Use of sugar kelp aquaculture in Long Island Sound and the Bronx River Estuary for nutrient extraction

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ABSTRACT: We previously demonstrated the suitability of seaweed aquaculture as a nutrient management tool, using the warm temperate rhodophyte *Gracilaria tikvahiae* McLachlan. The present follow-up study revealed an even higher nutrient bioextraction capacity in the cold-water species *Saccharina latissima* at 3 sites—the mouth of the Bronx River Estuary (Bronx, NY; BRE), western Long Island Sound (Fairfield, CT; WLIS) and central Long Island Sound (Branford, CT; CLIS), during winter and spring of the 2012–2013 growing season. These sites differ in temperature (BRE > CLIS > WIS), salinity (BRE < WLIS = CLIS) and nutrients (BRE >> WLIS = CLIS). We estimated that *S. latissima* could remove up to 180, 67 and 38 kg N ha^{-1} at BRE, WLIS and CLIS respectively, in a hypothetical kelp farm system with 1.5 m spacing between longlines. In the same hypothetical kelp farm system, the estimated carbon sequestration values are 1350 (BRE), 1800 (WLIS) and 1100 (CLIS) kg C ha^{-1}. The potential monetary values of N sequestration by the sugar kelp are up to $1600 (BRE), $760 (WLIS) and $430 (CLIS) ha^{-1}, if incorporated in the State of Connecticut Nitrogen Credit Trading Program and a carbon-pricing scheme. The potential economic values of C sequestration are $30–300 (BRE), $40–400 WLIS), and $24–240 (CLIS) ha^{-1}. These results suggest that seaweed aquaculture is a useful technique for nutrient bioextraction in urbanized coastal waters, such as LIS and BRE. Alternation of the warm- and cold-water species would maximize nutrient bioextraction and augment other ecosystem services, producing economic benefits for the region while helping to manage non-source eutrophication.

KEY WORDS: Nutrient bioextraction · Kelp aquaculture · *Saccharina latissima* · Estuary

INTRODUCTION

Eutrophication represents a severe threat to coastal ecosystems. Impacts include harmful algal blooms (HABs), hypoxia, fish kills and loss of underwater vegetation (Varekamp et al. 2014, Kim et al. 2015). Reduction of nutrient inputs from land-based sources has been the primary management strategy to ameliorate estuarine and coastal eutrophication (Paerl et al. 2014, Tedesco et al. 2014). Point sources of nutrients, which are associated with identifiable discharges, are relatively easy to identify and monitor in comparison to non-point sources, which include at-mospheric deposition and agricultural runoff. For example, recent management efforts, such as the Total Maximum Daily Load (TMDL) concept, have significantly reduced nitrogen (N) input into Long Island Sound (LIS). This reduction derives from the upgrading of wastewater treatment plants. However, a remaining challenge in many watersheds is the control of non-point sources, which are frequently the important sources of nutrients discharged to coastal waters (National Research Council 2000, US EPA 2011, Tedesco et al. 2014). A recent LIS study, for example, reported that atmospheric deposition of N may exceed 30 % of total N load to LIS (Moore et al. 2011).

Nutrient bioextraction using seaweed and shellfish aquaculture has been suggested as an additional tool to remove nutrients from urbanized estuaries (US EPA 2013, Tedesco et al. 2014). While the bioremediatory role of shellfish aquaculture has been extensively studied (Newell 2004, Lindahl 2011, Kellogg et al. 2013), few studies have examined seaweed aquaculture as an approach to nutrient bioextraction. Kim et al. (2014) recently grew a native warm-water species, *Gracilaria tikvahiae*, in the coastal waters of New York and western LIS (WLIS), and reported that nutrient bioextraction using seaweed aquaculture can effectively remove nutrients. The study found that *G. tikvahiae* could remove up to 94 kg N ha^{-1} during a 90 d growing season (July–October). Here, we present a follow-up study, applying a cold water winter season species to the aquaculture framework, thereby extending the nutrient bioextraction practice year round, and increasing total nutrient bioextraction through seaweed aquaculture. N concentration in LIS and New York City (NYC) estuaries is lowest during the summer months and begins to increase from late August to early September, with peaks during the winter months (January–February; Egan & Yarish 1990, Capriulo et al. 2002, http://lisicos.uconn.edu/). These data suggest that remediation strategies may be more effective if nutrients in LIS can be removed during the winter months before the spring phytoplankton bloom that accompanies peak nutrient levels.

Saccharina latissima, which is known as sugar kelp, is a cold temperate brown algal species. While similar kelp species (e.g. *S. japonica*) have been cultivated for many decades in Asian countries, *S. latissima* has only recently been successfully cultivated in the Atlantic Ocean (Buck & Buchholz 2004, Chopin et al. 2012, Wang et al. 2014). The goal of the present study is to evaluate the feasibility of growing the phaeophyte, *S. latissima*, for bioextraction of nutrients under different environmental conditions in urbanized estuaries like LIS and the coastal waters of NYC during its fall to spring growing season.

MATERIALS AND METHODS

Kelp seedstring preparation

Native *Saccharina latissima* seedstrings were produced using the nursery rearing technology developed at the University of Connecticut (Redmond et al. 2014). To develop the seedstrings, spores of wild-harvested specimens were collected from LIS in November 2012 to obtain a wide variety of genotypes. Reproductive sorus tissue was scraped gently and cleansed of epibionts, immersed in a dilute solution of Betadine®, rinsed and then wrapped in damp paper towels. The sorus tissue was stored overnight at 10°C in darkness. The following day, it was re-immersed in autoclaved natural seawater to stimulate release of flagellated meiospores (zoospores). After removing the spent sori, the spore-filled seawater was filtered through cheese-cloth to remove potential contaminants (Brinkhuis et al. 1987). Spore concentration was determined with a hemocytometer under a compound microscope, and adjusted via dilution with autoclaved natural seawater to ~4000 cells ml^{-1}. These zoospores were seeded directly on seedstrings (Korean type string: Guraron 24, 2 mm) wrapped around 38 × 6 cm PVC nursery spools, and placed in a seeding tank containing 10°C sterilized Provosoli's enriched seawater (PES) and 2 ml l^{-1} of germanium dioxide (GeO$_2$). After 24 h in the seeding tanks (dark, 10°C), the spools were then transferred to grow-out tanks containing sterilized PES (half strength) treated with GeO$_2$ and maintained at 10°C. Photosynthetically active radiation (PAR) was adjusted to 20 µmol m^{-2} s^{-1} at a 12 h light:12 h dark photoperiod, and then gradually increased to 100 µmol m^{-2} s^{-1} as sporophytes grew.

Open water cultivation

When plants reached 1 mm in size, *S. latissima* seedstrings were outplanted on two 50 m longline culture units at each of 2 near-shore sites (with ca. 100 m spacing between longlines) in Long Island Sound (WLIS, Fairfield, CT; 41° 06.882′ N, 73° 15.277′ W and central LIS [CLIS], Branford; 41° 12.772′ N, 73° 57.070′ W) and 1 site (with ca. 30 m spacing between longlines) at the mouth of the Bronx River Estuary (BRE; 40° 80.129′ N, 73° 87.122′ W). All longlines were sub-divided into removable 5 m culture units, and monitored for tangling and fouling whenever possible, but no biomass samples were taken until the final harvest. The kelp was cultured at 2 different depths (0.5 and 1.0 m) to determine the depth that maximizes productivity and nutrient bioextraction. To evaluate the productivity of cultured *Saccharina* at each site, the fresh weight biomass of kelp per longline (kg fresh weight [FW] m^{-1}) at final harvest was measured. Each month, water samples (n = 3) were also collected adjacent to the longlines at 1.0 m depth for inorganic nutrient analysis, using a Smart-Chem Discrete Analyzer (Westco Scientific Instruments). At each site, a temperature sensor (HOBO

data logger 64K - UA-002-64) was deployed at 1.0 m depth to monitor water temperature throughout the growing season. Salinity was also measured at the same depth using a refractometer (Fisher Scientific).

Measurements

Tissue carbon (C) and N contents were monitored monthly from February–May (or June at BRE). Thirty randomly selected fronds (3 to 6 fronds per 5 m culture unit) were removed monthly at each depth. The fronds were dried in an oven at 50°C, pooled into 5 dried samples (n = 5), and then ground using a tissue grinder (Model MM200 Grinder, Retsch). The tissue C and N contents were determined using a CHN analyzer (Series II, CHNS/O 2400 Analyzer, Perkin Elmer). Tissue phosphorus (P) content (n = 5) was analyzed at the Center for Environmental Science & Engineering Laboratory of the University of Connecticut, using the fronds at final harvest. The fronds of the sub-divided culture units (5 m) were weighed at harvest (n = 3 to 7) to estimate productivity at the different depths and sites. The amounts of C, N and P removed (mass per unit time) by the seaweeds were used to assess the value of seaweed bioextraction in improving nutrient removal, hence improving water quality. The C, N and P removal rates multiplied by the kelp biomass per meter of culture line yielded the total amounts of C, N and P sequestered by *Saccharina* at harvest (see Kim et al. 2014 for equation). The stable N isotope ratios in monthly collected samples at each site were also analyzed at the University of California Davis Stable Isotope Facility.

Statistical analysis

All analyses were conducted using Sigmaplot (v. 12, StatSoft) statistical and graphing software. Data was checked for homogeneity of variance prior to analysis. In the few cases where data did not meet this assumption, values were ln-transformed and retested. Temperature data was still heteroscedastic after transformation; thus, the data was examined using the Kruskal-Wallis non-parametric analogue of ANOVA. Data sets for which sampling dates were missing from one or more sites (or depths) were examined using only those data for which all treatments were represented. When analysis indicated non-significant difference between mean values, a power test was performed to evaluate the degree of confidence in the conclusion.

RESULTS

Temperatures at the 3 sites varied in a similar fashion (Fig. 1), decreasing until mid-February, then increasing until the end of the study (June). Over the period during which all 3 sites had temperature records (7 February–15 May 2013), temperatures varied significantly among the sites (Kruskal-Wallis $H = 230$, df = 2, p < 0.001). The Bronx site was the warmest, on average, over the experimental period (6.0°C), followed by the central (5.9°C) and the western (5.5°C) LIS sites; all site temperatures significantly differ from each other. Maximum water temperature was greatest at the Bronx site (15.2°C),
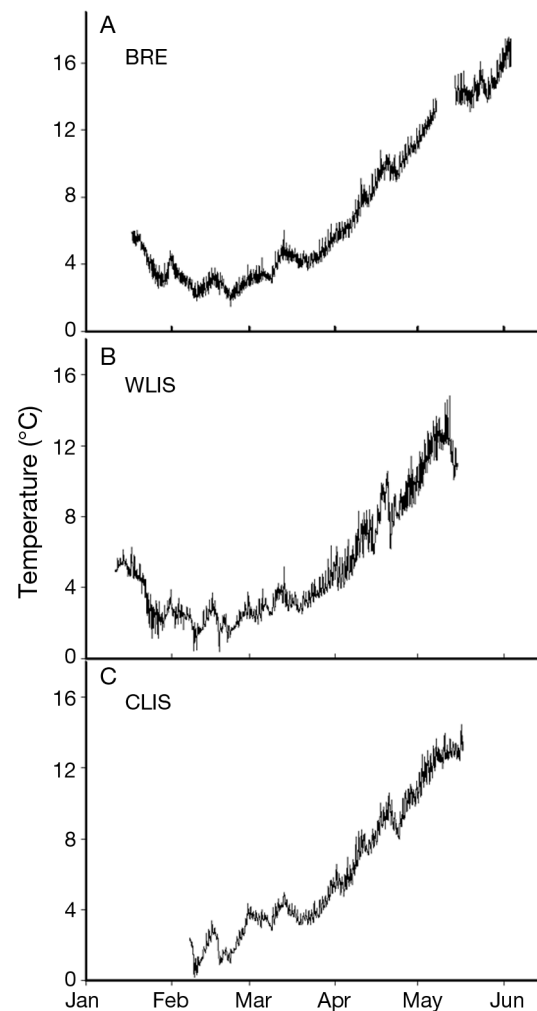

Fig. 1. Daily temperature means for 2013 at the 3 study sites (BRE: Bronx River Estuary; WLIS: Western Long Island Sound; CLIS: Central Long Island Sound) during the study. Temperatures at the 0.5 and 1.0 m depths were averaged, since the 2 depths differed by ~0.3°C for the majority of samples (90–94%)
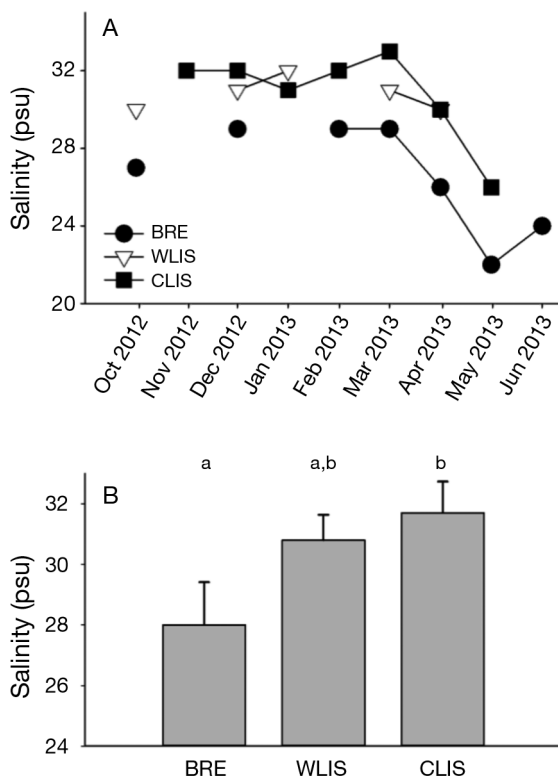
Fig. 2. (A) Mean (n = 3) salinities at the 3 study sites over the duration of the study. (B) Mean (n = 3) salinities from pooled data (Oct 2012–Apr 2013) for the 3 sites. Significant differences (p < 0.05) in mean values indicated by different letters (multiple pairwise comparisons via Dunn's method). Error bars are SD. See Fig. 1 for site abbreviations
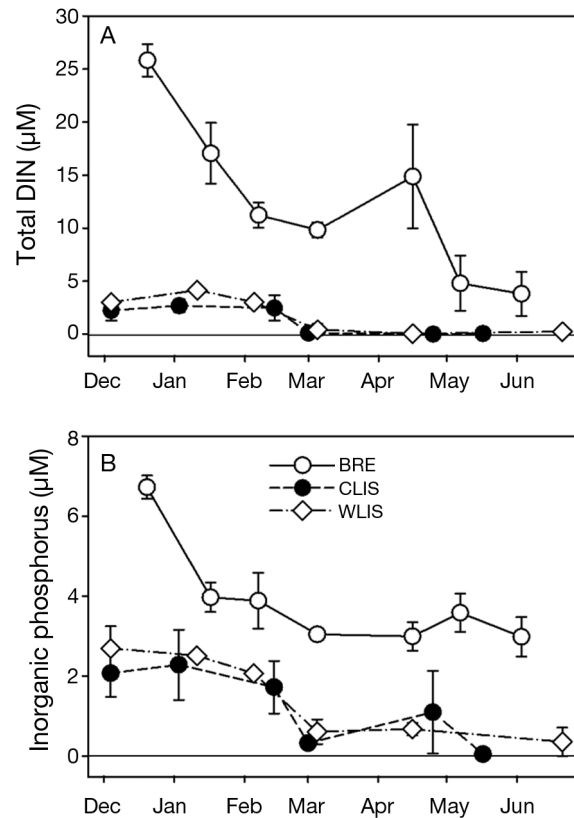

Fig. 3. Mean (n = 3) dissolved (A) inorganic nitrogen (DIN) and (B) phosphorus concentrations over the course of the study (Nov 2012–Jun 2013) for the 3 study sites. Error bars are SD. See Fig. 1 for site abbreviations

followed by the western and central LIS sites (14.9 and 14.0°C respectively).

The salinities at the 3 sites showed similar patterns: roughly constant from October 2012 to March 2013, then declining through May and June (Fig. 2). For analysis, data for each site were pooled across time from October 2012 to April 2013 (sample date did not significantly influence salinity over this period). The salinities at the 3 sites differed significantly (Kruskal-Wallis $H = 11.0$, df = 2, p = 0.004), with CLIS salinity (31.7 psu) being >BRE salinity (28.0 psu), and WLIS salinity (30.8 psu) being similar to salinities at both CLIS and BRE.

Total dissolved inorganic N differed among months ($F_{5,36} = 33.6$, p < 0.001) and sites ($F_{5,36} = 356$, p < 0.001), with the interaction term also being significant ($F_{5,36} = 16.1$, p < 0.001). The latter term indicated the difference in temporal pattern between the BRE site and the western and central LIS sites. Total inorganic P differed among sites ($F_{5,33} = 22.5$ p < 0.001) and months ($F_{2,36} = 113$, p < 0.001), but the interaction

term was non-significant ($F_{8,33} = 1.99$, p = 0.079); the power of the interaction test was relatively low (0.376) (Fig. 3).

Yield (kg FW m^{-1} longline) was first examined using a site × depth ANOVA for the BRE and WLIS sites. This analysis revealed no influence of depth (0.5 vs. 1.0 m) on total plant production. Hence, data from the 2 depths were combined. The ANOVA on depth-pooled data revealed no differences among sites (Fig. 4, $F_{2,20} = 3.00$, p = 0.072), with variation due to site representing only 23% of total variation. Yield at the CLIS site was only 62 and 69% of yields at the WLIS and the BRE sites respectively.

The most comprehensive tissue N data sets came from the BRE and the WLIS sites (CLIS lacked the 0.5 m depth) for February–May (Fig. 5). These data were first examined for site, sample date, and depth effects on tissue N content. Site ($F_{1,42} = 119$, p < 0.001) and date ($F_{2,42} = 31$, p < 0.001) were both highly significant terms, while depth ($F_{1,42} = 0.05$, p = 0.81) had no influence on tissue N content. Site and date also interacted significantly ($F_{2,42} = 26$, p < 0.001), reveal-
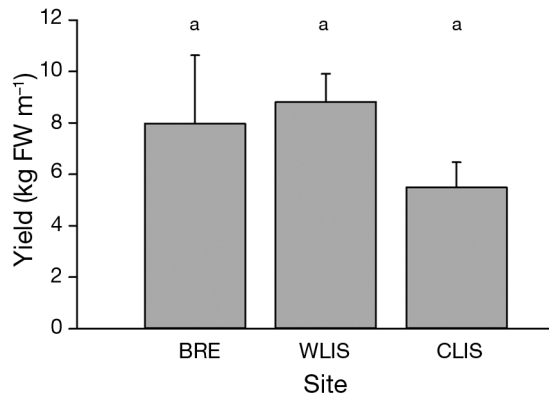
Fig. 4. Mean (±SD) yield (kg FW m⁻¹) at 1.0 m depth at the 3 Long Island Sound study sites over the duration of the study (depth, i.e. 0.5 vs. 1.0 m, did not have a significant effect on yield). Mean values were not statistically different (ANOVA, p = 0.072), and site explained only 23% of the total variation. n = 13, 6 and 4 for BRE, WLIS and CLIS, respectively. FW: fresh weight. See Fig. 1 for site abbreviations

ing the decline in tissue N at the WLIS site, and stasis in tissue N at the BRE site.

Since depth did not influence tissue N content in the above analysis, data for the 2 depths were pooled for each site from February–May. Site and month had significant main effects ($F_{2,69}$ = 58, p < 0.001; $F_{3,69}$ = 176, p < 0.001 respectively), and interaction effects were also significant ($F_{6,69}$ = 44, p < 0.001; Fig. 5). The latter term identified the difference in patterns between the BRE site (constancy of tissue N) and the western and central LIS sites (decline from ~4 to 1% N). The N content of samples collected in May were higher in the BRE samples (2.6% DW) than in the western and central LIS samples (1.0 and 1.2% DW respectively).

In general, tissue C content increased throughout the course of the study (Fig. 5); on average, C increased from 20.6% DW in February to 28.5% DW in May. Site, month and the site × month interaction all significantly influenced tissue C content ($F_{6,847}$ = 6.9, p = 0.002; $F_{3,69}$ = 30, p < 0.001; $F_{6,69}$ = 10.9, p < 0.001). The interaction term was driven by the low C content in April 2013. The tissue C contents in May (last overlapping sample) at the BRE, WLIS and CLIS sites were 26.8, 29.1 and 29.9% DW respectively.

Tissue P was quantified only on harvested tissue (i.e. end point measurements only). Site significantly influenced tissue P content ($F_{2,9}$ = 539, p < 0.001). While the tissue P concentrations at the 3 sites all differed statistically, the BRE site produced tissue with the highest P concentration (i.e. 70% more P [0.99% DW] than tissue from the CLIS site [0.59% DW], and 102% more P than tissue from the WLIS site [0.49% DW]).
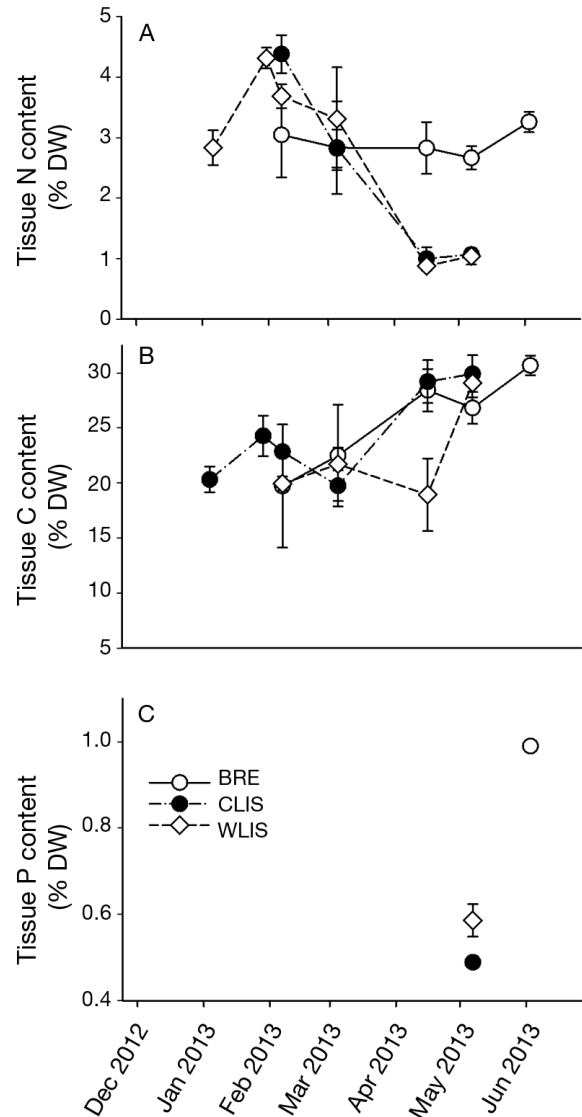

Fig. 5. Mean (±SD) tissue nutrient content (±SD, % DW). (A) Nitrogen and (B) carbon throughout the study, and (C) phosphorus at the end of the study (harvest sample only) for the 3 Long Island Sound sites. n for C, N content averaged 8 for BRE, 8 for WLIS, and 5 for CLIS; n for P content was 4. DW: dry weight. See Fig. 1 for site abbreviations

Integrating average total production (harvested biomass) and average tissue nutrient content at harvest provided overall estimates of nutrient bioextraction per meter of longline (Fig. 6). Depth appeared to influence the removal of N, C and P at the BRE site, where N and C removal at the 0.5 m depth were respectively 104 and 163% greater than the removal at 1.0 m depth (P removal was only estimated at both depths for BRE and WLIS, but only at 1.0 m for CLIS). At the WLIS site, N and C removal were respectively 13 and 11% greater at the 1.0 m than at the 0.5 m depth. The influence of site on nutrient bioextraction
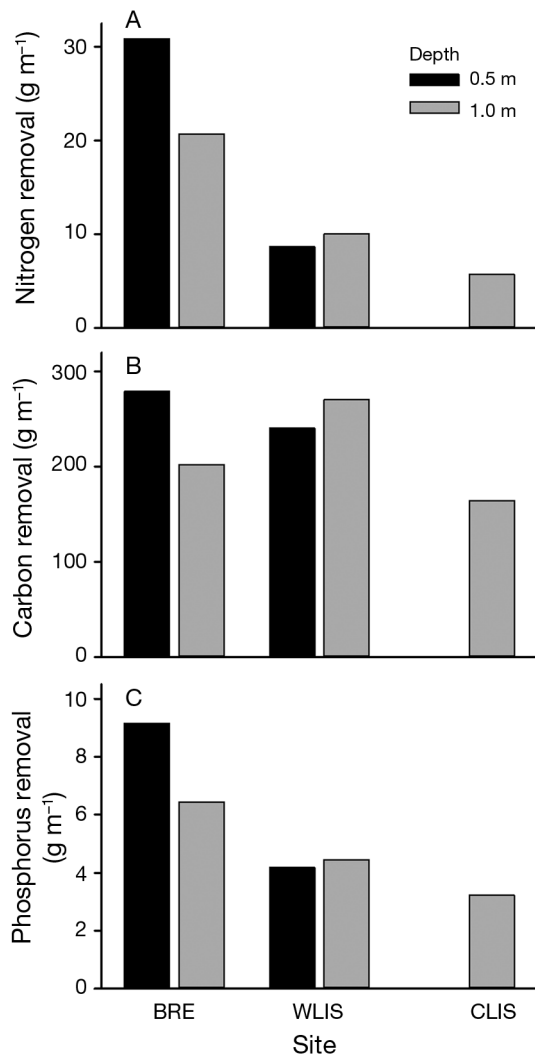
Fig. 6. Removal of (A) nitrogen, (B) carbon and (C) phosphorus by *Saccharina latissima* over the course of the study (i.e. biomass produced × nutrient content at harvest). Data are shown in g per meter of longline for 0.5 and 1 m depth at the BRE and WLIS and at 1 m only for CLIS. See Fig. 1 for site abbreviations

Fig. 7. Mean (±SD; n = 5) δ^{15}N values of tissue samples collected from the 3 Long Island Sound study sites over the course of the study. See Fig. 1 for site abbreviations

was examined using the full data set at 1.0 m depth. With the exception of C removal by WLIS kelp, BRE kelp always removed more nutrients than kelp at the other 2 sites, especially compared to CLIS kelp. This higher performance by kelp at the BRE site ranged from 23% more C removed than that at the CLIS site, to 99 and 262% more P and N removed respectively, compared to the CLIS site.

The δ^{15}N values for *Saccharina latissima* tissue samples also differed (Fig. 7). Statistical analysis of the February–May samples (obtained for all sites) revealed significant effects of site ($F_{2,24} = 577$, p < 0.001) and date ($F_{2,24} = 30$, p < 0.001), with a significant interaction between site and date ($F_{6,24} = 8.1$, p <
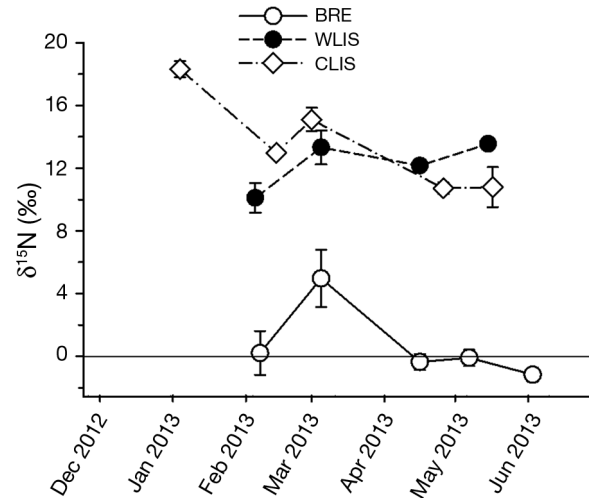
0.001). The interaction term was significant partly because the March δ^{15}N values were elevated compared to those of the other dates. The May samples (last date with values for all sites) for the 3 sites were all significantly different from each other, with tissue averages of −0.094 ± 0.51, 13.56 ± 0.02 and 10.80 ± 1.28‰ for the BRE, WLIS and CLIS respectively.

DISCUSSION

Nutrient bioextraction by seaweed aquaculture

The sugar kelp *Saccharina latissima* grew well under different environmental conditions and extracted nutrients from LIS and the BRE, demonstrating that nutrient bioextraction through seaweed aquaculture can be an effective coastal nutrient management tool in urbanized estuaries. Recently, Kim et al. (2014) estimated N removal expected from 1 ha seaweed farms. They reported that the warm temperate red seaweed *Gracilaria tikvahiae* removed 28 and 94 kg N ha^{−1} from the western LIS and the BRE sites respectively, if it was cultivated with 2 m spacing between longlines. In the present study at the same locations, *S. latissima* is estimated to respectively remove 70 and 180 kg N ha^{−1} at 0.5 m depth (data not shown), and 67 and 140 kg N ha^{−1} at 1.0 m depth, with 1.5 m spacing between longlines (Table 1). Together, seaweed aquaculture would have the potential to remove 98 and 274 kg N ha^{−1} yr^{−1} from the western LIS and the BRE sites respectively, if *Gracilaria* and *Saccharina* culture were

Table 1. Nutrient bioextraction at the 3 Long Island Sound study sites at 1 m depth for hypothetical *Saccharina latissima* farms with longline separation distances of 1.5 and 6 m

Site	1.5 m longline spacing			6.0 m longline spacing		
	N removal (kg ha^{-1})	P removal (kg ha^{-1})	C removal (kg ha^{-1})	N removal (kg ha^{-1})	P removal (kg ha^{-1})	C removal (kg ha^{-1})
Bronx River (BRE)	139	43	1357	35	11	344
Western Long Island Sound (WLIS)	67	30	1813	17	8	460
Central Long Island Sound (CLIS)	38	22	1102	10	5	280

alternated in different seasons. This is a conservative estimate for several reasons. The estimate of Kim et al. (2014) for N removal by *Gracilaria* was based only on a 90 d growing season (July–October), although *Gracilaria* farming can be started when the water temperature is >15°C (as early as May in our region; Capriulo et al. 2002). In addition, the yield of *S. latissima* in the present study (~9.3 kg m^{-2}) was only one half of the yield (18 kg m^{-2}) that we found during our preliminary cultivation in the 2011–2012 growing season in LIS. The yield in the 2012–2013 growing season was also lower than that reported from recent studies in Europe; Peteiro & Freire (2013) reported yields of 12–16 kg m^{-2}, while Sanderson et al. (2012) reported 28 kg m^{-2}. The estimate of N removal from our preliminary cultivation was up to 280 kg N ha^{-1}, with an assumption of 1.5 m spacing between longlines. The yield reduction in the 2012–2013 growing season was due to the January Nor'Easter, which is a severe regional scale winter storm in the northeast of North America that severely damaged the kelp farms. Taken together, these results suggest that N removal through seaweed aquaculture could be greater than the sum of the estimates of Kim et al. (2014) and the present study. The potential N removal from the WLIS site could exceed 320 kg N ha^{-1} yr^{-1} (280 and 40 kg ha^{-1} for *Saccharina* and *Gracilaria* respectively) and 430 kg N ha^{-1} yr^{-1} from the BRE site (280 and 150 kg ha^{-1} for *Saccharina* and *Gracilaria* respectively). This N removal performance by seaweed aquaculture is higher than or comparable to reported N removal capacities of either farmed or restored shellfish beds (77–556 kg N ha^{-1} yr^{-1}; Newell 2004, Newell et al. 2005, Kite-Powell et al. 2006, Higgins et al. 2011, Lindahl 2011, Kellogg et al. 2013).

In integrated multi-trophic aquaculture systems, the surface area requirement to reduce the negative impact of fish aquaculture has been estimated in many studies (Chopin et al. 2012, Sanderson et al. 2012, Kim et al. 2013a). For example, to remove N waste generated by the largest salmon farm in Nor-

way, a surface area of 56 ha would be required (Sanderson et al. 2012). Buschmann et al. (2001, 2009) also estimated that a salmon farm occupying 0.8 ha and producing 1500 metric tons (mt) yr^{-1} would need 50–60 ha of cultivation area, if both *G. chilensis* and *Macrocystis pyrifera* were co-cultured, to reduce the amount of N entering the ecosystem by 80%. In the present study, we also estimated the surface area needed to reduce a significant amount of N in LIS. Considering that the LIS target TMDL in 2014 is 22 million kg N yr^{-1}, at least 1.6–2.2 million kg N yr^{-1} could be removed by seaweed aquaculture if both *Gracilaria* and *Saccharina* were cultivated throughout the year in a 5100 ha area (~1.5% of the area of LIS). For more accurate estimates of long-term farm performance, it is also critical to collect additional productivity data from a broad range of locations over time to evaluate weather risk, as well as spatial and temporal variability in growth conditions.

Co-culture of seaweeds and shellfish may even enhance the nutrient bioextraction capacity of urbanized estuaries. Seaweeds and shellfish are in different trophic levels, requiring different nutrient sources for their growth (inorganic nutrients for seaweeds vs. organically bound nutrients for shellfish). Co-cultivation of seaweeds and shellfish enhanced the growth of both species when high concentrations of nutrients were available (e.g. adjacent to a finfish farm; Ridler et al. 2007, Chopin et al. 2008, Wang et al. 2014). Qian et al. (1996) also reported that *Kappaphycus alvarezii* and pearl oysters grew better when co-cultured than when cultured separately. It is probable that oyster culture promotes better water clarity, thereby increasing photosynthesis and growth of seaweeds (Newell 2004). Inorganic nutrients excreted into the water column by oysters might ordinarily accumulate and suppress oyster growth. However, these excreted nutrients can be bioextracted by seaweeds under co-culture conditions, thus improving the growth of both oysters and seaweeds (Ryther et al. 1975).

Seaweeds are an important CO_2 sink and the duration of net CO_2 removal can be extended if the biomass is used in environmentally friendly ways (Chung et al. 2013). Kim et al. (2014) suggested that large-scale seaweed farms can be a useful tool for CO_2 sequestration. They estimated that *Gracilaria* at the WLIS and BRE sites could remove up to 300 and 727 kg C ha^{-1} respectively, during the 90 d of the summer growing season. We also estimated that the sugar kelp can sequester 1800 (WLIS) and 1350 (BRE) kg C ha^{-1} (Table 1). Together, over 2100 and 2000 kg C ha^{-1} could be removed at WLIS and BRE, respectively, by growing the 2 species in alternation.

Salinity at the BRE site fluctuated depending on the tidal cycle and freshwater input, while salinity at the LIS sites was constant (Galimany et al. 2013, Wikfors 2013). The BRE site experienced higher salinity (~29 psu) during flood tides and received a significant amount of freshwater from the East and the Bronx Rivers during ebb tides, decreasing salinity to 22 psu (Galimany et al. 2013, Wikfors 2013). Lower salinity suppresses the growth of S*accharina* (Spurkland & Iken 2011, Nielsen et al. 2014). However, the tolerance range for one environmental factor may be influenced by other environmental factors. For example, when resource availability is sufficient, the range of tolerance for other resources may broaden (Hurd et al. 2014). The sufficient nutrient availability at the BRE site may increase the range of tolerance for temperature and/or salinity.

Economics of carbon and nitrogen sequestration

The economic values of C and N removal have been estimated by many different groups around the world (Folke et al. 1994, Buschmann et al. 1996, Chopin et al. 2001, Peters-Stanley et al. 2012). In the present study, we estimated the potential economic values of N and C removal via sugar kelp aquaculture, using the most recent market values for these 2 elements in the US ($11.04 kg^{-1} N, $6.00–$60.00 mt^{-1} C [as CO_2]; Stephenson & Shabman 2011, CDP 2013, CT DEEP 2013, Tedesco et al. 2014) and for N and C removal. The potential monetary values of N sequestration by the sugar kelp at the 3 sites are up to $1600 (BRE), $760 (WLIS) and $430 (CLIS) ha^{-1}. The corresponding potential economic values of C sequestration are up to $30–300, $40–400 and $24–240 ha^{-1}. Kim et al. (2014) estimated that the potential economic values of N and C sequestration by *G. tikvahiae* were up to $940 (BRE) and $311 (WLIS) ha^{-1} for N, and $20–200 (BRE) and $7–70 (WLIS) ha^{-1} for

C. If both species were cultivated in alternation year round, and seaweed aquaculture were incorporated into the CT Nitrogen Credit Trading Program and a carbon-pricing scheme (CDP 2013, CT DEEP 2013, www.cga.ct.gov/2015/TOB/H/2015HB-05690-R00-HB.htm), removal of N and C could represent >$3000 ha^{-1} of additional income for seaweed growers beyond the value of seaweed products. The economic values might be larger if the full growing season of *Gracilaria* were considered and storms were not a factor. In addition, the value of seaweed production to growers would likely be greater if biomass were feedstock for other products (e.g. food applications, animal feeds, cosmeceuticals, biofuel; Pereira & Yarish 2008, Corey et al. 2014, Johnson et al. 2014). It is important to note that, in order for seaweed aquaculture to be included in the CT Nitrogen Trading Program, an administrative action is required. The first steps of this requirement have recently been introduced into the CT State Legislature under House Bill 5690 dated January 2015.

Bioextraction by *Saccharina* varied at different locations. Among the 3 study sites, the BRE site showed the greatest nutrient removal by kelp. The inorganic nutrients at the BRE site were sufficient for the growth of *Saccharina* throughout the entire growing season. The dissolved inorganic nutrient concentrations at the western and central LIS sites dropped in March and remained at very low levels (nearly zero) until harvest, while the N and P concentrations at the BRE site were >3.8 and >3.0 µM respectively, even during the month with the lowest values (June 2013), due to the spring bloom (Lopez et al. 2014). The tissue N content (1.1 and 1% at WLIS and CLIS respectively) and C:N ratio (27.4 and 29.3 respectively) clearly indicated N limitation (Kim et al. 2007, Corey et al. 2012) at these sites at harvest, while the values at the BRE site (3.3% tissue N and 9.4 C:N) showed N sufficiency even at harvest. In general, the sugar kelp accumulated N from December to March, when ambient N levels were high. Fast growth from March to May was accompanied by a depletion of these reserves (Chapman & Craigie 1977, Egan & Yarish 1990). Harvesting is recommended before summer (Redmond et al. 2014) because N limitation and heat stress inhibit the growth and can even kill this alga (Lee & Brinkhuis 1986, Egan & Yarish 1990), returning nutrients to the ecosystem. In addition, warm temperature (>15°C) stimulates the growth of fouling organisms on the sugar kelp (Thorne-Miller et al. 1983). Interestingly, when sufficient nutrients were available for the growth of the sugar kelp, the active period was extended. Chap-

man & Craigie (1977) fertilized *Laminaria longicruris* (= *S. latissimi* f. *longicruris*) with sodium nitrate once a week throughout the summer months and found that the tissue N concentration was 20× higher than without N fertilization. The N augmentation elevated growth rates to as high as that of winter. The present study also suggests an extension of the sugar kelp gowing season under high nutrient conditions (i.e. BRE). The sugar kelp at the BRE site showed little tissue deterioration, and growth was still observed in June when water temperature was >16°C.

$\delta^{15}N$ signature

Kim et al. (2014) reported that wastewater treatment plants were the primary N source supporting the growth of *G. tikvahiae* at the WLIS and BRE sites. We also examined N sources for *S. latissima* by analyzing $\delta^{15}N$ in tissues at final harvest from each farm site. Non-impacted $\delta^{15}N$ values for *S. latissima* tissue range between 0 and 2‰ (Wang et al. 2014), although it may be as high as 6‰ (Fredriksen 2003). Treated sewage discharged by wastewater treatment plants typically has elevated $\delta^{15}N$ signature (10–20‰; McClelland et al. 1997) relative to overall marine $\delta^{15}N$ values (4–6‰) (Heaton 1986, Savage 2005, Kim et al. 2013b). Savage (2005) reported that sewage influence on brown macroalgal (e.g. *Fucus vesiculosus*) $\delta^{15}N$ values was most pronounced within 10 km from a wastewater treatment plant. In our study, wastewater treatment plants were close to each sugar kelp cultivation site: ~100 m west of the BRE site, 1 km east of the WLIS site, and 7 km west of the CLIS site. The $\delta^{15}N$ values indicated a clear impact of wastewater treatment plants at both LIS sites. Surprisingly, we found no influence of wastewater treatment plant discharge at the BRE site, even though it is closest to a wastewater treatment plant. More than half of the total discharge from New York City's wastewater treatment plants (ca. 5 million m³ daily) is discharged into the East River, and should influence the $\delta^{15}N$ signature of the sugar kelp. The lower $\delta^{15}N$ values of *Saccharina* grown at the BRE site are paradoxical. Given the short distance to the discharge from a wastewater treatment plant, one would expect tissue $\delta^{15}N$ values that are at least as high as those of the western and central LIS sites.

There are 3 possible explanations for the low $\delta^{15}N$ values in the sugar kelp grown at the BRE site. First, the sugar kelp might have obtained a significant amount of ^{15}N-depleted N from terrestrial runoff through East River and/or the Bronx River during the growing season. Fertilizers are depleted in ^{15}N (−3 to +3‰; McClelland et al. 1997, Kim et al. 2014). Second, atmospheric input of N, which is known to be ^{15}N depleted (−2‰ for atmospheric precipitation; Altabet 2006, Sigman et al. 2009, Moore et al. 2011), might be an important source of N for the sugar kelp. These 2 possibilities, however, cannot explain why Kim et al. (2014) found a clear signature of wastewater treatment plant discharge in *Gracilaria* grown at the same location during the 2012 summer to fall growing season. Alternatively, the release of N from sediments, which may become ^{15}N-depleted via bacterial metabolism in the sediment (Fairbanks 2004, Altabet 2006, Sigman et al. 2009), might have provided sufficient N to skew the $\delta^{15}N$ values of sugar kelp tissue from the large $\delta^{15}N$ values characteristic of wastewater. When water turnover times were rapid despite the primary treated sewage input, Waldron et al. (2001) found no sewage impact on seaweed $\delta^{15}N$ values in the Firth of Clyde, UK. The super storm Sandy in October–November 2012 and winter storms (e.g. January 2013, Nor'Easter) during the growing season at the Bronx site, might have resuspended surface sediments. This could have resulted in the washing away of the heavier N isotope (^{15}N) and subsequent release of ^{14}N-enriched N from the sediment, as well as the flushing of lighter N from terrestrial systems. However, additional studies are needed to evaluate the possible mechanisms driving the reduction in $\delta^{15}N$ values in the kelp tissue.

Advantages of nurtrient bioextraction

The advantages of sugar kelp aquaculture in highly urbanized estuaries such as Long Island Sound and New York estuaries are: (1) rare overlaps between the growing season of the sugar kelp and the period of heavy recreational boat activities; (2) non-overlap between the sugar kelp growing season and shellfish farming season, and (3) minimum maintenance effort for cultivation, hence minimum costs. However, challenges include natural disasters (e.g. Nor'Easters, storms) during the winter season as we experienced during our study period. Therefore, it is important to have an appropriate farm design and to determine appropriate locations considering environmental factors, e.g. waves, currents, nutrient conditions, salinity and sediment types (Goudey et al. 2015).

Although efforts to reduce nutrient input from point sources have been successful, the duration of

eutrophication-driven hypoxia in LIS has not decreased during the past 2 decades, ranging from 34 d in 1996 to 79 d in 2008 to 62 d in 2013 (http://longislandsoundstudy.net/indicator/duration-of-hypoxia/; Lopez et al. 2014). This lack of response to mitigation efforts is likely due to nutrient contributions by non-point sources, including atmospheric deposition and agricultural run-off that are difficult to manage (Tedesco et al. 2014). Nutrient bioextraction has been suggested as an additional nutrient management tool for urbanized estuaries (US EPA 2013, Tedesco et al. 2014). We have demonstrated the suitability of seaweed aquaculture as a nutrient management tool, using the warm temperate *G. tikvahiae* (Kim et al. 2014). This follow-up study revealed an even higher level of nutrient bioextraction capacity in the cold-water species *S. latissima*. Our work suggests that alternation of the warm- and cold-water species would maximize the nutrient bioextraction capacity of seaweed aquaculture throughout the year. As Galimany et al. (2013) and Kim et al. (2014) emphasized, nutrient bioextraction is not meant to replace current land-based management efforts, but to be an additional methodology. Site selection will also be critical in maximizing nutrient bioextraction capacity, since the nutrient removal capacity of seaweeds is site-specific. Additional ecosystem services provided by seaweed aquaculture include phytoremediation (seaweeds concentrating heavy metals from seawater into tissues), and habitat generation for invertebrates and/or juvenile or small vertebrates (Shimshock et al. 1992, Steneck et al. 2002, Chopin et al. 2012). Ecosystem services provided by seaweeds such as these often fall unnoticed by non-researchers, partly because the seaweeds are hidden underwater, and the services themselves are not yet accurately valued by economic markets (Barbier 2013, Costanza et al. 2014). The additional ecosystem benefits of seaweed aquaculture need both further study and dissemination via popular media channels.

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