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Article *in* Aquaculture · June 2014

DOI: 10.1016/j.aquaculture.2014.05.034

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Field scale evaluation of seaweed aquaculture as a nutrient bioextraction strategy in Long Island Sound and the Bronx River Estuary

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ARTICLE INFO

Article history:
Received 31 January 2014
Received in revised form 2 May 2014
Accepted 7 May 2014
Available online 17 June 2014

Keywords:
Nutrient bioextraction
Seaweed aquaculture
Gracilaria
Urbanized estuary
Nitrogen
Carbon

ABSTRACT

Nutrient bioextraction using *Gracilaria tikvahiae* McLachlan was tested at two sites: one off Fairfield, CT (LIS), and the other at the mouth of the Bronx River Estuary (BRE), during the summer and fall of 2011 and 2012. The estimates of nitrogen (N) removal by *Gracilaria* over a 90-day growing season were up to 28 and 94 kg N ha^{-1} at the LIS and BRE sites, respectively. In July 2012, *Gracilaria* grew up to 16.5% day^{-1} at BRE and 4.8% day^{-1} at the LIS site. Tissue N contents at the same periods were 3.7% (BRE) and 1.5% (LIS), respectively. These results demonstrate rapid assimilation of nutrients fueling the growth of new *Gracilaria* tissue at the BRE site, while nutrients appeared to limit growth at the LIS site during the summer months. The estimated C removal by *Gracilaria* at the BRE and LIS sites were up to 300 kg ha^{-1} (LIS) and 727 kg ha^{-1} (BRE), respectively. The potential economic values of N and C sequestration for the period examined in this study were as high as $311 (LIS) and $940 ha^{-1} (BRE) for N, and $5.51 (LIS) and $13.32 ha^{-1} (BRE) for C if seaweed aquaculture would be included in Connecticut's Nitrogen Trading Program. This represents a potential additional economic incentive for seaweed growers, beyond the direct value of seaweed products. The findings in this study showed that seaweed (*Gracilaria*) aquaculture can be a useful technique for nutrient bioextraction in urbanized coastal waters, such as the estuaries of New York City (BRE) and Long Island Sound.

1. Introduction

Anthropogenic flux of nutrients into coastal waters leads to coastal eutrophication, which can result in hypoxia, both conditions that threaten ecosystem health (Davidson et al., 2012; Diaz and Rosenberg, 2008; Ekau et al., 2010; GESAMP, 1990; Howarth et al., 2000; Kemp et al., 2009; NRC, 2000; Varekamp et al., 2014). Attempts to control or reverse coastal eutrophication have centered on the reduction of point and nonpoint sources such as wastewater treatment plants and runoff, respectively (Tedesco et al., 2014). Worldwide, approximately $164 billion is spent on water and wastewater treatments, with $27 billion spent in the U.S. (Sachs, 2008). Although source reduction of pollutants is clearly important, not all pollutants can be captured before return of treated wastewater to the environment. In addition, the current eutrophic status of estuaries, in particular, cannot be immediately reversed by source reduction because of decades to centuries long accumulation of nutrients in benthic sediments.

Wastewater contains elevated concentrations of inorganic nutrients, which could be used to support the growth of economically valuable seaweeds. Simultaneously, the seaweed biomass could be harvested as a source of valuable products. This ecosystem service role of seaweed aquaculture is not a new concept. McVey et al. (2002) pointed out the importance of the balanced ecosystem management and suggested that polyculture (now referred to as Integrated Multi-Trophic Aquaculture, or IMTA) can help restore ecosystem function. The ecosystem service performed by seaweeds, extraction of inorganic nutrients, is now referred to as nutrient bioextraction (Galimany et al., 2013; Rose et al., 2012; Tedesco et al., 2014). IMTA and nutrient bioextraction have recently received much attention by federal/state governments and agencies, ENGOs, and the public (Rose et al., 2010; U.S. EPA, 2013). The use of extractive aquaculture technologies for nutrient harvesting could provide the public with water quality improvement at relatively low cost while providing jobs and the enhancement of natural resources.

Many studies have estimated N removal by shellfish harvested from wild or cultured populations (Higgins et al., 2011; Kellogg et al., 2013; Newell, 2004). Newell et al. (2005) estimated that a wild grown oyster could remove 0.5 g N at harvest (150 g individual wet weight; ~7% tissue N and ~0.3% shell N). More recently, Higgins et al. (2011) estimated that 2-year old cultured oysters in the Chesapeake (76 mm length, ~30 g weight) removed 0.13 g of N (7.85% tissue N and 0.19% shell N) at harvest. However, few studies have estimated N removal via seaweed growth. The red seaweed *Porphyra yezoensis*, grown on a large seaweed farm (300 ha) in China, removed approximately 50 kg per ha of nitrogen

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http://dx.doi.org/10.1016/j.aquaculture.2014.05.034
0044-8486/

annually (He et al., 2008). Bowen and Valiela (2004) examined a potential bio-extraction of N via harvesting naturally abundant macroalgae. If the mean annual biomass of the standing stock of seaweed was harvested from Waquoit Bay, an estimated 15–66 kg N would be removed per hectare each year. Recently, Kim et al. (2013b) estimated approximately 58 kg N per ha per 5-month growing season could be removed by *Gracilaria* farming (alone) in Waquoit Bay.

Nutrients, especially nitrogen (N), have received increasing attention in Long Island Sound (LIS) and New York City (NYC) estuaries. Recent management efforts, such as the total maximum daily load (TMDL) concept, have helped significantly reduce nitrogen input into LIS by upgrading wastewater treatment plants. In Connecticut and New York, the nitrogen reductions, from both states combined falling from 31 to 22 Mkg N $year^{-1}$. This reduced the goal of the TMDL by 58.5% by 2014 (Tedesco et al., 2014). However, hypoxia still occurs during summer months in western LIS (Capriulo et al., 2002; Lopez et al., 2014; Varekamp et al., 2014). This suggests the need to manage other sources of nutrients, including atmospheric deposition, storm water discharge, excess fertilizer flows, etc., as well as taking steps to improve the current eutrophic status.

The fate and uptake of dissolved inorganic nitrogen derived from wastewater treatment plants can be traced using the ratios of stable isotopes, $^{15}N/^{14}N$. While the untreated wastewater carries a ^{15}N-depleted signature (i.e., nitrate- and ammonia-based fertilizers have $\delta^{15}N$ close to zero), treated wastewater typically has an elevated $\delta^{15}N$ signature (>20‰) relative to marine DIN $\delta^{15}N$ (4–6‰) (Heaton, 1986; Owens, 1987; Peterson and Fry, 1987; Savage, 2005). The shift in isotopic signature occurs because natural microbial processes in wastewater treatment strongly discriminate against the heavier isotope, producing ^{15}N-enriched wastewater (i.e., elevated $\delta^{15}N$ signature; Heaton, 1986; Owens, 1987). The increasingly elevated signature can be traced from primary producers, including phytoplankton and seaweeds, up through the food chain (Savage, 2005). The influence of sewage N on the $\delta^{15}N$ signature can be identified as far as 24 km downstream from the treated wastewater outfall, but is more significant within 10 km. For instance, *Fucus vesiculosus* grown in Himmerfjarden embayment, Baltic Sea, assimilated proportionally more wastewater treatment plant-derived N than other macroalgae growing further from the wastewater treatment plants (Savage, 2005).

Gracilaria is a red seaweed with over 120 species (Goff et al., 1994; Guiry, 2014). It has been used for human food, animal feed, and phycocolloids (agar and agarose) products (Pereira and Yarish, 2008). *Gracilaria tikvahiae* Mclachlan is the only *Gracilaria* species native to New England (Schneider et al., 1980; Sears, 1998). It is a warm temperate species, preferring temperatures from 15 to 30 °C (Bird et al., 1979). *Gracilaria* is an ideal candidate for extractive aquaculture (IMTA or nutrient bioextraction) due to its ease of propagation, relatively high growth rates, capability of storing high concentrations of nitrogen in its tissue, wide tolerance to a range of environmental conditions (salinity, temperature and nutrients), and its existing and potential commercial value (Hanisak, 1987).

The goal of this study was to evaluate the performance of the red seaweed *G. tikvahiae* in open water nutrient bioextraction farm systems when cultured at demonstration scales in LIS and the Bronx River Estuary (BRE).

2. Materials and methods

G. tikvahiae McLachlan (strain G-RI-ST_1) was cultivated on two 50-m long lines at two near-shore sites in Long Island Sound (LIS; Fairfield, CT; 41°06.882′ N/73°15.277′ W) and at the mouth of the Bronx River Estuary (BRE; Bronx, NY; 40° 48.047′N/73° 52.164′W). Two 50-m long lines (one at each depth) were deployed at two depths, 0.5 m and 1.0 m in 2011 and 0.25 m and 0.5 m in 2012 summer to fall growing seasons (the 1.0 m depth proved sub-optimal for growth). Twenty-gram bundles of *G. tikvahiae* thalli were inserted into nylon line. Each 50 m long line was sub-divided into 10 5-m units that were randomly placed at the two stocking densities. Two stocking densities (20-g FW bundles at every 20 cm (100 g FW m^{-1}) vs. at every 10 cm (200 g FW m^{-1})) were used to determine the optimal stocking density. To determine the optimum growth interval for the 5-m units, and thereby maximize the nutrient removal capacity of each culture unit, half of the units from each density were harvested at two harvest periods (short (8–19 days) vs. long (21–52 days)). Constant harvest intervals were not possible due to inclement weather.

Subsurface irradiance was measured using a LiCor LI-185A PAR meter (Lincoln, Nebraska, USA). A LI-193 spherical sensor was lowered from the surface to 6 m depth at 0.25 m intervals. Measurements were recorded as the sensor descended and then ascended and were averaged at each depth. Three light profiles were recorded on each date. Aerial irradiance was also measured contemporaneously. Temperature was also measured simultaneously at the same depths using YSI 556 MPS meter. Salinity was measured at 1.0 m depth using a refractometer (Fisher Scientific, Pittsburg, PA). Irradiance data sets were fitted in Excel with an exponential curve to estimate the diffuse attenuation coefficient for each light profile.

At each harvest, *Gracilaria* bundles were weighed after removing superficial water using a salad spinner to yield a consistent wet weight. After removing new tissue, the *Gracilaria* bundles were then reinserted into the long line at the initial weight (20 g). Growth rates were estimated using the equation:

$$\text{Growth Rate}\left(\%\ \text{d}^{-1}\right) = \frac{(\ln(\text{start biomass}) - \ln(\text{end biomass}))}{\text{elapsed time}} \times 100.$$

The new tissue collected from the *Gracilaria* culture units was dried in an oven at 55 °C to a constant weight (ca. 48h) and later ground to a uniform powder (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 contents combined with biomass data, the nitrogen and carbon removal was calculated using the following equation:

$$\text{N (or C) removal} = \frac{\text{g FW produced}}{\text{m} * \text{d}} * \frac{\text{g DW}}{\text{g FW}} * \frac{\text{g N (or C)}}{\text{g DW}}$$

The N stable isotope ratios in samples were also analyzed at the University of California Davis Stable Isotope Facility (Davis, CA; http://stableisotopefacility.ucdavis.edu/). Water samples were also collected at 1.0 m depth at each harvest and analyzed for inorganic nitrogen and phosphorus at NOAA Milford Laboratory for total nitrogen and phosphorus.

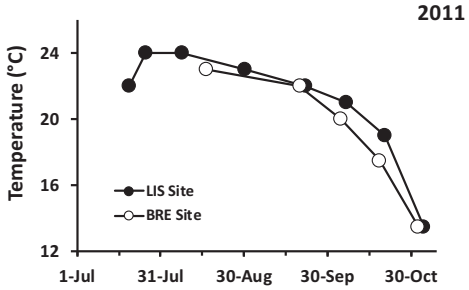

Fig. 1. Temperature over the course of the 2011 study at the LIS and BRE sites. Error bars (SD) are smaller than the symbols.

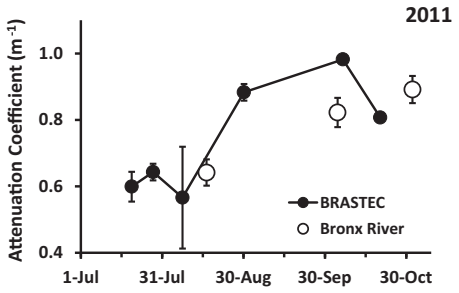

Fig. 2. Diffuse attenuation coefficients during 2011 for the two sites. The data for the first four dates average three replicate light profiles across 6-m depth, while the latter two dates are derived from only one profile. Curve fits using an exponential decay model were highly significant, with R^2 values greater than 0.93, and usually greater than 0.99.

3. Statistical analysis

All analyses were conducted using Statistica (v. 5) software. The open water field sites complicated the analysis. Although the experiments were designed to provide a fully nested data set, storm events (e.g., tropical storm Irene, hurricane Sandy) prevented some sampling trips and also caused tissue loss. In the latter case, when some replicate bundles showed negative growth rates while others along the same section of long line were positive, we took the conservative approach of discarding all data from that long line. Additionally, a reduction in funding during the second year necessitated modifications of the experimental design. In particular, data from the first year (2011) revealed that growth was light-limited at 1.0 m depth, particularly at the BRE site. As a consequence, depths evaluated in 2011 (0.50, 1.00 m) were altered for the 2012 season (0.25, 0.50 m). In addition, stocking density, evaluated in the first year (2011) by comparison of growth at inter-bundle spacing of 10 and 20 cm (200 and 100 g FW m^{-1}, respectively), revealed no difference in growth rate or tissue N or C concentration. Consequently, stocking density was restricted in 2012 to 10 cm spacing (200 g FW m^{-1}) only. Finally, harvest intervals longer than ca. 2.5 weeks resulted in too much new growth; the added drag of the new tissue often caused the breakage of bundles and loss of tissue from the long line, even under benign, non-storm conditions. These cases were evident in the highly variable, sometimes negative growth rates during what should have been optimal periods of growth. As indicated above, when this occurred, we chose the conservative option and omitted all growth rate and tissue production measurements from those dates. As a consequence of these factors, the data set did not include all factors at all times.

We report analyses of data from the first year that enabled us to select the second year treatments (i.e., 0.25, 0.5 m depths, low stocking density). To obtain useful information for broader application to other seaweeds at other sites, we chose to highlight the spatial (i.e., between site) and temporal (between year) variability in the growth rate, tissue N and C concentrations, and N and C bioextraction rates under the optimal conditions defined by our study (0.5 m depth, low (10 g FW m^{-1}) stocking density, short growth period). Doing this, we pool data across the growing season. As a consequence, the error terms are large (e.g., standard deviations were typically 35–45% of mean values for growth rate and tissue N content) and are less meaningful than the outcomes of the statistical analyses and the spatial and temporal patterns. For these reasons, we omit error bars from the figures.

Data were checked for homogeneity of variance prior to analysis. The tissue N, C, and $\delta^{15}N$ data sets all met this assumption. In several cases, growth rate data did not. This may have been due, at least in part, to fragmentation and loss of biomass that was not obvious when samples were collected. In these cases, data were ln-transformed and re-examined. In a few cases, even transformation did not remove the heteroscedasticity. However, in cases where data sets are relatively large and balanced among treatments, ANOVA is robust to violations of the assumption of equal variances (Underwood, 1997).

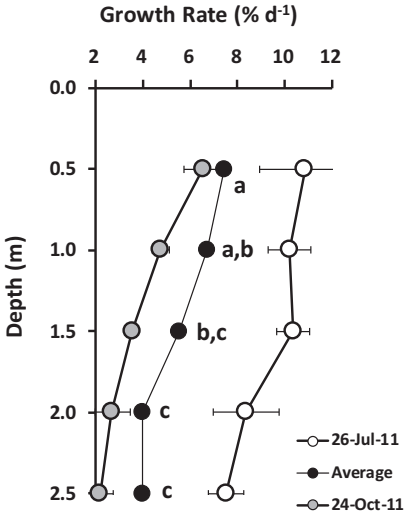

Fig. 3. Growth rate as a function of depth on vertical lines at the LIS site. The average over the six sampling dates is presented along with earliest data set (26-Jul-2011) and the latest data set (24-Oct-2011) to show the maximum and minimum growth rates. Letters indicate significant difference at the 0.05 level. Date (not shown) also had a significant effect, but did not interact with depth.

4. Results

4.1. Physical and chemical environmental data

Temperature changed over the course of the first year of the study (2011) in similar fashion at both sites (Fig. 1). Replicate temperature measurements produced error terms that were too small to appear outside the symbols. The slow decline from mid-July through early October was followed by a faster decrease through the final sample

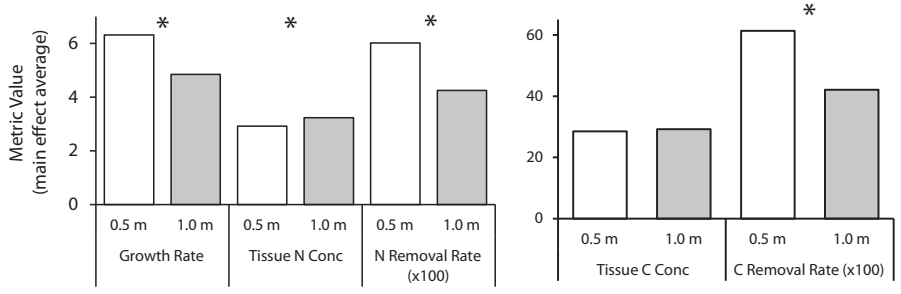

Fig. 4. Results of analysis of LIS (2011) growth rate on horizontal lines of 0.5 and 1.0 m depths. Units: growth rate (% day^{-1}); tissue N, C concentration (g g^{-1} DW); N, C removal rate (mg m^{-1} long line day^{-1}). Asterisks indicate significant difference between depths.

Table 1
Effect of depth on growth rate (% day^{-1}), tissue nitrogen (% dry weight), and nitrogen removal rates (mg m^{-1} long line day^{-1}) during 2011. Long lines placed at 0.5- and 1.0-m depths and tissue production measured during July–Nov (LIS) and Oct–Nov (BRE). Units: growth rate (% day^{-1}), tissue N, C concentration (g g^{-1} DW), N, C removal rate (100 × mg m^{-1} long line day^{-1}). **Boldface** text indicates significant difference between depths.

Site	Metric	Depths		F value	p value
		0.5 m	1.0 m		
LIS	Growth rate	**6.32**	**4.85**	**$F_{1,134} = 12.6$**	**0.00052**
	Tissue N	**2.92**	**3.23**	**$F_{1,153} = 4.57$**	**0.036**
	N removal rate	**6.02**	**4.25**	**$F_{1,153} = 12.6$**	**0.00051**
	Tissue C	28.49	29.20	$F_{1,153} = 3.00$	0.085
	C removal rate	**61.3**	**42.1**	**$F_{1,153} = 13.9$**	**0.00026**
BRE	Growth rate	**6.13**	**3.47**	**$F_{1,44} = 47.9$**	**<0.00001**
	Tissue N	**4.30**	**5.00**	**$F_{1,54} = 15.9$**	**0.0002**
	N removal rate	**8.91**	**4.08**	**$F_{1,53} = 11.6$**	**0.0013**
	Tissue C	**28.07**	**31.20**	**$F_{1,54} = 15.2$**	**0.00027**
	C removal	**58.1**	**24.6**	**$F_{1,95} = 13.8$**	**0.0048**

(4-November). Water clarity also varied across the 2011 season. Diffuse attenuation coefficients at the LIS site were significantly lower during July and early August than late August through late October ($t_{df=3} = 5.40$, $p = 0.0062$; Fig. 2). Data for the BRE site were not sufficient for similar statistical testing, nor for confident comparison with those from the LIS site since visits to the sites did not overlap in time. Salinity at the LIS site during the growing season was ranged from 26 to 30 psu in 2011 and from 30 to 33 psu over the same period in 2012 (data not shown). The salinity at the BRE site was slightly lower, and ranged from 20 to 25 psu in 2011 and 25 to 29 psu in 2012. Nitrogen and phosphorus concentrations in the LIS site were similar in both growing seasons ranged from 2.4 to 3.4 μmol L^{-1} and from 0.9 to 2.5 μmol L^{-1}, respectively, in July (data not shown). The nitrogen and phosphorus concentrations at this site started to increase from late August and were as high as 11.5 and 4.9 μmol L^{-1}, respectively, in LIS by the end of the growing season (November). The nutrient concentrations at the BRE site were higher than those at the LIS site (33–55 μmol L^{-1} of nitrogen and 5–19 μmol L^{-1} of phosphorus, respectively, during the months of August through October in 2011 and 2012).

4.1.1. Effect of depth

During the first year (2011) of the study, the effect of depth on seaweed performance was evaluated in two ways: three vertical lines with bundles at five depths at the LIS site (0.5–2.5 m) and horizontal lines set at two depths at both the LIS and BRE sites (0.5 and 1.0 m). The vertical lines revealed a strong, significant effect of depth on growth (averaged across the entire growth period; Fig. 3); average growth rates of *Gracilaria* declined from 0.5 m through 2.0 m, and then remained constant at 2.5 m. Fig. 3 also shows the vertical growth rate profiles for mid-summer and early fall to provide an indication of the range in growth rate that can be expected across the growing season.

Table 2
Comparison of LIS and BRE sites in 2011 and 2012 (analysis of years performed separately) on growth rate (% day^{-1}), tissue nitrogen and carbon (% dry weight), and nitrogen and carbon removal rates (mg m^{-1} long line day^{-1}). Long lines were placed at 0.5-m depth, with production measured from coincident samples in 2011 (Oct) and 2012 (Aug, Oct). Units: growth rate (% day^{-1}), tissue N, C concentration (g g^{-1} DW), N, C removal rate (100 × mg m^{-1} long line day^{-1}). **Boldface** text indicates significant difference between sites, except for δ^{15}N for which comparison was among the two sites and the nursery-produced tissue.

Year	Metric	Sites		F value	p value
		LIS	BRE		
2011	Growth rate	6.32	6.13	$F_{1,92} = 0.14$	0.71
	Tissue N	**2.83**	**4.46**	**$F_{1,89} = 56.7$**	**<0.00001**
	N removal rate	**6.02**	**8.91**	**$F_{1,106} = 9.10$**	**0.0032**
	Tissue C	28.38	29.04	$F_{1,89} = 0.99$	0.32
	C removal rate	61.3	58.1	$F_{1,106} = 0.18$	0.67
2012	Growth rate	**5.14**	**9.12**	**$F_{1,55} = 21.3$**	**0.00002**
	Tissue N	**3.15**	**4.17**	**$F_{1,55} = 12.8$**	**0.00074**
	N removal rate	**4.11**	**19.06**	**$F_{1,42} = 15.7$**	**0.00029**
	Tissue C	27.50	27.79	$F_{1,55} = 0.12$	0.73
	C removal rate	**41.2**	**195.9**	**$F_{1,42} = 13.4$**	**0.00069**
	δ^{15}N	**9.84**	**10.72**	**$F_{1,81} = 30.0$**	**<0.0001**

Data from the horizontal lines, pooled across the growing season at the LIS site, indicated a significant influence of depth on growth rate and tissue N concentration, and on N and C removal rate (Fig. 4; Table 1); on average over the course of 2011, growth rates 30% higher at 0.5 m than 1.0 m, while N and C removal rates were 42% and 46% higher (even though N concentrations were 10% lower at 0.5 m than at 1.0 m).

At the BRE site, depth significantly influenced all metrics (growth rate, tissue N and C concentration, N and C removal rates; Fig. 5; Table 2); on average over the course of 2011, growth rates averaged 77% higher at 0.5 m than 1.0 m, and N and C removal rates were 118% and 136% higher. Tissue N and C levels were 14% and 10% lower at 0.5 m than at 1.0 m.

4.1.2. Comparisons between LIS and BRE sites

In 2011, the tissue N and N removal rates were the only metrics that differed between sites, with average tissue N concentrations 58% higher at the BRE site than LIS, and N removal rate 48% higher at the Bronx River. Growth rates did not differ statistically between sites during 2011, nor did tissue C levels or C removal rates (Figs. 6 & 7; Table 2). During the following year (2012), the sites differed qualitatively and quantitatively. The two sites were statistically different in growth rate, tissue N concentration, and N and C removal rates (Figs. 6 & 7; Table 2). In all cases, the metric averages were higher at the BRE site than at the LIS site. During 2012, N and C removal rates at the Bronx River site greatly exceeded those of the LIS site (365% and 375% greater, respectively). The 2012 inter-site differences were driven by much higher Bronx River growth rates (+77%) and tissue N concentrations (+32%).

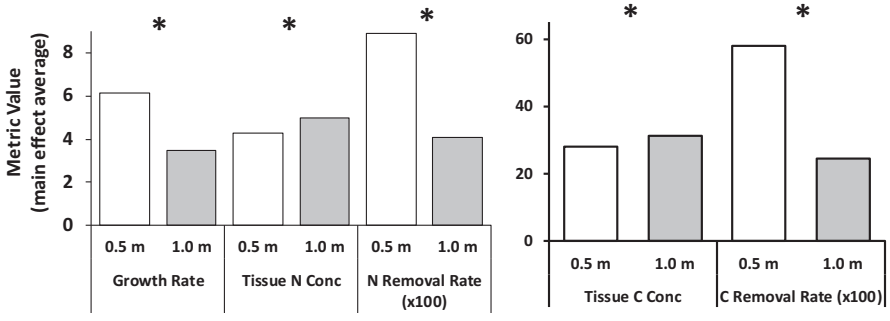

Fig. 5. Results of analysis of BRE site (2011) growth rate on horizontal lines of 0.5- and 1.0-m depths. Units: growth rate (% day^{-1}); tissue N, C concentration (g g^{-1} DW); N, C removal rate (mg m^{-1} long line day^{-1}). Asterisks indicate significant difference between depths.

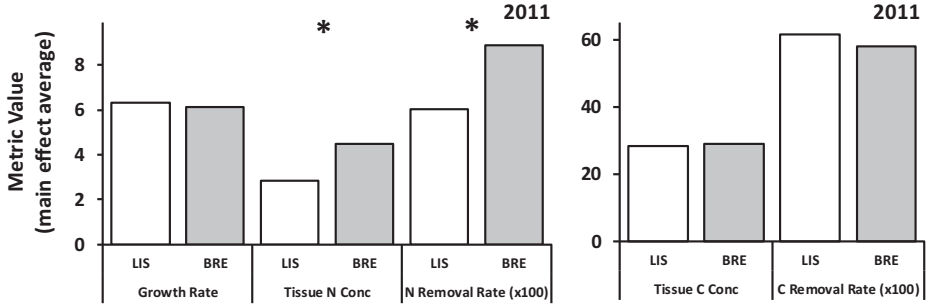

Fig. 6. Intersite comparisons in 2011 at 0.5-m depth. Units: growth rate (% day^{-1}); tissue N, C concentration (g g^{-1} DW); N, C removal rate (mg m^{-1} long line day^{-1}). Asterisks indicate significant difference between sites.

The $\delta^{15}N$ signature of the 2012 tissue samples also differed significantly between sites, and from the initial (pre-outplant) isotopic signature (Table 2; Fig. 8). The BRE site $\delta^{15}N$ values were significantly (25%) larger than those of LIS samples, though the difference was driven by the final date in the analysis. When data from the last sampling date (16-Oct-2012) was omitted, the $\delta^{15}N$ values did not vary significantly between the LIS and BRE sites ($t_{20} = 1.517$, $p = 0.072$). At both sites, $\delta^{15}N$ values increased slightly, though not statistically significantly, over the first three dates. Field-grown samples from both sites were 10–11 parts per thousand heavier than the signature of the nursery samples.

4.1.3. Comparisons between 2011 and 2012

At the LIS site, 2011 and 2012 differed for growth rate, tissue C, and N and C removal rates (Fig. 9, Table 3). In all cases, metric averages were higher in 2011 than in 2012 (+57%, +8%, +66%, +69%, respectively). With the exception of tissue C content, the differences between years at the BRE site were qualitatively similar to those at the LIS site; metric averages for growth rate and N and C removal rates were higher in 2012 than in 2011 (+49%, +63%, +158%, respectively; Fig. 10, Table 3).

5. Discussion

Our study demonstrated that *Gracilaria* aquaculture can be a useful technique for nutrient bioextraction in urbanized coastal waters, such as the estuaries of New York City (BRE) and Long Island Sound. To place this conclusion in better context, we apply the results to a hypothetical one-hectare seaweed farm in Long Island Sound. Assuming 2 m and 4 m spacing between long lines situated at 0. 5-m depth, with a stocking density of 200 g FW m^{-1} and a short (i.e., <21 days) harvest period, aquacultured *Gracilaria* would remove 29–94 kg N ha^{-1} from the BRE site and 13–28 kg N ha^{-1} from the LIS site during a 90-day growing season (July–October). Since these estimates only encompass part of the full May–October growing season, total realized bioextraction would be much greater. The greatest extraction, a function of ambient temperature, light, and N concentration, will likely occur during May–July. With global climatic change, the *Gracilaria* growing season will be extended, resulting in even greater bioextraction by *Gracilaria* (Harley et al., 2012; Ugarte et al., 2010).

The performance of *G. tikvahiae* reported here is comparable that of bivalves, considered a useful tool to offset terrestrial nutrient sources. Nitrogen removal by bivalves, either aquacultured or restored, has been extensively studied (Higgins et al., 2011, 2013; Kellogg et al., 2013; Lindahl, 2011; Newell, 2004; Newell et al., 2005). For examples, mussels (*Ischadium recurvum*) could remove 217 kg N ha^{-1} year^{-1} (estimate in restored mussel reef; Kellogg et al., 2013) in Chesapeake Bay. Oysters (*Crossostrea virginica*) removed 331 kg N ha^{-1} per up to two years (Higgins et al., 2011) when farmed, and 556 kg N ha^{-1} year^{-1} from a restored oyster reef in Chesapeake Bay (Kellogg et al., 2013). Farmed oysters removed an estimated 296 kg N ha^{-1} year^{-1} from Waquoit Bay (MA; Kite-Powell et al., 2006). The Kite-Powell et al. (2006) model, however, might have overestimated the removal because their estimate was based on the wild oyster measurements of Newell et al. (2005). Utilizing the equation provided by Higgins et al. (2011), conservative estimates of N removal by oysters would be 77 kg ha^{-1} year^{-1}.

Recently, a large seaweed farm in an embayment, Hangzhou Bay, China was examined for nutrient bioremediation potential. Huo et al. (2011) cultivated a red alga, *Gracilaria verrucosa* on 63 250-m long lines with 3 m spacing between the long lines and reported a significant reduction of nutrients in the embayment; NH_4^+ and NO_3^- were reduced by 54% and 76%, respectively, and PO_4^- by 49%. Huo et al. (2011) also reported that concentrations of red tide species in the region (e.g. *Skeletonema costatum*, *Prorocentrum micans* and *Prorocentrum donghaiense*) were significantly reduced when *Gracilaria* was farmed. In addition, species diversity and richness increased after *Gracilaria* was cultivated in that embayment. These results suggest that seaweed aquaculture can perform not only nutrient bioextraction, improving water quality, but also provide ancillary ecosystem services in urbanized estuaries.

Aquacultured seaweeds are also a significant CO_2 sink (Chung et al., 2013). Worldwide productivity of harvested aquatic plants in 2011 was

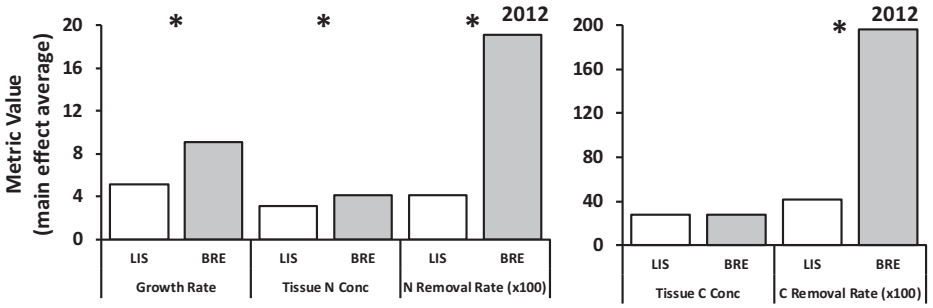

Fig. 7. Intersite comparisons in 2012 at 0.5-m depth. Units: growth rate (% day^{-1}); tissue N, C concentration (g g^{-1} DW); N, C removal rate (mg m^{-1} long line day^{-1}). Asterisks indicate significant difference between sites.

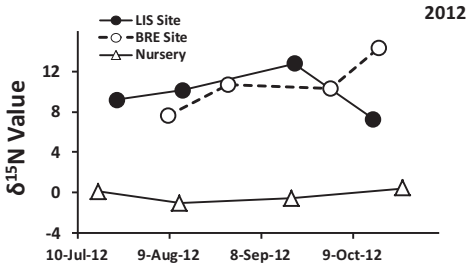

Fig. 8. Nitrogen stable isotope values (as $\delta^{15}N$; ‰) at the LIS and BRE sites, and tissue from the nursery before outplanting.

7.1×10^6 (brown seaweeds), 10.8×10^6 (red seaweeds), 0.02×10^6 (green seaweeds) and 3.0×10^6 tons (miscellaneous aquatic; FAO (Food and Agricultural Organization of the United Nations), 2012), sequestering approximately 1.3×10^6 tons of carbon, with assumptions of 80% tissue water content and 30% tissue carbon content (Abreu et al., 2011; Corey et al., 2013; Kim et al., 2007, 2008, 2013a). Large-scale seaweed cultivation may also be a useful tool for CO_2 absorption and sequestration because of its well-known, low-cost technologies and the harvest could be used for multiple products (Buchholz et al., 2012; FAO (Food and Agricultural Organization of the United Nations), 2012; Pereira and Yarish, 2008).

The estimated C removal by *Gracilaria* at the BRE and LIS sites were up to 727 kg ha^{-1} and 300 kg ha^{-1}, respectively (Table 4). To estimate the potential economic values of N and C removal via *Gracilaria* aquaculture, current market values for the two elements ($11.04 kg^{-1} N; $5.00 mt^{-1} C (as CO_2); CT DEEP, 2013; Stephenson and Shabman, 2011; Tedesco et al., 2014; http://www.arb.ca.gov/cc/capandtrade/capandtrade.htm) are multiplied by N and C removal (Table 4). The potential economic values of N and C sequestration for the period examined in this study range from $147–$311 (LIS site) to $330–$940 ha^{-1} (BRE) for N, and from $2.59–$5.51 (LIS) to $6.38–$13.32 ha^{-1} (BRE) for C. These values would be larger when the full growing season (May–Oct) is considered, and represent source of potential additional income for seaweed growers beyond the value of seaweed products.

The present study highlighted spatial and temporal differences in the metrics measured (growth rate, tissue N and C contents, N and C bioextraction rates), suggesting the importance of site selection to maximize the capacity for nutrient bioextraction by *Gracilaria*, as well as the existence of inter- and intra-annual variability in performance. For example, inter-annual differences in performance were apparent in both sites, but the pattern was not the same. Performance in 2011 exceeded to that in 2012 at the LIS site, while the opposite pattern (2012 > 2011) was observed at the BRE site. In addition, within 2012 at the BRE site, July growth rates were up to 16.5% day^{-1}, decreasing to 4.9% day^{-1} in October as water temperature and day length decreased, to below 0% day^{-1} as the physical environment deteriorated further. During the same time period, the growth rate at the LIS site was ranged from 2.5 to 5.6% day^{-1}, without a clear temporal effect until the early

Table 3
Comparison of years 2011 and 2012 (analysis of sites performed separately) on growth rate (% day^{-1}), tissue nitrogen and carbon (% dry weight), and nitrogen and carbon removal rates (mg m^{-1} long line day^{-1}). Long lines placed at 0.5-m depth, with production measured from coincident samples in 2011 (Oct) and 2012 (Aug, Oct). **Boldface** text indicates significant difference between sites.

Site	Metric	Year		F value	p value
		2011	2012		
LIS	Growth rate	**6.32**	**4.06**	$\mathbf{F_{1,83} = 12.4}$	**0.00072**
	Tissue N	2.83	2.60	$F_{1,82} = 0.75$	0.40
	N removal rate	**6.82**	**4.11**	$\mathbf{F_{1,82} = 10.7}$	**0.0016**
	Tissue C	**28.38**	**26.15**	$\mathbf{F_{1,82} = 10.3}$	**0.0019**
	C removal rate	**69.6**	**41.2**	$\mathbf{F_{1,82} = 14.2}$	**0.0003**
BRE	Growth rate	**6.13**	**9.12**	$\mathbf{F_{1,54} = 12.1}$	**0.00099**
	Tissue N	4.45	4.17	$F_{1,54} = 1.17$	0.28
	N removal rate	**11.7**	**19.1**	$\mathbf{F_{1,49} = 5.41}$	**0.024**
	Tissue C	29.04	27.79	$F_{1,52} = 1.83$	0.18
	C removal rate	**76.0**	**195.9**	$\mathbf{F_{1,49} = 11.8}$	**0.0012**

November decline. This spatial variation in the growth may derive from different inorganic nutrient regimes at the two sites. The inorganic nutrient concentrations at the BRE site remained high, >33 μmol L^{-1} of nitrogen and >5 μmol L^{-1} of phosphorus throughout the growing season, while the nutrients appeared to limit growth rates at the LIS site during summer months (<3.4 μmol L^{-1} of nitrogen and <2.5 μmol L^{-1} of phosphorus) for the growth of *Gracilaria*. The C:N ratio in *Gracilaria* also supported this. In the BRE site, the C:N ratio was low throughout the growing season, ranged from 5.3 to 8.6, suggesting N enriched environment (Corey et al., 2012, 2013, 2014; Kim et al., 2007, 2013a). However, the C:N ratio at the LIS site was over 16 in July and August suggesting nitrogen limitation, and then the ratio dropped down to 7.0 in October (Figs. 6 & 7). At the LIS site, it is likely that the phytoplankton respond more rapidly than do seaweeds to temperature and day length increases, resulting in limited nutrients for *Gracilaria*. As temperature and day length begin to decrease, phytoplankton abundance also decreases, with a concomitant increase in water column nutrients (Capriulo et al., 2002; Egan and Yarish, 1990; Lopez et al., 2014). However, the decreasing temperature and day length also reduce growth rates of *Gracilaria* (Bird et al., 1979; Lapointe et al., 1984).

However, this interpretation does not fully explain the growth pattern in the BRE site. The highest growth rate found in the present study (16.5% day^{-1} in July) at the BRE site is, in fact, the highest growth rate ever reported in *G. tikvahiae* and also far exceeds or is comparable to that of other aquacultured *Gracilaria* species including *Gracilaria chilensis*, *Gracilaria vermiculophylla* and *G. verrucosa* (i.e. 4–16% day^{-1}: Abreu et al., 2009; Buschmann et al., 2001; Huo et al., 2011; Troell et al., 1997). This exceptional growth rate at the BRE site is likely stimulated by the bottom-up mechanism of elevated levels of inorganic nutrients in water column throughout the summer months. The BRE site is located at the confluence of the East River and the Bronx River in New York City. At least six wastewater treatment plants, including the three largest plants in New York City, discharge over 4 billion L day^{-1}

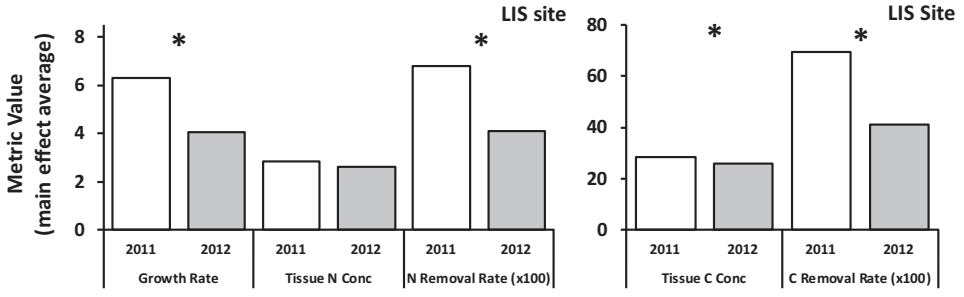

Fig. 9. Inter-year comparisons at the LIS site (0.5-m depth). Units: growth rate (% day^{-1}); tissue N, C concentration (g g^{-1} DW); N, C removal rate (mg m^{-1} long line day^{-1}). Asterisks indicate significant difference between years.

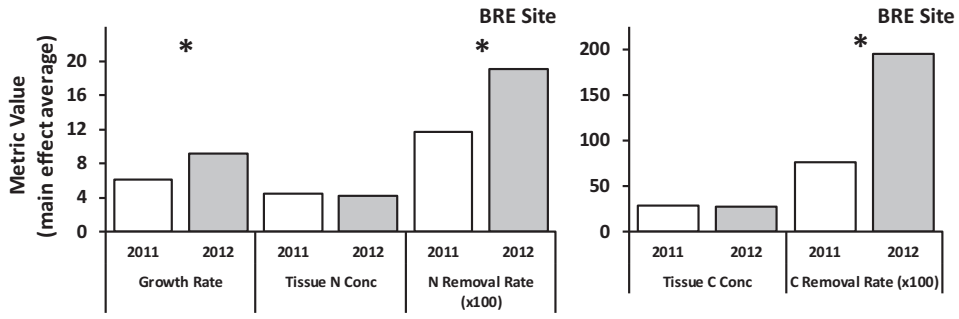

Fig. 10. Inter-year comparisons at the BRE site (0.5-m depth). Units: growth rate (% day^{-1}); tissue N, C concentration (g g^{-1} DW); N, C removal rate (mg m^{-1} long line day^{-1}). Asterisks indicate significant difference between years.

of treated sewage to the East River (NYC Department of Environmental Protection), elevating the nutrient concentration at the BRE site. In addition, the contribution of nutrients from non-points sources through the East River and the Bronx River watersheds should also contribute to elevated concentrations of nutrients at that site (Varekamp et al., 2014). *Gracilaria* may also benefit at the Bronx River estuary site from reduced competition with phytoplankton because the high silt load from benthic sediments reduces phytoplankton production (Galimany et al., 2013).

We determined the primary source of nitrogen that *Gracilaria* absorbed from each farm site by analyzing δ^{15}N in tissues. At the BRE site, a large wastewater treatment plant is located <100 m west of the long lines. At the LIS site, a wastewater treatment outfall is located <1 km east from the farm site. Therefore, impacts from the wastewater treatment plants were expected at both farm sites. *Gracilaria* grown in the nursery tanks showed very low values of δ^{15}N (−1.1 to −0.5‰), not surprising since the N for the nursery system was supplied by a commercial ammonia and nitrate-containing fertilizer. However, in 2012, the δ^{15}N values in *Gracilaria* grown at both farm sites were higher than the δ^{15}N values in the marine dissolved inorganic N (4–6‰; Owens, 1987; Peterson and Fry, 1987). The δ^{15}N values were 7.6–13.3‰ at the BRE site and 7.3–12.8‰ at the LIS site, suggesting the impact of the wastewater treatment plants at each site.

Attempts to improve water quality in urbanized estuaries have primarily focused on the management of land-based sources of nutrients, such as wastewater treatments, fertilizer applications, storm water run-off, etc. Nutrient bioextraction using seaweed aquaculture represents an additional approach, removing nutrients by enhancing nutrient processing in coastal systems (Tedesco et al., 2014). The findings in this demonstrate-scale study have showed that seaweed (*Gracilaria*) aquaculture could be included as part of a suite of management tools to minimize nutrient impacts in urbanized coastal waters. Nutrient bioextraction by seaweeds and bivalves could effectively aid in the restoration of ecosystem services and a cost effective, affordable and equitable solution (Kellogg et al., 2013; Tedesco et al., 2014). However, it is important to emphasize that nutrient bioextraction cannot be expected to replace current land based management efforts but, rather, acts as an additional methodology. To increase the applicability of this approach, additional native seaweed species need testing for nutrient bioextraction performance. In particular, the inclusion of cold water winter season species into the aquaculture framework would extend the practice year round, and may also provide different downstream products (e.g., biofuels, fertilizer, alginates if from kelps). In this latter context, appropriate applications of harvested seaweed (human food, hydrocolloid, animal feed, fertilizer, cosmetics, biofuel, etc.) should be determined based on the sites and temperature-dependent species.

Acknowledgments

We wish to thank the students and volunteers in the Seaweed Marine Biotechnology Laboratory of the University of Connecticut and Purchase College who assisted in the field and laboratories. We also thank the staff, faculty and students of Bridgeport Regional Aquaculture Science and Technology Education Center (especially John Curtis) and Rocking the Boat (especially Adam Green) for their assistance in the open water farm sites and laboratories. We thank Ms. Eileen Wands and Kevin Keane, HDR/HydroQual and Dr. Carter Newell, Maine Shellfish R&D, for their assistance with permitting and farm installation at the BRE site. We also thank Dr. Shannon Meseck (NOAA Fisheries Northeast Fisheries Science Center Milford Laboratory) for the water analysis, and Dr. Paul Mankiewicz (Gaia Institute, NY, NY), Drs. Gary Wikfors and Julie Rose (NOAA Fisheries Northeast Fisheries Science Center Milford Laboratory) for the project planning and invaluable discussions on project design. Much appreciation also goes to Lynn Dwyer (Northeast, Eastern Partnership Office, National Fish and Wildlife Foundation, Patchogue, NY), Joseph E. Haas (Environmental Protection Bureau, New York Attorney General, NY), Mark Tedesco and Joe Salata (Long

Table 4
Predictions of nitrogen and carbon removal and their economic values within a one-hectare *Gracilaria tikvahiae* farm at the two sites in Long Island Sound and the Bronx River Estuary. Model uses optimum conditions (0.5-m depth), 10 cm spacing of seaweed bundles (100 g FW m^{-1} long line), short harvest period (<21 days), a standard 90-day culture period, and current rates for N and C sequestration ($11.04 kg^{-1} N, $5.00 mt^{-1} CO_2, respectively).

Site	Year	Average N removal rate		N removal kg N [90-day season]$^{-1}$ ha^{-1}	Economic value
		kg N day^{-1} m^{-1}	g N day^{-1} ha^{-1}		
LIS	2011	0.0627	314	28.2	$311
	2012	0.0295	148	13.3	$147
BRE	2011	0.0664	332	29.0	$330
	2012	0.1890	995	93.5	$939
		Average C removal rate		C removal kg C [90-day season]$^{-1}$ ha^{-1}	Economic value
		g C day^{-1} m^{-1}	g C day^{-1} ha^{-1}		
LIS	2011	0.668	3340	300	$5.51
	2012	0.314	1570	141	$2.59
BRE	2011	0.773	3870	349	$6.38
	2012	1.61	8070	727	$13.32

Island Sound Study, U.S. EPA, Stamford, CT) for the guidance and invaluable assistance throughout the duration of the project. This study was supported by grants to C. Yarish, J.K. Kim and G.P. Kraemer from the U.S. EPA Long Island Sound Study's Long Island Sound Futures Fund, New York State Attorney General's Bronx River Watershed Initiative Grant Program, National Fish and Wildlife Foundation (NFWF/Legacy Grant Project IDs: 1401.10.024266 and 8012.08.030370) and a grant to C. Yarish from Connecticut Sea Grant College Program (R/A-38).

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