for sterilization of metal instruments to avoid any contamination of cultures.

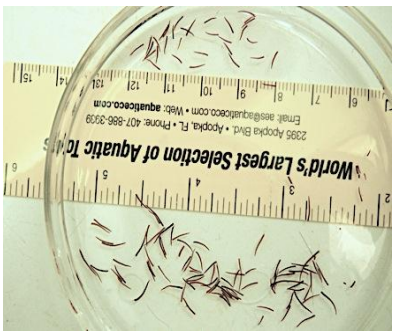
Collected fronds from the field should be processed in a separate room from your culture isolation working space for the initial rinsing. The fronds are initially placed in the first container, and then fronds are individually selected and rinsed in a series of vessels by grasping with large forceps, submerging and shaking vigorously underwater several times. This can be repeated several times in a series of vessels where the last should contain the fronds ready for tip isolation. If there are still epiphytes present on the tissue, gentle scrubbing with cotton balls, cotton-tipped swabs, or paper towels can remove any clinging organisms. *Gracilaria* is a euryhaline algae (able to tolerate a wide range of salinities), so a quick rinse (30-60 seconds) in clean, deionized freshwater can be used as a final cleansing step. A small section of the frond can then be removed for tip isolation, and placed in a small dish with sterilized seawater and moved to the clean working space (Fig. 17).

Figure 17. Cleaning wild-collected *Gracilaria* (from left to right): 1) Initial rinse in clean seawater; 2) Selection of clean, healthy fronds and second rinse; 3) Scrubbing and selection of tips for isolation

To isolate tips, a clean, sterilized work surface is essential. The work area should be set up with sterilized jeweler's forceps (fine tipped tweezers), a sharp scalpel or razor blade, ethanol, and a flame source. A small section of the parent frond is placed in a Petri dish with sterile seawater, and healthy tips are excised with a scalpel or razor blade. Once all tips from a section are cut, the excess frond is removed and the tips are further cleansed. It is useful to cut the tip a little longer than what is needed, about 1mm, in order to grasp the cut end of the tip with jeweler's forceps (Fig. 18).

Once a number of healthy tips have been excised, each tip is individually cleaned in sterile seawater. A series of Petri dishes can be set up in the working area to clean individual tips. A tip is placed in sterile seawater, grasped at the cut end with fine tipped forceps, and wiped down with a sterile cotton-tipped swap. This is followed by an agar drag through a prepared agar plate (see index for instructions), which will pull off any additional microscopic contaminants. A scrubbed tip is grasped, again, at the cut end, and dragged through the agar gel at least three times. Each drag should go through an unused portion of the agar gel. When the agar gel is used up, plates should be properly disposed of. The tip should now be free of contaminants, and can be placed in sterilized seawater prepared with Von Stosch's Enrichment media (VSE) and germanium dioxide (GeO_2). Multiple tips should be isolated from each frond in order to increase chances of obtaining a clean culture, because even all of these careful steps will not guarantee a unialgal culture. To maximize success, always check your culture under a microscope.

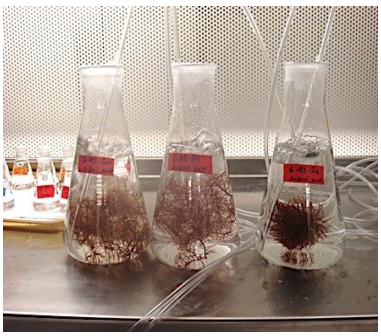
A. Excising Healthy Tips



B. Tip collection

C. Agar Drag for cleansing tips

D. Initiation of culture from tips



E. Scaling up cultures

F. Scaling up cultures

Figure 18. Isolating and cleaning tips for establishing a unialgal culture.

Individual tips can be isolated and cultured separately in small Petri dishes or flasks. They should be placed in VSE seawater with GeO_2 under low light (10-20 µmol photons m^{-2} s^{-1}), with a 12:12, L:D photoperiod, at 20°C (=68.8°F). Lower light may discourage the growth of any remaining epiphytic contaminants. Tips will begin to elongate in about a week or two. Once tips have begun to grow and appear clean, light aeration can be applied to cultures to increase growth rates. Cultures should be changed once every two weeks initially, then once per week as growth rates increase. Once tips begin to elongate and branch into larger fronds, they should be transferred to larger and larger containers to encourage growth (Fig. 18).

Once a clean culture is established, it can be expanded through fragmentation, by breaking up one frond to start many new fronds with the fragments. The growing environment is optimized to increase growth rates by gradually increasing light levels (up to 250 µmol photons m^{-2} $s^{-1)}$, increasing growing area (larger containers/tanks), and increasing frequency of media changes (to increase availability of nutrients). Clean glass culture bottles, jars, flasks or carboys of varying sizes are excellent for expanding or maintaining your cultures. Once a sufficient biomass is reached, the fronds can be transferred to larger indoor or outdoor tanks to expand further. To preserve or maintain original culture strains over long periods of time in the laboratory, growing environments are minimized, reducing light, reducing temperature, space, and frequency of media changes.

Spore Isolation

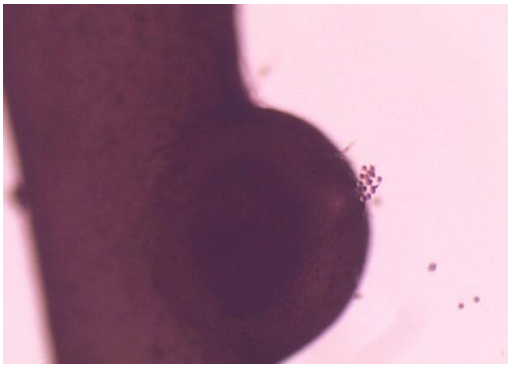


Figure 19. Carpospore release from a cystocarp formed on the surface of a female thallus.

Clean cultures can also be initiated from either carpospores or tetraspores. Both types of spores can be released and isolated in the same way, though each will give rise to a different phase in the life cycle. Carpospores are obtained from mature cystocarps, which are apparent as bumps on the female thallus (Fig. 19). These are easily identifiable, being obvious without the aid of a microscope. A microscope, however, is needed to observe the presence of the tetrasporangium on the thallus of the tetrasporophyte. These appear as small reddish spots scattered throughout the cortex of the thallus.

To release spores, it is important to clean the parent frond well by shaking, scrubbing, and rinsing in sterilized seawater. Reproductive branches can be removed and wiped clean with a cotton-tipped swab and followed by an agar drag. A gentle desiccation period can be utilized to stimulate release of spores by wrapping the branch in damp paper towels, placing in a Ziploc® bag, and storing for a few hours or overnight in darkness or dim light. If fronds are mature, the desiccation period can be skipped and placed in seawater for release. To release spores, short sections of the fronds can be placed in sterilized seawater in a Petri dish over glass slides and

kept under low light at 20°C. Release may take place at once, or can occur over several days. Spore release can be checked under a dissecting microscope.

Once there are spores released in the water, a small sample can be removed from the Petri dish and placed in a fresh dish with new media. Individual spores can then be selected with a very fine-tipped Pasteur pipette under a microscope. Selected spores are placed on cut glass slides (25mm x 25mm) or on coverslips in small Petri dishes with sterilized VSE seawater. Dishes can be kept undisturbed at 20°C under 30 µmol photons m^{-2} s^{-1} light, with a 12:12, L: D photoperiod. Spores will settle within 12-24 hours after release, adhere to the glass slide, and begin to divide. After the initial division, the diameter will begin to increase as a multi-cellular disc is formed, a few days after settlement. The center of the disc will then undergo further cell division to create a raised dome in the center, but this is a slow process, taking up to 2 months to form a 2-3 cm cylindrical axis. This is the beginning of the new frond (Fig. 20).

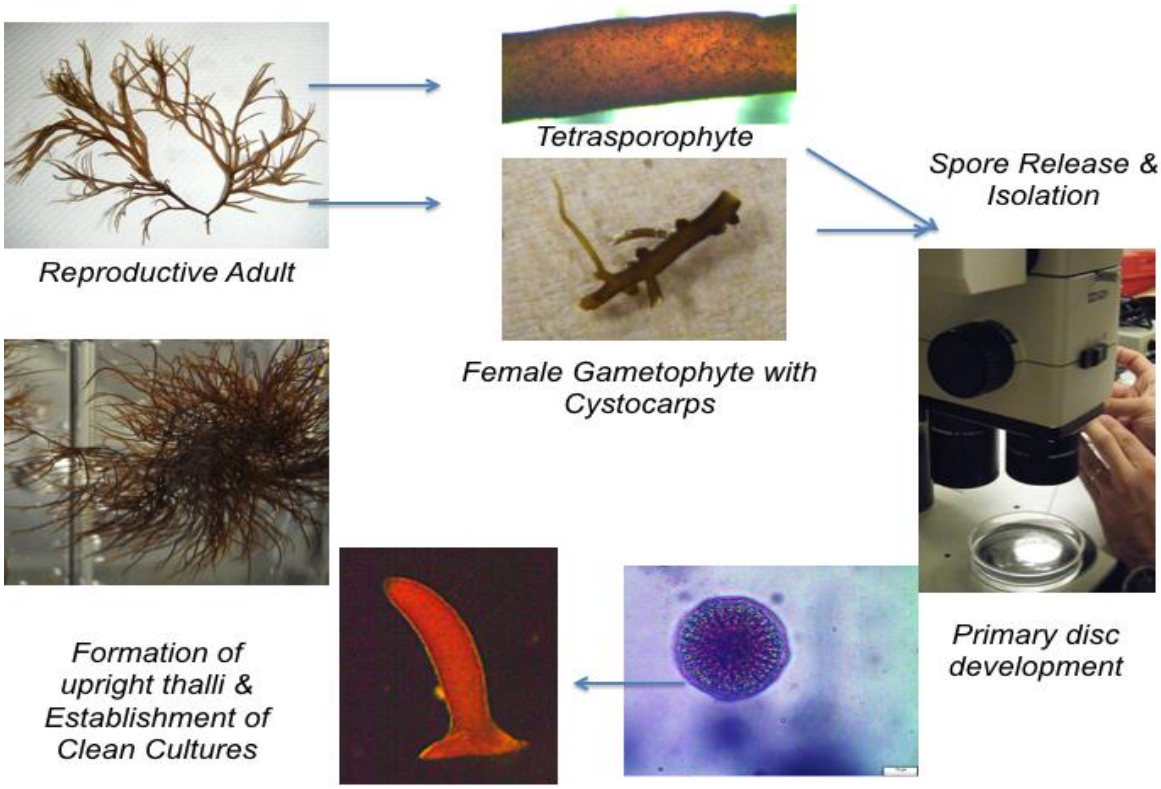


Figure 20. Establishment of clean *Gracilaria* laboratory cultures from spores

Spore initiation will result in an attached frond, and this can easily be transferred to new dishes by moving the glass slide, or the disc can be carefully scraped off the slide for an unattached frond. Once the first shoot begins to grow, light can slowly be increased, aeration can be added and sufficient water changes and larger containers can increase growth rates (Fig. 21). While spore isolation reduces the opportunity for contamination, the period of development is much longer than tip isolation. It can take 2-3 months to reach the size of an isolated tip. Spore

isolation allows the grower to start new cultures from a known point, however, for more control over their cultures. If a culture is started from a carpospore, the grower can be certain that the resulting frond is a tetrasporophyte, and if started from a tetraspore, it is certain that the resulting frond is a haploid gametophyte, though it is impossible to determine the sex until the frond is mature.

Figure 21. *Gracilaria* lab culture. Establishing new fronds and building biomass.

Biomass Production

Asexual Propagation

The most common method of *Gracilaria* cultivation is through vegetative propagation. This is a simple process that allows the grower to start many new fronds from the tips of any single frond. This is a form of clonal propagation, where all of the new fronds started from the initial "parent" will be genetically identical, which is useful for consistency in production. This is probably the easiest form of propagation, but it results in unattached, free floating fronds, which lends itself well to tank cultivation, but presents some challenges for any type of attached culture.

Tank Culture

Figure 22. Building biomass for *Gracilaria* culture: Laboratory scale, small tanks, medium tanks, large outdoor seawater tanks.

Gracilaria does very well in a tumbled tank culture system (Fig. 22). This is due to its ability for unattached vegetative growth over long periods of time, ease of propagation, and high growth rates under ideal conditions. In this type of system, fronds are stocked in a tank of appropriate size and are given light, nutrients, and aeration. This type of system is intensive, requiring an input of energy (light, nutrients, and water movement) for culture. While tank culture may require more input energy, it allows for full control of the growing parameters as well as contamination control. It is the best method for developing 'seedstock' for further out-planting, and will result in the highest quality fronds for edible or cosmeceutical/nutraceutical/pharmaceutical markets.

Aeration is a critical component of the tank culture, and is responsible for delivering several critical elements to the system. Aeration should be well distributed throughout the tank to deliver sufficient water movement to constantly 'tumble' the fronds around the tank. This allows for a higher stocking density, as the constant movement reduces shading limitations, increases light exposure, and increases photosynthetic efficiency throughout the culture.

Aeration also increases availability of carbon dioxide and stabilizes the pH, which can rise to very high levels in an actively photosynthesizing culture. Water movement created by aeration simulates natural water currents, which are important for delivering nutrients to the surface of the fronds. Seaweeds will take in nutrients from the surrounding water, and if there is no water current to replenish the water around the thallus, a "boundary layer" of nutrient-depleted water will be established, and growth will be inhibited. Water current is very important for sufficient nutrient delivery to all fronds in any culture, especially an intensive culture with a high stocking density. Current can also be important for reducing contamination on the fronds, as it may make it more difficult for other organisms to settle on the surfaces of the fronds if the water around the fronds is turbulent.

Any type of air blower can provide aeration, though the air should be filtered before entering the cultures (Fig, 23). The distribution of air can be established by running rigid tubing or PVC pipes throughout the tanks to create full circulation. Air should be moving up from the bottom of the tanks on the outside and the center of the tank to ensure there are no 'dead spots' where fronds could accumulate.

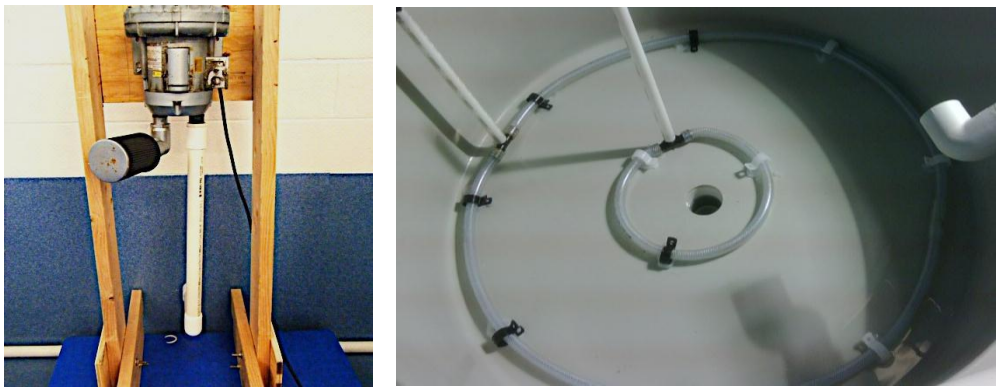


Figure 23. Aeration system for tank culture. A blower with air filter and aeration tube arrangement for a round tank.

The addition of sufficient nutrients to a culture is essential to obtain maximum growth rates and biomass yields. In a tank culture, this can represent a system expense, though the coupling of seaweed tanks with other 'waste' streams for alternative nutrient supplies is a possibility, as *Gracilaria* has a high bioremediation potential. Some alternative nutrients may include waste from other cultured animals (fish or shellfish), sewage, or fermentation residue from anaerobic digesters. In the absence of an alternative fertilizer, conventional land-plant fertilizer may be added. Fertilizer should be added in small daily increments to avoid ammonia toxicity. *Gracilaria* is able to take up nutrients in excess of their immediate needs and store these 'reserves' in their tissue for use in nutrient depleted situations. This storage capacity is useful to the farmer, for fronds can be 'fertilized' by soaking in high-nutrient media for 6-12 hours, then transferred to another site that may be low in nutrients. The seawater media in a culture should be changed on a regular basis to remove possible contaminants and to provide fresh nutrients. Larger cultures can be monitored for nutrients levels and half of the water volume changed on a regular basis to conserve inputs.

Light should be provided to the culture system in such a way to maximize growth while minimizing cost. Initially, lighting inside the laboratory can be provided with fluorescent lights or greenhouse growing lights, and light should be placed on a photoperiodic timer. As the culture system is expanded, natural lighting should be utilized, though fronds should be protected from extreme exposure by covering with neutral density screening. A light meter is an essential tool for determining and adjusting light levels as appropriate. Light (given non-limiting levels of nutrients) is an important parameter for controlling pigment levels in *Gracilaria*, and a controlled environment allows the grower to adjust light levels for desired pigment levels or the production of photo-protective pigments. This may or may not be important, depending on what the final product is being sold for. Light, aeration, and nutrients all work together to produce the final product. It is important to ensure that all fronds are exposed to enough light through constant turn over and tumbling, and nutrients need to be provided at non-limiting levels to allow for optimal growth rates, given sufficient light.

The culture is easily expanded by breaking apart, or fragmenting, the fronds, which will continue to branch and grow into new fronds (Fig. 24). The culture should be kept at a density that will maximize growth with efficient use of the resource inputs (e.g. 2-4 g L^{-1}). As the culture expands, the density can be reduced by removing fronds or by moving to a larger container or tank. This is an efficient way to grow up a large amount of initial biomass for further cultivation in a field setting or in larger tanks. For more in-depth discussion of lab and tank rearing technologies see Craigie and Shacklock (1985), Craigie (1990) and Craigie *et al*. (1999).

Figure 24. Building biomass in a tank culture through fragmentation of fronds.

Suspended Rope Culture

In order to reduce input costs of culturing seaweeds, field culture is an option, wherein seaweed fronds are placed out in the coastal environment to take advantage of the available natural resources. This is an extensive farming practice, where inputs and labor are relatively low, compared to the highly controlled intensive laboratory and tank culture. Suspended rope culture is a relatively simple fixed grow-out system, comprised of a floating longline held in place by anchors and buoys. *Gracilaria* plants are attached to the line by entwining or tying, and are further grown out on the lines (Figs. 25, 26). *Gracilaria* can be outplanted on longlines when temperature rises above 15 ˚C (60 ˚F). As biomass increases, fronds can be harvested by 'trimming' the outer portion of fronds and leaving fronds to re-grow to cut again every two to four weeks, or by a total harvest, by taking in the whole frond. Culture sites will vary considerably in their conditions, and local prevailing light, temperature, nutrient and salinity conditions should be determined for optimal growth (Fig. 27).

Figure 25. *Gracilaria* bundle inserted in line for field culture.

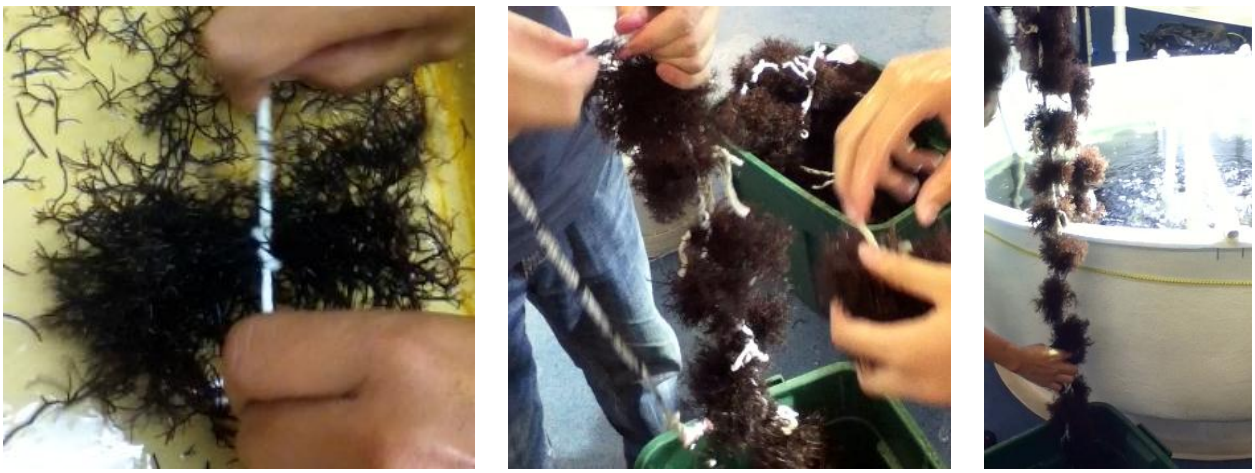


Figure 26. Inserting *Gracilaria* bundles onto a longline for out-planting.

Problems associated with field culture of *Gracilaria* include grazing, fouling, and environmental stress. Grazing by herbivorous fish or other predators is not as much as a problem in the Northeast as in the warmer climates, but smaller grazing animals are a possibility. Fouling is the biggest challenge in an open water grow-out situation. Many different types of fouling organisms may settle on the lines, including epibionts, such as tunicates, hydroids, bryozoans, mussels, worms, and amphipods, and epiphytes, such as other green, red, and brown seaweeds

or microalgae. There are several methods available for minimizing fouling. These include controlling depth, stocking density, and out-planting or harvest time. Depth can be adjusted, either up or down, to minimize settlement or survival of particular organisms, which tend to be more abundant at a particular depth. Increasing stocking density and maximizing growth rates will allow the fronds to outcompete or exclude potential fouling organisms. All organisms in the Northeast, including fouling organisms, have a seasonally controlled life cycles. Once the life cycle is understood, timing the outplanting of the seaweed lines and harvest of the crop can be a very important method for avoiding fouling settlement windows.

Extreme weather events can pose a hazard to the farm, either by causing damage to the infrastructure or by stressing the fronds. Farms should be sited in protected areas, and should be strong enough to withstand storms or high wind events. Flexibility in the design of a farm is important to minimize losses. An example of farm risk management would be the ability to bring lines in for storage in holding tanks during dangerous storms, or being able to adjust depth so that lines could be lowered to avoid extreme wave action or runoff events. *Gracilaria* is a stress tolerant species, but extreme changes in light, salinity or temperature can stress a culture unit and reduce growth and production.

Figure 27. *Gracilaria* open water farm (left). Deployment and cultivation of *Gracilaria* on longlines in the open water farm (middle). The harvesting of *Gracilaria* bundles.

Bottom Culture

Bottom culture is a popular culture technique in warm temperate climates, but may be difficult in the Northeast due to leasing area limitations. Bottom culture involves either growing fronds that are 'seeded' onto rocks and spread on the bottom in a shallow area, or by attaching fronds to lines that are strung on stakes and suspended just above the bottom. The technique of suspending lines from stakes is very similar to the suspended line culture, but is a little less flexible with depth adjustment. Access, however, may be easier in shallow sites.

Sexual Propagation:

Spore seeded substratum (carpospore or tetraspore)

Spore seeding is an alternative means of *Gracilaria* propagation. Instead of fragmenting a frond to produce new fronds, spores from a mature carposporophyte or tetrasporophyte are seeded onto a substratum, allowed to develop into juvenile fronds, and placed out into the growing area. The advantage of spore seeding is the convenience of attached fronds, while the disadvantage is the long incubation period to grow the settled spores into young fronds. The development of a juvenile frond 1-3cm in height can take two months in culture. Spore-

originated fronds can also exhibit a higher level of polymorphism, since all fronds are not genetically identical, as with the asexually propagated fronds. This will increase capacity for adaptation and survival in fluctuating or changing environments, but may not be ideal for a specific commercial purpose.

Spores can be released over the desired substrata (seed line or rocks) by laying prepared, mature spore-bearing thalli on a screen suspended in water. Spores are released over a 2-4 day period at 20°C, and once released, spores are allowed to settle undisturbed for 24-42 hours in dim light. If seeding both sides of a line wound on a frame, the frame is inverted and the same release and settlement period is done for the other side. The seeded substratum is then kept at 20°C under low light (20-40 µmol photons $m^{-2}s^{-1}$) for approximately two months for development of the juvenile fronds. Culture media (VSE) should be changed every two weeks to start, then more often as fronds start to develop. Gentle aeration can be applied for water movement. Once juvenile fronds are visible, they can be transferred to the culture site. A protected site should be chosen to avoid frond loss in high-energy environments.

Spore seeding of rocks may be a way to reseed or restock natural populations of *Gracilaria*. Once the fronds are established, the seeded rocks can be broadcast on the bottom of a protected shallow area, in order to help reestablish natural populations or supplement harvests.

Mixed Culture Methods

Several different culture methods may be employed in the culture of *Gracilaria*, and best practices will depend on the facilities available to the grower. Fronds grown in tanks may be used to stock long lines, and additional spore seeding of ropes may be possible to supplement the harvest and increase productivity. Other types of grow out systems are possible, including net tubes instead of lines, or floating cages stocked with unattached fronds. A 'spray culture' is another option, where recirculating water is sprayed continuously over fronds that do not have to be submerged in a tank of seawater. Fronds grown in areas or time periods of low levels of nutrients in the field can be 'fertilized' by placing in land-based tanks with high levels of nitrogen for 6-12 hours, then placed back into the field. Successful culture and production will depend on the grower's flexibility, inventiveness, and good management practices. For more in-depth discussion of cultivation techniques the reader is urged to consult Yarish and Pereira (2008).

Suggested Readings

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Appendix

Lighting

- Light Resources: Bulbs, Information, and Distributors
- GE Lighting: www.gelighting.com
- Philips: www.usa.lighting.philips.com
- Sylvania: www.sylvania.com

Handheld Quantum Light Meters

- Apogee Instruments www.apogeeinstruments.com
- LI-COR Environmental www.licor.com

Light measurements

- µE=µmol photons m^{-2} s^{-1}=(lux)(0.013)
- Light measurements conversions http://www.egc.com/useful_info_lighting.php

Supplies

Artificial Sea Water

- Instant Ocean (www.instantocean.com)
- Tropic Marin Sea Salt (www.tropic-marin.com)
- Ultramarine Synthetica www.waterlife.co.uk/seaquariums/ultramarine.htm
- Sigma-Aldrich Dry Sea Salt Mixture (www.sigmaaldrich.com)

Pre-mixed Enrichment Stocks

- National Center for Marine Alga and Microbiota NCMA (formerly CCMP) https://ncma.bigelow.org/
- Culture Collection of Algae and Protozoa (CCAP) www.ife.ac.uk/ccap
- Sigma-Aldrich f/2 media (www.sigmaaldrich.com)
- f/2-AlgaBoost, ES enrichment stocks (www.algaboost.com)

Aquarium Equipment & Supplies

Tanks, pumps, aeration, tubing, filters, lighting, etc

- Deep Blue Professional (www.deepblueprofessional.com)
- Aquatic Ecosystems Inc (www.aquaticeco.com)
- Lifeguard Aquatics (www.lifegardaquatics.com/)
- Frigid Units, Inc (www.frigidunits.com)
- Emperor Aquatics, Inc. (www.emperoraquatics.com)
- Aqualogic (www.aqualogicinc.com)
- Polytank, Inc. (www.polytankco.com)
- Ironfish Aquaculture Directory (www.ironfish.org)

- Percival Scientific (www.percival-scientific.com)
- Omega Engineering Inc. (www.omega.com)
- Hydroponic and Greenhouse Suppliers (www.sunlightsupply.com, www.hydrofarm.com, etc)

Laboratory

Agar Media for Preparation of Agar Plates

- To prepare sterile agar plates, use new disposable plastic Petri dishes or sterilized glass Petri dishes.
- An agar solution is prepared by adding 1.5% agar powder to a 50:50 sterilized seawater/fresh water solution in a flask.
- The media should be microwaved or heated on a stir plate to both fully dissolve the agar powder and to sterilize the solution (about 5 minutes). Cover the opening of the flask with tinfoil upon removal.
- Prepare a clean working space free of any air currents to pour hot agar into plates. An open flame in the working space is used to sterilize the neck of the flask by passing it through the flame before pouring.
- While pouring the agar solution, open the Petri dishes as little as possible, holding the cover at an angle, and keep the lid over the dish. Cover the poured dish. Place agar plates on an undisturbed surface to cool and set. Agar medium will set into a stiff gel at room temperature.
- Stack cooled and hardened agar plates upside down in the refrigerator. Do not freeze. Plates are stacked upside down to prevent condensation from dripping down onto the agar surface.

Haemocytometer counts for cell density

Haemocytometer and coverslips

A bright line haemocytometer is a specially etched glass slide made for taking blood cell counts, but is also useful for spore density calculations because it allows for an estimation of number of cells per milliliter of spore solution. The center of the haemocytometer slide contains two loading wells, each leading to a number of etched blocks on top of the slide which are apparent under the microscope. A haemocytometer comes with a special cover slip, and this should be placed over the center of the etched glass. There are two small wells on both sides of the glass slide, under the cover slip. These are loaded by placing a well-mixed drop of the sample solution in each well with a fine tipped pipette. The drop containing the spores will be pulled over the series of blocks, each with a different grid pattern. The middle block is divided up into 25 gridded squares, representing 10^{-4} ml. This is the block that should be counted. The haemocytometer should be viewed at 100x or 200x, and one square at a time should be counted. It may be easier to view the etched blocks under the microscope by turning down the light and closing the aperture of the microscope to increase contrast. The eyes should be methodically moved from the left to the right, and a consistent way of counting the cells on lines should be established. Cells on dividing lines are only counted in each square from the top (or bottom) and the left (or right). Consistency is very important for getting cell counts. A handheld clicker can be used if the density is very high, or the solution can be diluted by adding more seawater to count a more manageable sample.

Density Calculation:

Count the number of spores found in the 25 blocks that make up the middle block of the haemocytometer. This number represents the amount of cells in 10^{-4} ml, so to find the density of spores per ml, just add 4 zeros to your count.

Example:
Count = 40 spores
Just add 4 zeros to your count = 400,000 spores / mL
Now to find the total amount of spores available, multiply this by the total amount of spore solution that you have: Ex: 100ml * 400,000 spores = 40,000,000 spores / 100 mL
To determine the amount of spore solution to add to your spools for inoculation, determine the total amount of seawater being used for the inoculation:
Ex: 6 Liters = 6,000 mL

Spools should be inoculated at 2,000-5,000 spores/mL. To find the total amount of spores needed for your inoculation, multiply your desired density by the total amount of seawater being used.
Ex: 2,000 spores/mL * 6,000 mL = 12,000,000 spores total
Now you can divide the total amount of spores by the number of spores per mL in order to determine how many mL of spore solution you should add to inoculate your spools.
Ex: 12,000,000 spores / 400,000 spores ml^{-1} = 30 mL spore solution

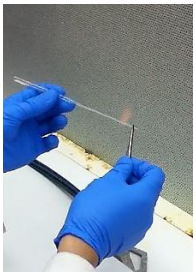
Germanium Dioxide

Diatoms are a common type of contamination in seaweed cultures, but can be eliminated with addition of a saturated solution of germanium dioxide to culture media. A saturated stock solution can be prepared by dissolving 250mg of GeO_2 per 1 Liter of deionized water. This stock solution is then added to culture media at a concentration of 2mL/L seawater. Stock solutions should be stored in a refrigerator and properly labeled. Brown algae are also sensitive to high concentrations of GeO2, so this concentration should not be exceeded when culturing kelp. One to two weeks of treatment is usually sufficient to eliminate diatoms in a culture.

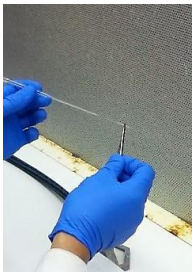
Micropipette Preparation

Micromanipulation by micropipette allows for the selection and isolation of microscopic spores under a microscope. Micropipettes can be prepared in the laboratory using disposable glass Pasteur pipettes.

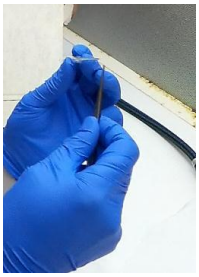
Holding the top of the pipette in one hand, and the small-bore end with a pair of steel forceps, hold the end of the pipette over an open flame to soften the glass until malleable. Take the pipette out of the flame and pull the ends apart, stretching the glass to form a very small bore. Snap the end off, and carefully place aside. Several of these micropipettes can be made up beforehand for isolation work under the microscope.



1) Hold glass pipette over flame until soft



2) Pull ends apart to stretch glass



3) Snap off end

VON STOSCH'S ENRICHED SEAWATER MEDIUM

von Stosch's Enrichment (as cited by Ott, 1966)

The seawater should be filtered (Whatman's #1) to remove large organic particles and sand. Then sterilize by autoclaving (time: 100 ml requires 10 minutes; 2 liters requires 40 minutes; 3 liters requires 50 minutes; and 5 liters requires 70 minutes). To each liter of seawater, then add the following:

Salts	1 liter of seawater
(1) Na $N0_3$	42.50 mg
(2) Na_2HP0_4 $12H_2O$	10.75 mg
(3) $FeSO_4$ $7H_2O$	278.00 ug
(4) $MnCl_2$ $4H_2O$	19.80 ug
(5) Na_2EDTA $2H_2O$	3.72 mg
Vitamins	
(6a) Thiamine-HCl	0.20 mg
(6b) Biotin	1.00 ug
(6c) B12	1.00 ug

It is convenient to prepare a stock solution of each salt in distilled water; of such concentration that 1 ml of the stock solution gives the required concentration of each ingredient. The three vitamins may be incorporated in the same stock solution, which should be refrigerated. The salts and vitamins after preparation into stock solutions should be filter sterilized.

I. To make stock solutions use deionized distilled water and clean volumetric flasks.

II. Filter each stock solution through separate 0.22 um Millipore filters. Each solution will have to be sterilized separately.

III. Aseptically pour filtered volume of liquid into autoclaved stock bottles.

1 liter stock solution	2 liter stock solution
(1) 42.500 grams	85.000 grams
(2) 10.750	21.500
(3) 0.278	0.556
(4) 0.0198	0.039
(5) 3.720	7.440
(6a) 0.200	0.400
(6b) 0.001	0.002
(6c) 0.001	0.002

Modified Von Stosch Enrichment (VSE) for use with red algae			
****To sterilized seawater, add 1 mL per 1 L of seawater of prepared solutions 1-6 after combining 3 and 4 together prior to addition****			
Solution Components			
Ingredients			
Solution 1: Nitrogen	**Quantity (grams)**	**Quantity (X2)**	**Quantity (X3)**
Deionized water	1L	2L	3L
Ammonium chloride (NH_4Cl)	26.75 g	53.49 g	80.24 g
Solution 2: Phosphate	**Quantity (grams)**	**Quantity (X2)**	**Quantity (X3)**
Deionized water	1L	2L	3L
Sodium Phosphate. Dibasic, 12-Hydrate, Crystal ($Na_2HPO_4*12H_2O$)	0.4 g	0.8 g	1.2 g
Solution 3: Iron* (Combine with 4 immediately prior to addition to seawater)	**Quantity (grams)**	**Quantity (X2)**	**Quantity (X3)**
Deionized water	1L	2L	3L
Ferrous sulfate ($FeSO_4*7H_2O$)	0.278 g	0.556 g	0.834 g
Solution 4: EDTA *(Combine with 3 immediately prior to addition to seawater)	**Quantity (grams)**	**Quantity (X2)**	**Quantity (X3)**
Deionized water	1L	2L	3L
Disodium Ethylenediamine Tetraacetate (Na_2EDTA)	3.72 g	7.44 g	11.16 g
Solution 5: Manganese	**Quantity**	**Quantity (X2)**	**Quantity (X3)**
Deionized water	1L	2L	3L
Manganese Chloride ($MnCl_2$)	0.0198 g	0.0396 g	0.0594 g

Solution 6: Vitamins* (Store in freezer)	**Quantity**	**Quantity (X2)**	**Quantity (X3)**
Deionized water	1L	2L	3L
Thiamine	0.2 g	0.4 g	0.6 g
Biotin	0.001 g	0.002 g	0.003 g
Vitamin B12	0.002 g	0.004 g	0.006 g

Notes on VSE preparation:

- **The original source of nitrogen for VSE is sodium nitrate ($NaNo_3$). This can also be used at 42.5 g per 1 L of deionized water. The original source of phosphate for VSE is Na_2 β-glycerophosphate. This can be substituted at 6.48 g per L of deionized water.**
- Prepare all six solutions separately using clean sterilized volumetric flasks, clean pipettes, digital balance, and mix with magnetic stirring bars.
- Filter sterilize each solution using a 0.2μm filter and a vacuum pump assembly. Vitamins should not be heat sterilized.
- Media solutions should be stored in the refrigerator, vitamins (solution 6) should be stored in the freezer and thawed for use
- All chemicals should be dated when received and when opened on the bottle
- All solutions should be clearly labeled at every step, and aseptic technique should be used in preparation
- It is easiest to make up large amounts of the solutions initially, then aliquot out usable amounts in smaller bottles (well sealed) for convenience. The prepared bottles can then be kept ready in the refrigerator for water changes.
- Full strength is 1ml/L
- Germanium dioxide is another, separate component that is added to cultures to prevent growth of diatoms. The solution can be prepared in advance and refrigerated. This solution is added at 2mL/L of water.
- All glassware should be sterilized, and the working space should be very clean and include a flame of some sort to prevent contamination of this high nutrient media.

Nutrient Media Supplies List

Media preparation	
Glassware: (Pyrex)	
	Volumetric flask (1000mL; measuring)
	Erlenmeyer flasks (3000mL; mixing and storing)
	Graduated cylinders (500 or 1000 mL)
	Storage flasks and bottles
	Digital scale (0.0001 g; 3 decimal places)
	Weigh paper/boats
	Metal chemical spoon or spatula (measuring)
	Safety goggles and gloves
	0.2 micron filter for media sterilization (Corning Disposable Sterile Bottle Top Filter, 150mL Funnel, #25965-45)
	Pyrex screw-cap media storage bottles (media storage)
	Clean work space
	Flame (for sterile technique; Bunsen burner, etc)
	Sterile pipettes, 10mL (measuring; disposable plastic or glass autoclavable)
	Pipette bulb or motor
	pH meter and associated buffers
	Stirring plate and magnetic stir bars
	Parafilm

General Culture System Materials and Estimated Costs		
Item	Base	
Seawater System		
Seawater filters—3-step cartridge system, down to 1 micron		Online, Hardware stores
Filter cartridge housings	40	Pentek Blue
Filter cartridges (20, 5, 1 micron size)	5	Pentek
Seawater Holding Tank System		
500 gallon plastic holding tank	400	Ace Roto-mold
UV Light for seawater sterilization	400	Smart UV Sterilizers/Emperor Aquatics
External water pump to circulate water	200	Marinedepot.com
Round Polyethylene or Fiberglas Tanks, various sizes	200-2000	Aquatic Ecosystems, tank suppliers
Polycarbonate or glass 10L clear autoclavable Carboys	50-200	Laboratory, Homebrewing suppliers
Flasks		Laboratory suppliers
Petri Dishes		
Forceps		
Microscope		
Pasteur pipettes	20 (box of200)	www.sigmaaldrich.com
Temperature and Power Alarm and Auto Dialer	330	Omegaphone. www.omega.com
Lights		
CW-HO Fluorescent Lamps - High Output - Cool White		Greenhouse growers supply
Light Bulbs & Fixtures	200	Lighting Suppliers
Photoperiodic timers	30	Aquarium, Hardware supply
Aeration		
Aquarium aeration pumps	30	Aquarium Supply
Aeration tubing, small diameter, 25'	6	Aquarium Supply
Rigid Aeration tubing for large tanks, large diameter	30	Aquarium, Aquaculture Supply

Nutrient Bioextraction:

opportunities for nutrient management in Long Island Sound

C. Yarish, J.K. Kim S. Redmond & T. Dowding (University of Connecticut, Stamford), J. Curtis (Bridgeport Regional Aquaculture Science and Technology Education Center), G.P. Kraemer (Purchase College), and A. Green (Rocking The Boat)

NUTRIENT BIOEXTRACTION is an environmental management strategy by which nutrients are removed from an aquatic ecosystem through the harvest of enhanced biological production, including the aquaculture of marine algae and/or suspension-feeding shellfish

How does nutrient bioextraction work ?

Seaweeds remove inorganic nutrients from water and shellfish (oysters, clams, mussels) filter organically bound particles rich in nutrients. The combination of these two groups of organisms will extract both inorganic and organic bound nutrients, and therefore, could be a powerful tool in cleaning up nutrient-enriched areas.

Why is nutrient bioextraction being considered in Long Island Sound?

Long Island Sound has a long history of acting as a giant receptacle for human pollution. Its waters are consistently high in nutrients from waste water treatment plants (point source) and land runoff (nonpoint source). The Bronx River, is a natural freshwater river that empties into the East River of New York City. The East River receives enormous quantities of waste water (point source) and nonpoint source run-off .

Seaweed Aquaculture for Nutrient Bioextraction in LIS

Our ultimate goal is to design, demonstrate, and promote the bioextraction of inorganic nutrients from coastal waters using native seaweeds (the red seaweed, *Gracilaria tikvahiae* & the brown sugar kelp, *Saccharina latissima*). Nutrient extraction for bioremediation was tested using *G. tikvahiae* at two sites: off Fairfield, CT (LIS), and at the mouth of the Bronx River estuary (BRE), during the summer and fall of 2011. *Gracilaria* at the BRE site grew 11.8% and 10.0% d^{-1} at 0.5m and 1.0m deep, respectively, in Aug. Growth rates at the LIS site were 5.9% and 6.0% d^{-1} at the same depths. We have designed a *hypothetical* nutrient bioextraction 1 hectare *Gracilaria* farm system that assumes 4 m spacing between longlines. Our *hypothetical* one hectare nutrient bioextraction farm system at the LIS site could remove 2.6 kg N ha^{-1} mon^{-1} from Aug-Oct., and 5.5 kg N ha^{-1} mon^{-1} in Sept. and Oct. at the BRE site. During Aug. at the BRE site, nitrogen could be removed at 10.3 kg N ha^{-1} mon^{-1}. These results suggest that nutrients were rapidly assimilated and used to fuel the growth of new *Gracilaria* tissue at the BRE site, while nutrients appeared to limit the growth of *Gracilaria* at the LIS site during July and Aug. A winter crop, the sugar kelp (*Saccharina)* was farmed at the LIS during the winter of 2012 for a related CT Sea Grant Program related project. After outplanting juvenile kelp (<1mm), we have found that our aquacultured kelp grew as much as 3.0 m in length and yield of 18 kg m^{-1} after 5 months (Dec.-May). These results suggest that seaweed aquaculture can be a useful technique for nutrient bioremediation in urbanized coastal waters. Since N removal varied with site and season, seaweed bioremediation might be best applied at nutrient hot spots in LIS and New York estuaries. In collaboration with T. Dowding & colleagues (UCONN School of Business), an optimization model is being developed to determine the value of both nutrient bioextraction and the commodities (including biofuels, animals feeds, etc.) derived from the harvested seaweed as a method to maximize potential multiple revenue streams while reducing any residual biomass waste to zero. This pilot scale nutrient bioextraction research is being funded by the Long Island Sound Futures Fund, The New York State Attorney General's Bronx River Watershed Initiative Grant Program, the National Fish and Wildlife Foundation & The Connecticut Sea Grant College Program. For more information email charles.yarish@uconn.edu and/or visit http://www.longislandsoundstudy.net/issues-actions/water-quality/nutrient-bioextraction/; http://www.cptv2.org/allthingsct/episode/savin-rock-west-haven; http://www.theday.com/article/20120311/NWS01/303119895/1070/FRONTPAGE; http://www.nbcconnecticut.com/video/#!/on-air/as-seen-on/Brastec--A-Kelp-Farm/157580315); or http://seagrant.uconn.edu/publications/magazines/wracklines/fallwinter11/dream.pdf.

Potential applications and uses of biomass

Ecosystem services
-Habitat Improvement
-Nitrogen credit

Sea vegetables

Hydrocolloids and Cosmeceuticals

Biofuel

Project partners

University of Connecticut · Bridgeport Regional Aquaculture Science & Technology Education Center · Purchase College State University of New York

Project Sponsors

Sea Grant Connecticut · Long Island Sound Study · State of New York Attorney General · Environmental Protection Agency

This is the FIRST pilot scale nutrient bioextraction research at an ecosystem level. If the pilot farms are successful in LIS and the Bronx River (NYC), the science will be applicable for other urbanized estuarine systems.

http://www.cptv2.org/allthingsct/episode/savin-rock-west-haven

CPTV program, 'All Things Connecticut,'

http://www.theday.com/article/20120311/NWS01/303119895

Seaweed farming in the Sound: The beginning of something big?

March 11, 2012

By Judy Benson

Scientists and business interests say 'Yes'

Spend time talking to Charles Yarish and Paul Dobbins, and you'll start to believe seaweed farming could be the answer to some of the world's most intractable problems.

For starters, it could provide a highly nutritious, sustainable food source to a hungry planet; it could be transformed into biofuel that removes heat-trapping carbon dioxide even as it cleans offshore waters of pollutants; and it could create environmentally friendly economic opportunities for coastal communities including Long Island Sound.

Cultivation of this "virtuous vegetable," as Dobbins has dubbed it, is a multimillion dollar worldwide industry, supplying key ingredients for medicines, cosmetics, fertilizers and food products ranging from sushi wrappers to ice cream thickeners.

Dobbins is president of Ocean Approved, a year-old kelp farming company on the Maine coast, that sells frozen kelp to restaurants and speciality food stores, growing the sinuous green ribbons in about 8 acres of offshore beds supplemented with plots tended by local shellfishermen and lobstermen.

"We sold a very successful mussel farming business to start this, because we saw a tremendous opportunity with a tremendous product," Dobbins said.

It is now the only commercial seaweed grower in the United States (most of the world's cultivation now takes place in Asia). But if Yarish, the University of Connecticut professor who helped Ocean Approved get started, is right, seaweed farming has potential for major domestic expansion. He's put a heaping measure of enthusiasm and research skills - both his own and that of UConn colleagues in the marine sciences, engineering and business departments - toward making that happen.

Pollution filters

"These are my babies," said Yarish last week, smiling as he peered into a 13 -liter jar of juvenile gracilaria plants, bubbling in seawater he trucks from UConn's Avery Point campus in Groton to his lab at UConn's Stamford campus. The waters in the eastern Sound, he explained, are cleaner than in the western Sound, and therefore better for the gracilaria, kelp and the other seaweed types growing in his lab. His mature seaweed plants, however, are thriving in a research plot about a half-mile offshore from Bridgeport harbor.

His down-coastal connections to Avery Point also reach the labs of two marine sciences professors based there, Senjie Lin and Jamie Vaudrey. Vaudrey is studying Long Island Sound embayments that could benefit from the pollution filtering functions of seaweed farming, while Lin's work entails DNA and gene structure analysis to help identify the best seaweed strains for cultivation for particular areas and purposes.

Connecticut Sea Grant, also at Avery Point, has funded Yarish's research for several years, most recently with a grant that supported the first crop of cultivated kelp harvested from the Sound last month. But Sea Grant, a federally funded program to foster coastal research, outreach and education, is now moving from behind-the-scenes financial support into promotion of seaweed farming, said Tessa Getchis, associate extension educator at Sea Grant.

Sea Grant is overseeing the preparation of an instructional manual about seaweed farming, and doing outreach to potential new seaweed growers, she said. Seaweed farming could be a means of diversifying the Long Island Sound aquaculture industry, offering shellfishermen and lobstermen a way to supplement their incomes by using the same boats and marine knowledge they possess.

"We just met with some shellfishermen to determine what their level of interest is," Getchis said. "I don't know that there's room in Long Island Sound for many large-scale operations, but I definitely see potential for some smaller scale ones. There's just so much demand now for local and sustainable food sources."

Permits are being sought for a startup commercial seaweed farm in central Long Island Sound, and she also has had inquiries from potential growers in Rhode Island and Massachusetts.

From the Bronx to the Cape

At the Stamford UConn campus, business professor Tim Dowling is assigning his students to develop plans to maximize the economic viability of seaweed farming.

"I tell my students, 'You've got one ton of seaweed, what are you going to do with it?'" Dowling, who specializes in sustainable industries, said. "We're working on optimizing the revenue streams, so there's no waste."

Possibilities include selling the premium parts of the harvest for human food, and scraps for products ranging from animal feed to nutrition supplements.

Deriving income for seaweed farmers through the state's nitrogen trading program, which would assign a dollar value to the amount of nitrogen a seaweed plot absorbs from the waters, is also being explored, Dowling said.

Yarish, who has been involved in seaweed research for decades, is eager to do what he can to ensure that this budding interest in seaweed farming blossoms both locally and beyond. He readily shares his expertise as well as seed stock from his lab. In addition to the work in his own plots and lab and with Ocean Approved, he also has worked in the Bronx River on a project that pairs shellfish farming with seaweed-growing as a means of cleaning an urban waterway.

He also is supplying seaweed stock for a project in Waquoit Bay in Cape Cod and has made overtures to two culinary institutes about preparing dishes with locally grown seaweed. In addition, he has partnered with the Bridgeport Regional Aquaculture School and Technology Education Center on Long Island Sound to involve high school students in seaweed cultivation.

Easy to grow

Grown in buoyed plots off anchored lines that can extend a mile or more and are hauled out at harvest time, the seaweed grown in the Sound, Yarish said, must be fast growing and adaptable to wide ranges in water temperatures and other conditions.

"In Long Island Sound," Yarish explained, "we're doing very precise work, studying the best depth and density (for the plants) and frequency of harvest."

The plants are analyzed periodically to assess their nutrient uptake, growth rates and other properties. After harvest, the seaweed is dried and packaged. Most of the world's edible seaweed is sold as a dried product.

As a crop, seaweed is relatively easy to grow, said Dobbins, the Ocean Approved president. It doesn't require weeding or watering, so isn't as labor intensive as some land crops, and grows in relatively shallow, near-shore waters where sunlight can penetrate to the plants. One of the main issues that faces seaweed farmers, he said, is working out the bureaucratic issues involving use of marine property and permitting with regulators from the state and the U.S. Army Corps of Engineers.

Yarish's plot is the only permitted seaweed farming site in Long Island Sound. But Kristen Bellantuono, environmental analyst with the state Department of Environmental Protection's Office of Long Island Sound Programs, said her office is reviewing one permit application from a new grower, and is prepared for more. The key issues considered in granting a new permit, she said, are whether the lines and buoys cordoning off the seaweed plot would interfere with established lanes for recreational and commercial boating and fishing.

"We don't want to create any user or navigational conflicts," she said.

Yarish believes Connecticut may need to establish policies to work these issues out systematically rather than on a case-by-case basis, such as by enacting marine spatial planning - something like zoning for the sea - that would designate particular areas as suitable for specific uses such as seaweed farming. He also noted that some types of commercially valuable seaweed, such as kelp, are grown and harvested from late fall through early spring, when recreational boating and fishing traffic is at its ebb.

"Because I've been around the Sound for 35 years, I know all the sites from the far east to the far west," he said. "We have a huge number of sites that have potential (for seaweed cultivation). It's as far as the coastal managers would like to see it. The idea is to do this in Long Island Sound in a way that makes sense for Connecticut and New York."

http://ww2.ctmirror.org/story/14641/long-island-sound-legislation-stalled-washington-politics

Long Island Sound legislation stalled by Washington politics

December 6, 2011

By Jan Ellen Spiegel

Charlie Yarish knows exactly what it will take to get the technique he developed to clean the waters of Long Island Sound up and running.

"Additional funds," he said. "That's the bottom line."

But as Yarish, an ecology and marine sciences professor at the University of Connecticut, is learning, money in this time of tight finances, delayed federal budgets and, often, antipathy toward environmental issues is diminished at best and unavailable at worst.

For dozens of projects whose aim is cleaner water for Long Island Sound, as well as the land around it, that means waiting while two key pieces of federal legislation remain caught in the uncertainties of Washington's political battles.

One is the now combined Long Island Sound Restoration and Long Island Sound Stewardship Acts -- both overdue on their reauthorizations. The other is the appropriation for the Clean Water State Revolving Fund -- which provides some of the money for municipal wastewater, sewage and other infrastructure projects to help the state meet federal requirements for cleaning the Sound.

Complicating the politics of both is that most of their funding funnels through the Environmental Protection Agency. The EPA is a frequent target of cuts, if not wholesale elimination, by most of those seeking the Republican presidential nomination as well as a hefty chunk of Republicans in Congress.

"We're fighting for appropriations in a very tough climate," said Sen. Joe Lieberman, I-Conn., in a pre-Thanksgiving conference call on a day in which nearly a dozen-and-a-half environmental advocates from Connecticut and New York had fanned out on Capitol Hill spreading the word of their Long Island Sound cause.

"If you want an example where government really did something very significant at relatively low cost, this is it," Lieberman said. "It's not only a shame, but dumb, not to invest."

But even if both pieces of legislation start moving down the congressional pipeline, their funding seems to be settling at levels far less than either the state's delegation or the Sound's advocates would like.

The Long Island Sound Restoration Act, which generally covers water concerns, has been without authorization since Sept. 30, 2010. Authorization for the Long Island Sound Stewardship Act, which handles land issues, lapsed Sept. 30, 2011.

The combined reauthorization lays out priorities and suggested funding. The money for the Long Island Sound Program that finances it comes through appropriations for the EPA and is administered through its office, the Long Island Sound Study. With leveraging, the program has funded hundreds of projects, including beach cleanups, restoration projects, land stewardship and as much as $2 million annually for state projects, including about $775,000 for the state Department of Energy and Environmental Protection's 20-year ongoing water quality monitoring program.

Funding keeps dropping

Since fiscal 2010, funding for the Long Island Sound Program has decreased from $7 million to $5.3 million. For the current fiscal year, which has been running on continuing resolutions since it began Oct. 1, President Obama and House Republicans actually agree on a $2.962 million funding level -- a 44 percent cut. The Senate prefers something closer to $3.7 million.

Funding prospects are equally grim for the Revolving Fund, established in 1987 under the Clean Water Act. In fiscal 2010, the state received nearly $25 million and in fiscal 2011, more than $18 million.

That money was paired with state funds, which increased significantly in Gov. Dannel Malloy's budget ($658 million over two years, though it has not yet been approved by the Bond Commission). The state funds are to help cities and towns upgrade sewage and wastewater facilities to reduce nitrogen levels in the Sound. The state has been under federal mandate to reduce nitrogen discharges into the Sound to a prescribed level by 2014.

But while Obama has recommended a slight increase, from $1.52 billion to $1.55 billion, and the Senate recommended level-funding, the Republican-controlled House slashed funding to $689 million.

Few think, even with full funding, that the state will meet its 2014 requirement, and missing it could mean significant fines for out-of-compliance communities. Despite that, it's the authorizations that are more worrisome to advocates.

In past years, lack of authorization posed little concern, and programs were regularly funded without it. But in the current fiscal climate, advocates worry that no authorization could turn into a congressional license to kill.

"Reauthorization is the sole place for a lot of these projects," said Leah Schmalz, director of legislative and legal affairs for Save the Sound, a program of the Connecticut Fund for the Environment.

Schmalz noted that even if the Revolving Fund is cut, the state still contributes.

"The worry that we have if reauthorization doesn't take place is that it definitely shows a lack of interest to the people doing the appropriating."

Schmalz also pointed out that there is new language in the authorization that does things like require studies on the effects of sea level rise and marine spatial planning -- how to designate use areas for sometimes competing interests on and in the water.

The authorization also requires a pilot project on Yarish's cleansing technique, called nutrient bioextraction. It uses seaweed and shellfish to essentially sop up excess nitrogen.

Yarish ran a small study this summer using about $100,000 from the Long Island Sound Futures Fund, one of the main funding programs paid for with federal money from the Long Island Sound Study. But to turn it into an operation that would actually make a dent in the problem, Yarish says would take about $1 million. And, he pointed out, with potential byproducts from the nitrogen-soaked seaweed -- such as animal feed, fish feed and even biofuels, plus the jobs they can create -- it's a win-win-win.

"This is all part of the exciting thing of taking a problem and finding the solution," he said. "And along the way it's having economic value that is eventually turning into jobs."

'A zero sum game'

Mark Tedesco, director of the EPA's Long Island Sound Study office, said the Future's Fund has at least twice as many proposals as it can handle.

And the state has about 30 projects worth $15 million ready or nearly ready to go, said Betsey Wingfield, chief of DEEP's Water Protection and Land Reuse Bureau.

Sandy Breslin, director of governmental affairs for Audubon Connecticut, and a participant in the one-day lobbying last month, said what confounds her is a lack of realization that the authorizations and the Revolving Fund are not only linked by their goal to clean the Sound, but that they are also economic drivers.

"What we saw this summer was a wholesale attack on the EPA," she said referring to debate in the House during which the Revolving Fund became a prime target. "What's really concerning to us in the environmental community is the belief that environmental regulations are a bedrock cause of our economic problems."

It's the opposite, she said. Environmental projects create immediate jobs that have a multiplier effect of more jobs. All of this improves the economy.

"Pitting the environment against the economy is a zero sum game," she said. "The loser in that will be the people of Connecticut and the nation."

http://newyork.cbslocal.com/2012/08/21/commercial-seaweed-farm-coming-to-the-long-island-sound/

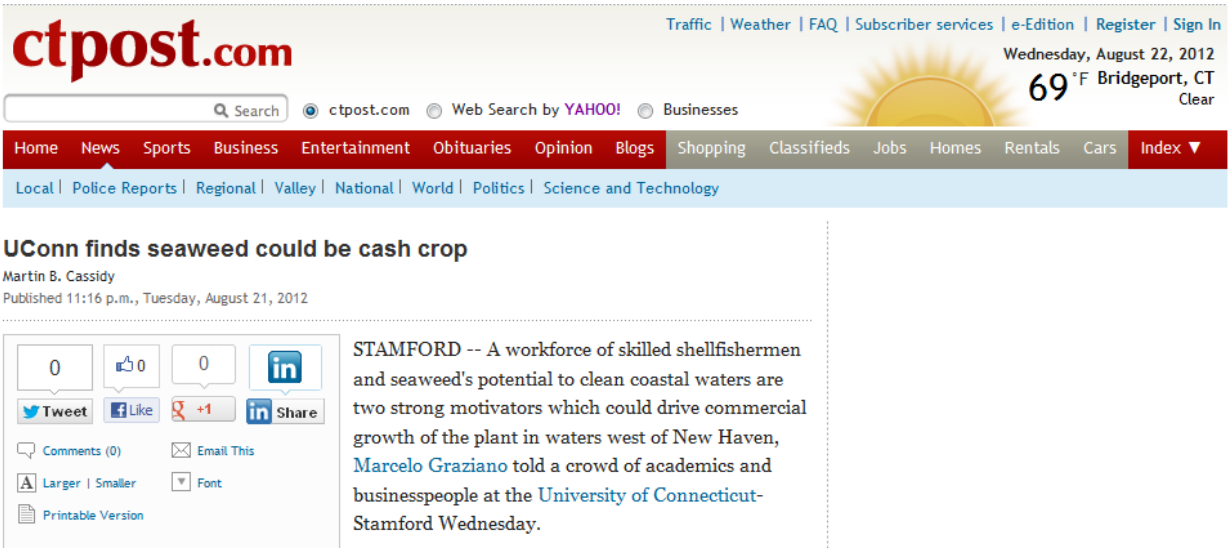
August 21, 2012
WCBS 880 Connecticut Bureau Chief Fran Schneidau reports

Commercial Seaweed Farm Coming To The Long Island Sound

STAMFORD, Conn. (CBSNewYork) – Aquaculture scientists at the University of Connecticut are poised to produce dividends from their decades of work to clean up the waters of Long Island Sound.
This fall, the first-ever commercial seaweed farm will open in the Long Island Sound.
The science team's decades of work has helped rid the waters of nitrogen and, as a byproduct, leaves behind edible seaweed and kelp.
"All that kelp went for sea vegetables. Most of it, actually, went into the Whole Foods markets around the east coast," team leader Professor Charles Yarish told WCBS 880 Connecticut Bureau Chief Fran Schneidau.
Yarish said the project is a win-win.

“We grow seaweed that we put into the system online so we can manage it. We give it a haircut every few weeks, harvest it at the end of our harvesting season, and we’re extracting nutrients out of the coastal waters,” Yarish told Schneidau.

Professor Yarish said his team is expanding the technologies and over time, the quality of the water in the Long Island Sound while continue to improve while also generating a new industry that produces everything from vegetables to biofuels.



http://www.ctpost.com/local/article/UConn-finds-seaweed-could-be-cash-crop-3805535.php

http://www.stamfordadvocate.com/local/article/UConn-finds-seaweed-could-be-cash-crop-3805257.php

UConn finds seaweed could be cash crop

By Martin B. Cassidy

Published 11:16 p.m., Tuesday, August 21, 2012

STAMFORD -- A workforce of skilled shellfishermen and seaweed's potential to clean coastal waters are two strong motivators which could drive commercial growth of the plant in waters west of New Haven, Marcelo Graziano told a crowd of academics and businesspeople at the University of Connecticut-Stamford Wednesday.

"Those shellfishermen are already using their boats to farm shellfish in the coastal areas, so there is little additional cost in terms of training a labor force to farm seaweed," said Graziano, a doctoral degree candidate in UConn's geography program.

In the near future, shellfishermen choosing to grow seaweed could turn a profit selling it for use in animal feed or for production of fertilizer, said Tim Hidu, a UConn business student.

Hidu said future conversion of a Connecticut crop of seaweed into granular fertilizer would generate a consistent demand for the product and could be sold for $2.31 a pound, a profit of just over 20 percent.

"The great thing about that is you can probably sell about just as much as you create," he said. "It also has a large number of plant hormones that would help your plants to grow."

On Tuesday afternoon, a team drawn from UConn's business school, engineering department and the Stamford seaweed research lab heralded the potential benefits of commercial seaweed farming to absorb nutrients, primarily nitrogen, in Long Island Sound and provide ecologically friendly fertilizers, biofuel and foods.

The economic analysis was the result of a partnership between UConn's Stamford campus, its business school and its Connecticut Center for Economic Analysis to assess the potential success of a seaweed farming industry in state waters.

The effort concluded that by using the 24,000 hectares of water area considered moderately suitable or better to cultivate seaweed in the Sound, Connecticut could produce more than 69,000 tons of it annually.

During the presentation, Tim J. Dowding, associate director of the business school's Business Accelerator announced the research effort helped the Thimble Island Oyster Co. gain approvals from the state Department of Energy & Environmental Protection to operate a commercial seaweed farm.

Charles Yarish, a UConn professor of ecology and evolutionary biology and marine science who has spent decades researching seaweed cultivation, said efforts by himself and others have already helped develop commercially viable, fast-growing seedstocks of seaweed's gracileria genus as well as kelp.

Yarish said he has expanded the seed plant operation, now growing them in 1,000 and 4,000 gallon tanks at the state's Bridgeport Aquaculture School.

Yarish said he aims to help several interested companies win permit approval to start growing gracileria and kelp for food within the next year, focusing on areas with better water quality in the Long Island Sound west of New Haven.

"What we're looking at is a form of marine agronomy, because we're really farming the waters," Yarish said.

Bren Smith, the owner of Thimble Island Oyster Co., said he contacted Yarish about the idea of seaweed farming after he lost 80 percent of his oyster crop and 40 percent of his gear in the wake of Hurricane Irene.

Beginning in October, Smith will begin growing kelp on his 60-acre oyster farm, attaching the plants to lines on which he also grows oysters and scallops in cages.

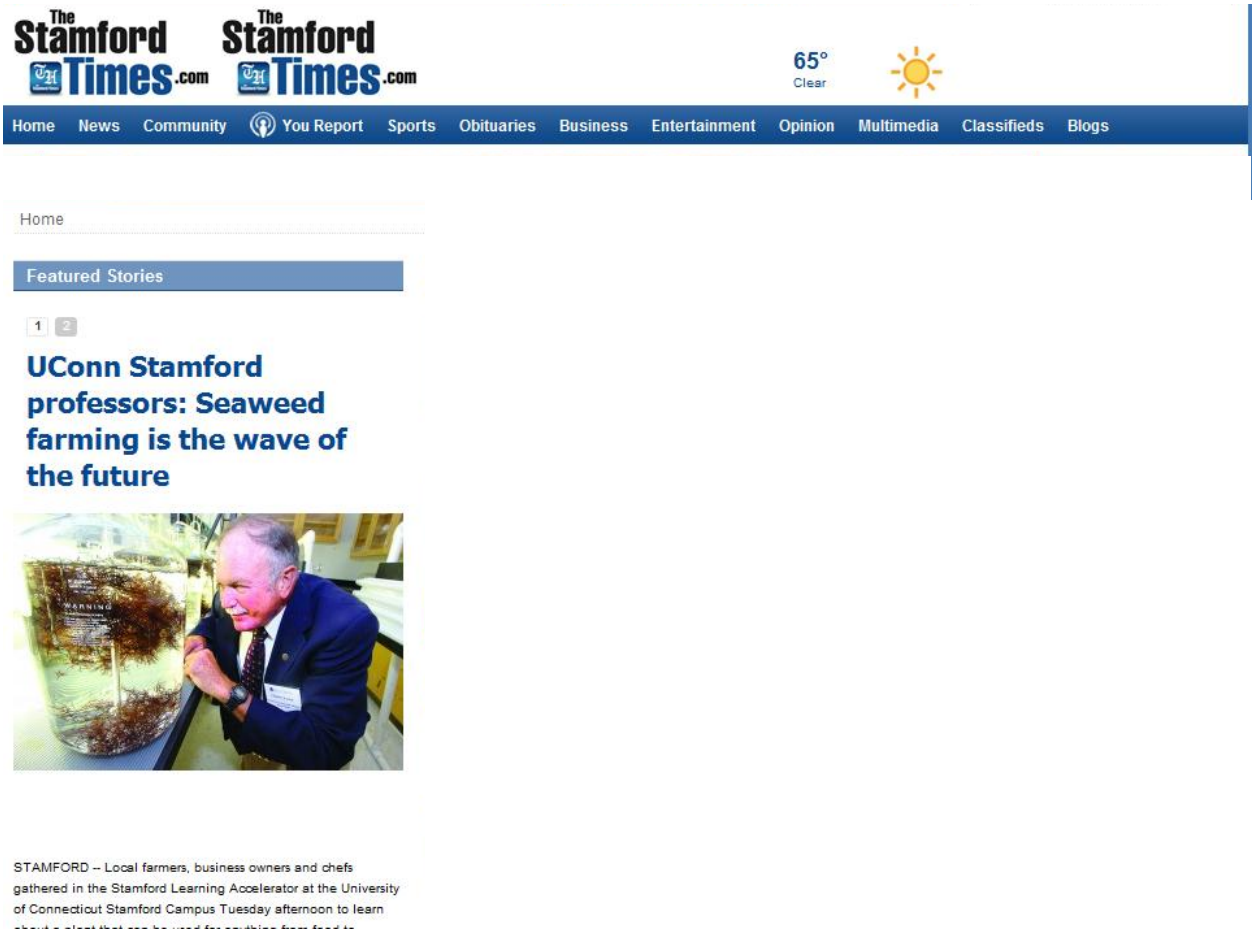
"There is some risk in being the first mover in this but there is also a tremendous upside in terms of benefit," Smith said. "With the fierceness of storms that is the new normal, and I have to adapt and try to figure out ways to be competitive."

After a tour of Yarish's lab, including two walk-in freezers where he is growing seaweed strains, attendees Tuesday dined on sushi from Miya's Restaurant in New Haven using tilapia from the aquaculture school, and clams and oysters also farmed in Connecticut waters.

Ariana Bain, sustainability director for Miya's, said a growing number of environmentally conscious consumers are interested in greater assurance their food choices are a "net positive" for the environment.

Once Connecticut seaweed is commercially available, she said growing consciousness of its environmental benefit in removing nitrogen from the water will encourage people to want to consume it.

"People get frustrated when they are trying to make choices that they hope don't have negative impacts on the environment," Bain said. "We're looking forward to being able to use Connecticut seaweed in our dishes and offer them as part of our menu items."



http://www.thehour.com/stamford_times/news/uconn-stamford-professors-seaweed-farming-is-the-wave-of-the/article_9c96b3b7-dcdf-5b69-9461-62fec52cbd63.html

UConn Stamford professors: Seaweed farming is the wave of the future

Posted: Tuesday, August 21, 2012 9:04 pm
By KARA O'CONNOR

STAMFORD -- Local farmers, business owners and chefs gathered in the Stamford Learning Accelerator at the University of Connecticut Stamford Campus Tuesday afternoon to learn about a plant that can be used for anything from food to biofuel, according to UConn professor Charles Yarish.

"Seaweed is a crop that can be used for a variety of things, and the best part is we can grow it right here in Long Island Sound," said Yarish. "Seaweed can be used as a table vegetable, a fertilizer, animal feed, a

biofuel and can be used as a natural tool for fighting the effects of pollution in the sound by reducing nitrogen in the water. It's an amazing plant."

Yarish and a team of a faculty and students from UConn Stamford's Learning Accelerator and International Business Accelerator program presented research Tuesday that examines the economic viability of growing seaweed in Long Island Sound. The team of faculty and students from the School of Business have been working on the research for more than a year, said Yarish.

"We are really excited to finally reveal our findings on aquaculture or seaweed farming," said Yarish. "Seaweed farming is going to be something that is not only environmentally sustainable, but will also help create many jobs in the state."

Bren Smith, a Guilford resident and owner of the Thimble Island Oyster Co., will be launching the first commercial seaweed farm in the state of Connecticut. Smith, who has been a shellfish farmer for the past 9 years, said he first thought of taking up seaweed farming after Tropical Storm Irene hit in August of 2011.

"After Irene, 80 percent of my crop was gone," said Smith. "So I started thinking about a crop that I could grow that would be resistant to those kinds of storms that are common in this area. That's when I met professor Yarish and he got me very interested in seaweed farming."

Smith said he will grow the seaweed on a floating long line above his oysters, clams and mussels.

"Before meeting professor Yarish, I would have never envisioned growing vegetables at sea," said Smith. "But kelp is one of the fastest growing crops in the world; it improves water quality and has a wide range of commercial uses after harvest. It's an ocean farmer's dream crop."

Ariana Bain, a consultant for Miya's Sushi, located in New Haven, said seaweed is starting to become very popular in restaurants because of the "net positive factor."

"This is the idea of regenerative or net positive eating," said Bain. "It has zero pesticides, it helps the environment and it's good for you. When you are trying to feed the world, seaweed is the solution. This is a delicious food that is actually beneficial to you and the environment, so people can do good in the world by eating it."

Yarish said that although Smith will open his farm in the fall of 2012, a lot of research still needs to be done on seaweed farming.

"Our team still needs to explore other potential commercial uses for seaweed from Long Island Sound," said Yarish. "We need to develop a business plan to manufacture fertilizer and animal feed in the U.S, analyze commercial production costs and conduct a quantitative evaluation of economic benefits of environmental remediation. We still have a lot of work to do."

UCONN Seaweed lab

Alex von Kleydorff | Posted: Tuesday, August 21, 2012 10:56 pm

Hour Photo/ Alex von Kleydorff. Professor of Ecology and Evolutionary Biology and Marine Sciences at UConn Stamford, Charles Yarish Ph.D., with some of the seed stock for kelp, grown in the school's laboratory.

An integrated multi-trophic aquaculture site in the Bay of Fundy, Canada, with salmon cages (back left) and mussel and seaweed rafts.

The Seaweed and Shellfish Solution

Using Nature's Filters to Help Curb Pollution and Fish Farm Waste

BY RENEE CHO

© THIERRY CHOPIN

When runoff from fertilized lawns, agricultural fields and sewage treatment plants reaches our rivers and coasts, it causes a water pollution problem known as eutrophication. In other words, unhealthy levels of nitrogen and phosphorus. These excess nutrients stimulate algal blooms. And these blooms, in turn, starve the water of oxygen, creating a dead zone where no creatures can survive. Some blooms are even toxic to humans.

One way to "bioextract" these nutrients that shows promise involves the use of shellfish and seaweeds. Charles Yarish, Ph.D., professor of ecology and evolutionary biology at the University of Connecticut, is one of the researchers involved in a $2.4 million project to help clean up the Bronx River in New York City using mussels and seaweeds, funded by the Long Island Sound Futures Fund (longislandsoundstudy.net).

Nitrogen and phosphorus exist in organic (plant and animal residue) and inorganic (mineralized) forms. As shellfish filter water, they remove organic nutrients, while seaweeds take up inorganic nutrients. This project will use the native red seaweed *Gracilaria* in summer and fall, and kelp, which thrives in winter. Because no large source of young seaweeds exists, Yarish and his colleagues are initially cultivating a seedstock of the seaweeds in their labs.

This summer and fall, a 20' by 20' raft of ribbed mussels and four seaweed lines will be placed at the mouth of the Bronx River in coordination with the National Oceanic and Atmospheric Administration (NOAA). Yarish estimates that the mussels will filter organic nutrients from up to 5.07 million liters of water daily;

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Charles Yarish with his *Gracilaria* (red seaweed) seedstock.

"[Blooms] starve the water of oxygen, creating a dead zone where no creatures can survive."

with seaweeds filtering the inorganic nutrients. Research suggests that the two species might also increase oxygen, improving water quality and fish habitats. Once the mussels and seaweeds have done their work they will be removed periodically to extract excess nutrients, and undergo extensive testing. "We want to find out what's being taken up by the shellfish and the seaweed, and what impact the system has on the local environment," says Yarish. If successful, the strategy could be used in other nutrient hot spots around the U.S.

Yarish will also be conducting a parallel *Gracilaria*-only project at an open water research site on Long Island Sound belonging to the Bridgeport Regional Aquaculture Science & Technology Education Center in Connecticut, a high school for marine science and aquaculture technology.

Both projects must yield environmental and economic benefits to be viable. Because the Bronx River site is near a wastewater treatment plant, no edible species can be cultivated there;

Thierry Chopin at an aquaculture site in Canada.

© MANAY SAWHNEY

however the ribbed mussels and seaweeds (and the Connecticut *Gracilaria*) will be evaluated for their economic potential for use as biofuels, agar (a component of red algae used in labs), biochemicals, animal feed, and nutrient extraction. The Connecticut *Gracilaria* will also be assessed as a possible food product by some of the Bridgeport students who run a seafood market at the Aquaculture school.

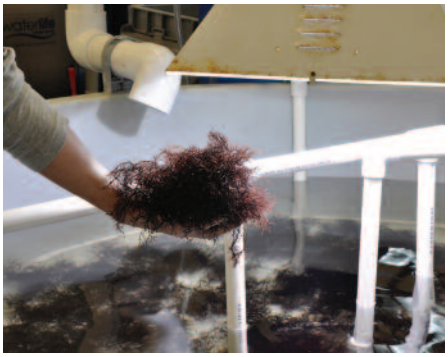
Combining species from different food web levels to perform ecosystem services is also the basis of integrated multi-trophic aquaculture (IMTA), an ancient practice that is being revived to make aquaculture more sustainable. Farmed aquatic organisms represent the fastest growing source of animal protein, responsible for over half of global seafood consumption. But the expanding sector of industrialized aquaculture, comprised mostly of fishmeal-fed monocultures of finfish and shrimp, fouls waterways with uneaten feed and fish waste containing nitrogen and phosphorus, resulting in pollution of the sea bottom and eutrophication.

Yarish's colleague Thierry Chopin, Ph.D., professor of marine biology at the University of New Brunswick, is working on IMTA with Cooke Aquaculture (cookeaqua.com) which produces and sells millions of pounds of salmon and trout in New Brunswick, Canada. Blue mussels placed downstream from the

salmon pens consume midsize organic particles, while kelp downstream from the mussels take up inorganic nutrients. Sea urchins and sea cucumbers have been added to the mix to consume larger particles on the ocean floor, and Chopin envisions eventually adding more species to the managed ecosystem according to their ability to consume different sized particles or when they flourish.

IMTA increases aquaculture's sustainability and profitability. Recently, Loblaw, Canada's largest food retailer, began selling Cooke's IMTA salmon in Ontario and Quebec as WiseSource™Salmon. Cooke's mussels are sold for food, and its seaweeds are used in restaurants and cosmetics manufacturing. Cooke is also testing seaweeds as a partial protein substitute in salmon feed, which could potentially generate more income.

IMTA can be done in marine or fresh water, temperate or tropical regions, and in tanks. "It's a theme and has variations…like music," says Chopin. "IMTA is the theme, and the variations are the many regions and species." Chile, Turkey, South Africa, Israel, Norway, Ireland, Scotland and China also practice IMTA, each region selecting its species according to economic potential, habitat and cultural context.



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Tank-grown Gracilaria.

Establishing the true value of IMTA would require calculating the economic value of nutrient removal, savings in feed that would otherwise be wasted, enhanced productivity of a healthier ecosystem, crop diversification and sustainably produced food crops. Chopin won't declare IMTA the silver bullet for aquaculture's environmental problems yet, but its prospects hold promise. E

RENEE CHO *is a freelance environmental writer in New York City and a regular contributor to The Earth Institute's* State of the Planet *blog.*


GRUNDENS


Gracilaria tikvahiae
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