

Reductions in distribution, photosynthesis, and productivity of eelgrass *Zostera marina* associated with oyster *Crassostrea virginica* aquaculture

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ABSTRACT: This study provides the first quantitative description of the influence of suspended bag oyster aquaculture (SBOA) of *Crassostrea virginica* on the distribution, morphology, physiology, and productivity of eelgrass *Zostera marina*. We explored possible mechanisms related to decreased light availability, nutrient stress, organic loading, and physical damage from bags or boats. Surveys at multiple spatial scales across a 125 km range of Atlantic Canadian bays and estuaries demonstrated consistent declines in eelgrass biomass by as much as 79% at commercial culture leases versus reference areas. Reductions in biomass were negatively related to both oyster stocking density and the age of culture leases. We further observed reductions in the capacity of eelgrass photochemistry at culture sites, accompanied by primary production declines of 37.9%. Our data suggest that these reductions in *Z. marina* distribution, productivity, and photosynthetic rates are primarily occurring as a result of light limitation caused by shading from aquaculture equipment.

KEY WORDS: *Zostera marina* · *Crassostrea virginica* · Eelgrass · Oyster · Aquaculture · Photosynthesis · Pulse-amplitude modulated fluorometry · Atlantic Canada

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INTRODUCTION

Bivalve culture in Atlantic Canada is dominated by suspension culture in subtidal regions, in contrast to the dominance of bottom culture (stock grown directly on or in the substrate, or in bags or trays) and off-bottom culture (stock placed on tables, trestles, stakes, longlines) of bivalves in the intertidal zone along the west coast of North America (Dumbauld et al. 2009). Suspended bag culture of eastern oysters *Crassostrea virginica* is the dominant method of oyster aquaculture in New Brunswick. New Brunswick culturists of eastern oysters increased production 6-fold between 2000 and 2007 to estimated yields of 2 to 4 t ha⁻¹ yr⁻¹ (Bastien-Daigle et al. 2007) and a total annual produc-

tion of ca. 881 t (Fisheries and Oceans Canada 2010). Considerable expansion is expected in the near future (Mallet et al. 2009). Using values from Dumbauld et al. (2009) converted to common units, current New Brunswick oyster aquaculture yields are comparable to those of Baynes Sound, British Columbia, Canada (5.48 t ha⁻¹ yr⁻¹; Jamieson et al. 2001) while being substantially lower than areas such as Thau Lagoon, France (8.00 t ha⁻¹ yr⁻¹; De Casabianca et al. 1997) and Toten Inlet, Puget Sound, Washington, USA (13.36 t ha⁻¹ yr⁻¹; Dumbauld et al. 2009).

At present, eastern oysters are almost exclusively cultured in the immediate nearshore regions of southern Gulf of St. Lawrence (sGSL) bays and estuaries at water depths of less than 5 m. The concentra-

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tion of these aquaculture activities directly overlaps with the distribution of eelgrass *Zostera marina*. Recent observations of suspended bag oyster aquaculture (SBOA) leases in New Brunswick suggest negative effects of culture on *Z. marina*. Aerial photographs of leases throughout the region show what appear to be substantial areas of substrate almost completely denuded of *Z. marina* (Fisheries and Oceans Canada unpubl. data).

A number of mechanisms may explain declines in eelgrass at culture leases. These include decreased light availability, nutrient stress, organic loading, or physical damage from bags or boats. Rumrill & Poulton (2004) demonstrated that 35% reductions in light levels below *Crassostrea gigas* culture lines were partially associated with decreased eelgrass cover and density. Likewise, a number of studies have shown that farmed bivalves may lead to organic enrichment of the sea bottom (Grant et al. 2005), and increased nutrient release and respiration (Hatcher et al. 1994, Thom et al. 1994, Barranguet 1997, Giles & Pilditch 2006) in both the benthic and pelagic environments due to degradation of biodeposits and bivalve excretion. Finally, a number of studies have suggested that physical damage to seagrasses may result from husbandry activities such as placement of aquaculture structures (Everett et al. 1995), or harvesting by hand (Cabaço et al. 2005) or mechanical means (Peterson et al. 1987).

As no known published studies exist on the interactions between SBOA and eelgrass, this study provides the first quantitative description of the influence of this expanding culture technique on the distribution, morphology, physiology, and productivity of *Zostera marina*. The goal of this study was to quantify the magnitude, footprint, and generality of the hypothesized *Z. marina* decline in SBOA areas while providing insight into possible mechanisms related to decreased light availability, nutrient stress, organic loading, physical damage from bags or boats, or some combination of these factors. We predicted that eelgrass at culture leases throughout the sGSL would exhibit reduced density, biomass, photosynthetic capacity, growth rates, and areal productivity relative to eelgrass in reference sites beyond the influence of oyster culture. We also expected eelgrass at culture leases to demonstrate enhanced photochemical efficiency in response to light limitation by having wider and longer leaves (Ochieng et al. 2010). Ultimately, we predicted these changes in distribution, morphology, and physiology would result in net losses of primary productivity in areas of suspended oyster aquaculture.

MATERIALS AND METHODS

Oyster culture in Atlantic Canada

The culturing techniques employed for eastern oysters have seen recent innovations that allow for faster growth and improved market appeal. Previously, oyster culture on leases in New Brunswick involved bottom seeding (i.e. spreading oysters directly on the substrate) or off-bottom techniques in which oysters, held in plastic mesh bags, were placed on trestles in the intertidal and shallow subtidal zone. These practices have since shifted to the suspension of oysters in mesh bags from lines (Fig. 1) at, or just below, the water's surface in the shallow subtidal zone (~0.3 to 5.0 m depth, chart datum) from approximately April to mid-October. For the remainder of the year, these lines of oysters are sunk to just above or directly on the substrate for overwintering to pro-

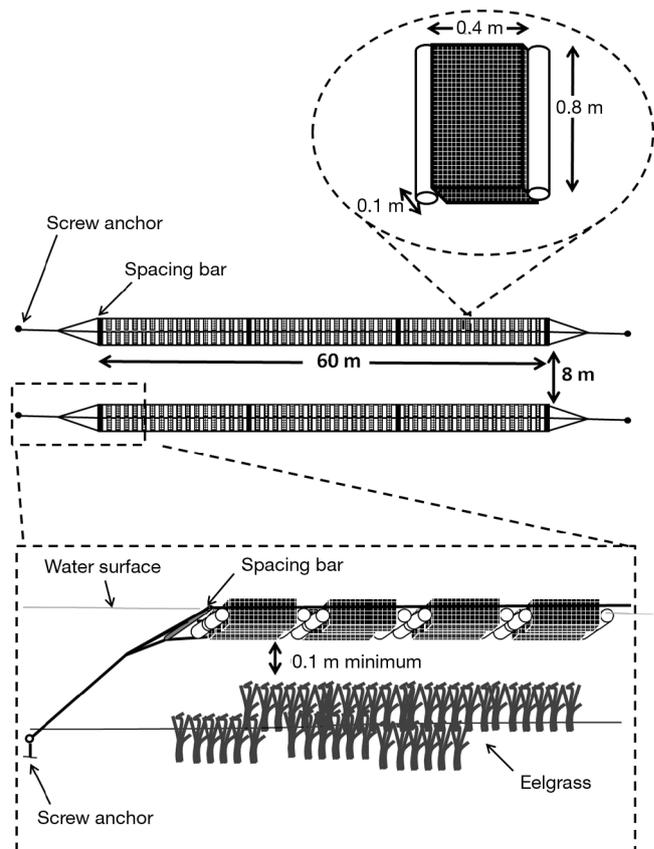


Fig. 1. Typical arrangement, spacing, and anchoring of oyster bags and lines on a New Brunswick suspended oyster aquaculture lease. Elliptical inset shows dimensions of an individual oyster bag with attached floats. Rectangular inset shows a 3-dimensional side view of oyster bags, lines, and anchoring points relative to eelgrass during lowest low tides

tect stock from ice and severe weather. Bags are either moved to the deepest section of the lease (DE), moved off-site to a separate overwintering location within the same lease (OS), or moved entirely to an alternate lease (AL) (Table 1) to ensure sufficient water depths (Transport Canada 2007). For a detailed description of Atlantic Canadian oyster culture practices, refer to Bastien-Daigle et al. (2007).

Study sites

We limited study sites to water bodies of similar depth, area, bottom type, water temperature, salinity, and level of anthropogenic influence along 125 km of northeastern New Brunswick coastline (Fig. 2). Four separate surveys were conducted to estimate the influence of SBOA on eelgrass across spatial scales: (1) a focused gradient survey to assess the potential ‘footprint’ effects on eelgrass distribution at a small number of culture leases; (2) a synoptic multi-bay survey comparing culture leases and reference sites across many bays to assess regional trends in eelgrass distribution, condition, and productivity; (3) a detailed survey comparing culture leases and reference sites in a single bay with a high density of culture leases to assess bay-scale trends in eelgrass distribution and condition; and (4) a detailed fluorometry survey comparing culture leases and reference sites in a single bay with a high density of culture leases to assess bay-scale trends in eelgrass photochemistry (Table 1).

Lease characteristics such as stocking density, distance from shore, and number of years in operation were also kept as similar as possible to minimize variance among sites (Table 1). We also aimed to avoid study design confounding related to overwintering practices. When sampling culture leases where oyster bags were overwintered on-site (DE; Table 1), rather than at a secondary location (OS or AL; Table 1), efforts were made to restrict sampling to areas within these leases where oysters were not placed on the bottom. Oyster stocking densities were estimated as a function of the number of lines of bags present on each lease assuming industry standard oyster biomass per bag, ratios of oyster year classes in production stock, and number of bags per line, as per Doiron (2008).

Table 1. Characteristics of leases sampled during the various surveys in this study. CD = chart datum; DE = deep edge; OS = off-site; AL = alternate location; GS = gradient survey (2006); MB = multi-bay survey (2007); BSS = Bay St. Simon survey (2007); FL = fluorometry survey (2009)

Bay	Lease ID	Coordinates	Lease characteristics				Year of suspended culture initiation	Overwintering	Survey sampling			
			Water depth (m, CD)	Surface area cultured (ha)	Estimated stocking density (kg m ⁻²)	Distance from shore			GS	MB	BSS	FL
Bay St. Simon north	AM2	47° 44.904' N, 64° 48.840' W	0.6	2.83	0.42	380	2002	DE	X	X	X	
	MG	47° 45.056' N, 64° 50.164' W	0.3	2.63	0.29	250	2003	DE	X	X	X	
Bay St. Simon south	JP	47° 43.706' N, 64° 46.892' W	0.6	1.38	0.70	180	2003	OS			X	
	ZN	47° 43.844' N, 64° 47.129' W	0.6	4.05	0.60	170	2003	OS	X	X	X	
	HS	47° 44.352' N, 64° 46.489' W	0.6	2.00	1.05	170	2001	AL	X	X	X	
	HN	47° 44.614' N, 64° 46.515' W	0.6	2.77	0.97	140	2001	AL	X	X	X	
	PVA	47° 43.254' N, 64° 45.649' W	0.3	2.99	0.88	340	2002	AL	X	X	X	
	PVS	47° 42.728' N, 64° 46.596' W	0.6	7.11	0.67	500	2002	AL	X	X	X	
Tabusintac	AM1	47° 43.251' N, 64° 46.041' W	0.6	5.53	0.68	400	2001	DE	X	X	X	
	THI	47° 20.914' N, 64° 56.774' W	0.3	5.15	0.44	50	2002	OS	X	X	X	
	DOI	47° 20.903' N, 64° 57.263' W	0.3	0.63	1.19	200	2002	OS	X	X	X	
Neguac	BA	47° 15.187' N, 65° 03.746' W	0.3	2.32	1.39	280	1999	AL	X	X	X	
	SI	47° 13.912' N, 65° 04.896' W	0.3	2.63	1.22	310	1999	AL	X	X	X	
Richibuctou	YR	46° 42.544' N, 64° 52.159' W	0.3	1.26	0.68	140	2000	OS	X	X	X	
	SG	46° 43.106' N, 64° 53.581' W	0.4	5.17	0.50	150	2002	OS	X	X	X	

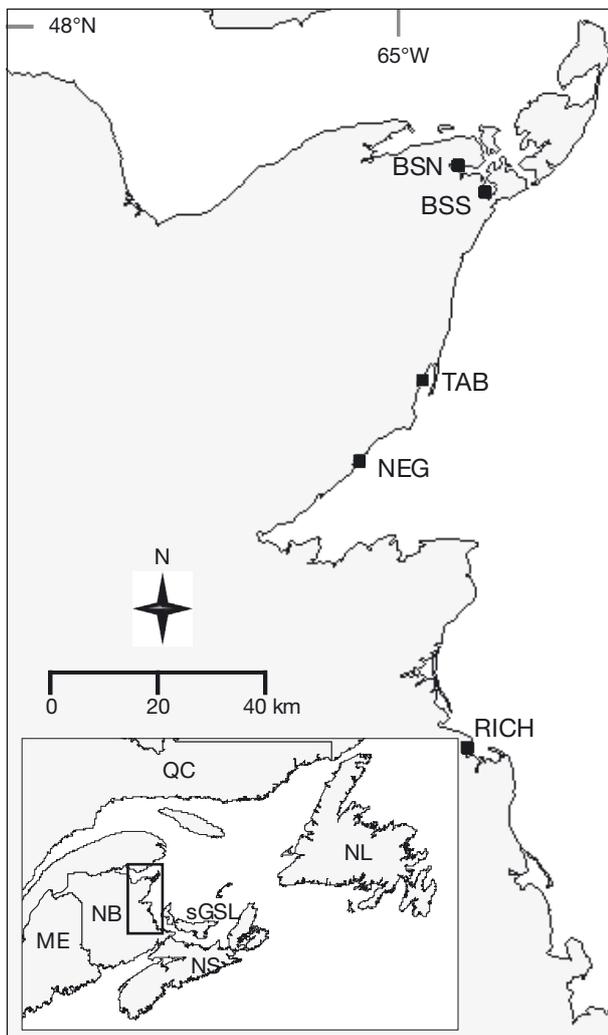


Fig. 2. Map of study area with sample bays indicated by black squares. Inset shows map of Atlantic Canada with study area outlined with black rectangle. sGSL = southern Gulf of St. Lawrence; ME = Maine, USA; NB = New Brunswick; NS = Nova Scotia; NL = Newfoundland; QC = Quebec; BSS = Bay St. Simon south; BSN = Bay St. Simon north; TAB = Tabusintac Bay; NEG = Neguac Bay (NEG); RICH = Richibucto Bay

Gradient survey (2006)

To establish the footprint of potential eelgrass effects at culture leases we assessed the eelgrass structure response variables (shoot density, above-ground biomass, and canopy height) at one SBOA lease in each of Bay St. Simon south (BSS), Tabusintac Bay (TAB), and Richibucto Bay (RICH) during August 2006 (Fig. 2). We followed a gradient-type design (Green 2005) with randomized 0.25 m² quadrat samples (n = 6) taken from each of 5 distances: directly under oyster lines within leases

(UND, 0 m), between oyster lines within leases (BW, 0 m), and at 'reference' distances of 25, 100, and 500 m away from culture lease boundaries. We collected samples from sites of similar depth to minimize variance due to the influence of light attenuation on photosynthesis (Kirkman 1996). A 2-way analysis of variance (ANOVA) assessed the effects of the orthogonal fixed factors Bay (3 levels: BSS, TAB, RICH) and Distance (5 levels: UND, BW, 25 m, 100 m, 500 m), and the interaction term Bay × Distance (see Table 2).

SCUBA divers cut all eelgrass shoots within these quadrats just below the meristem and returned samples to the lab in dark, moist containers. We washed all shoots in fresh water to remove sediment and dead tissue, and gently scraped away epiphytes and sessile fauna with a razor blade. We counted all shoots and measured canopy height (± 1 cm). We then dried the plant tissue and epiphytes separately for a minimum of 8 h at 60°C and transferred each to a dessicator until weighed to determine eelgrass above-ground biomass and epiphyte biomass, respectively (Duarte & Kirkman 2001).

Multi-bay productivity survey (2007)

In addition to eelgrass structure response variables (shoot density, above-ground biomass, and canopy height) we assessed leaf growth rates in quadrats to infer whether eelgrass was potentially limited by light availability at culture leases. In July 2007, we surveyed paired lease SBOA and reference sites (n = 2 'plots') in BSS, Bay St. Simon north (BSN), TAB, Neguac Bay (NEG), and RICH. Reference sites for each lease were located at least 300 m from the lease boundary, parallel to shore at the same water depth.

At each site, SCUBA divers marked 10 shoots (>30 cm total height) *in situ* by making a hole with a hypodermic needle in the leaf sheath ~4 cm above the meristem (Gaeckle & Short 2002). After a period of 21 to 22 d, SCUBA divers harvested the marked shoots and returned the samples to the lab in dark, moist containers. We determined leaf growth rates according to the plastochrone method (Short & Duarte 2001, Gaeckle & Short 2002). We washed all shoots in fresh water to remove sediment and dead tissue and gently scraped away epiphytes and sessile fauna with a razor blade. Marked shoots were identified and separated at the meristem into individual leaves and the number of new unmarked leaves recorded. The leaf plastochrone interval (P_1) was cal-

culated by dividing the growth period by the number of new unmarked leaves. The youngest mature leaf (generally the 3rd leaf) was then dried for 24 h at 60°C and weighed. Leaf growth rate ($\text{g dry weight shoot}^{-1} \text{d}^{-1}$) was then calculated by dividing the dry weight of the youngest mature leaf by P_L (Short & Duarte 2001, Gaeckle & Short 2002). Shoot density, above-ground biomass, and epiphyte biomass were determined using the methods described above. Finding locations within SBOA leases with more than 10 shoots per quadrat for growth marking proved difficult for the majority of leases sampled. While every effort was made to place quadrats within eelgrass patches that best represented the overall eelgrass condition for each culture lease, it is important to highlight that the lease-reference comparisons for shoot density and above-ground biomass data are biased. A 3-way mixed-model ANOVA assessed the effects of the factors Bay (5 levels: BSS, BSN, TAB, NEG, RICH; fixed), Lease (2 levels: Lease, 300 m; fixed), Plot (2 levels: 1, 2; nested within Bay; random), and the Bay \times Lease interaction term (see Table 3). As single quadrats were sampled for each site to facilitate increased replication of leases and bays, no test of the Lease \times Plot(Bay) interaction was possible or desired. The Lease \times Plot(Bay) interaction term was then used as the mean square estimate denominator in the calculation of F -ratios for the factors Lease and Plot(Bay).

Bay St. Simon detailed survey (2007)

We examined eelgrass structure, morphology, and leaf tissue nutrient concentrations to assess potential impacts of nutrient availability on eelgrass in August 2007. We chose to focus sampling in BSS as this area has the highest number of oyster culture leases of any bay in the region as well the most homogenous environment in terms of lease characteristics (Table 1). Based on these reasons and the fact we could sample many sites, we assumed this bay provided the highest probability for detecting aquaculture-related nutrient responses in eelgrass, if any were to exist.

SCUBA divers attempted to harvest all shoots within a random 0.25 m^2 quadrat randomly positioned within paired lease and reference sites ($n = 9$). At sites where eelgrass was present in samples ($n = 6$), we measured plant morphometrics as well as carbon and nitrogen content of eelgrass leaves. After morphometric processing, we oven-dried all shoots in glass scintillation vials at 50°C for 48 h and ground the dried tissue to a fine powder using a mortar and

pestle. The Stable Isotopes in Nature Laboratory (SINLAB; University of New Brunswick, Fredericton, NB) analyzed subsamples of 1.10 mg with a continuous-flow isotope-ratio mass spectrometer (Finnigan Mat Delta Plus, Thermofinnigan) equipped with a ThermoQuest elemental analyzer (Carlo Erba NC2500). Runs of an elemental standard, acetanilide ($n = 8$), resulted in a mean (\pm SD) percent carbon value of $71.56 \pm 1.78\%$ and a mean percent nitrogen value of $10.41 \pm 0.26\%$. The precision of the mass spectrometer over time was evaluated using repeated analyses of random samples ($n = 2$) resulting in a mean SD of 0.21 for percent carbon (range = 0.15 to 0.27) and a mean SD of 0.00 for percent nitrogen.

At each sample site, SCUBA divers also collected a sediment core (5 cm diameter \times 10 cm deep) immediately adjacent to eelgrass quadrats to evaluate organic matter content, water content, and silt and clay composition as explanatory variables. Sediments were immediately transported to the laboratory and samples frozen at -20°C until being processed. We placed an approximately 100 g subsample in a pre-weighed aluminum dish, dried it for 48 h at 60°C and re-weighed it to obtain estimates of water content. We then ashed subsamples at 500°C for 3 h and re-weighed them to determine organic content. To determine percent silt and clay composition, we sorted sediments in a mechanical sieve shaker for 10 min, weighed the samples, and compared $>63 \mu\text{m}$ (sand) and $<63 \mu\text{m}$ (silt and clay) fractions. Paired one-tailed t -tests were used to assess differences between lease and 300 m reference areas.

Fluorometry survey (2009)

In late September 2009, we conducted a survey of the *in situ* photosynthetic status of eelgrass exposed to SBOA. Using a pulse-amplitude modulated (PAM) fluorometer (DIVING-PAM, Heinz Walz), we sampled a selection of the same paired lease and reference sites ($n = 5$) in BSS as described above.

At each culture lease and 300 m reference site we determined maximum quantum yield (F_v/F_m , where F_v is the variable fluorescence and F_m is the maximum fluorescence yield) and maximum potential efficiency of Photosystem II (PSII) photochemistry measured after dark-adaptation (Maxwell & Johnson 2000) for eelgrass shoots ($n = 5$) at random points along a 10 m transect that was established parallel to shore (0.6 m depth, chart datum). At each distance, SCUBA divers cleaned epiphytes from the youngest

mature leaf of eelgrass shoots and placed this leaf in a leaf clip (DIVING-LC, Heinz Walz) 5 cm above the leaf sheath. They then dark-adapted these leaves for a minimum of 15 min before exposure to a 0.5–1.0 s burst of saturating light ($>2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) (Beer et al. 2001).

We also measured effective quantum yield, ϕ_{PSII} (Genty et al. 1989), an instantaneous measure of the efficiency of PSII photochemistry under light conditions. SCUBA divers took measurements from the epiphyte-free youngest mature leaf 5 cm above the leaf sheath on eelgrass shoots ($n = 11$) on a cloudless day with calm waters between 10:00 and 14:00 h to avoid potential confounding associated with leaf age, light refraction due to wave activity, variation in sun angle, and diurnal cycling of photosynthesis (Durako & Kunzelman 2002). Leaf tissues were held flat and perpendicular to the tip of the instrument's measuring optical fiber at a fixed distance of 5 mm using a sample holder (DIVING-USH, Heinz Walz), which also permitted simultaneous measurement of incident photosynthetic photon flux density (PPFD) immediately adjacent to the section of leaf surface measured for ϕ_{PSII} . We also calculated electron transport rate (ETR)—an instantaneous approximation of the rate of electrons pumped between Photosystem I (PSI) and PSII—for each shoot ($n = 11$) using the PAM fluorometer as $\text{ETR} = \phi_{\text{PSII}} \times \text{Incident PPFD} \times 0.44 \times 0.5$, where 0.44 is the absorption factor for *Zostera marina* leaf tissue (Beer et al. 1998) and 0.5 is the factor accounting for the assumed equality of photon absorption between PSI and PSII (Beer et al. 1998, Maxwell & Johnson 2000). Paired one-tailed *t*-tests were used to assess differences between lease and 300 m reference areas.

Statistical analyses

For univariate comparisons, we tested for differences ($\alpha = 0.05$) using ANOVA, linear regression, and paired *t*-tests. We assumed all data were independent and determined that normality assumptions were satisfied by visual inspection of residual plots. We assessed homogeneity of variances for using Cochran's test (Zar 1999) and response variables were power transformed using the Box-Cox method to meet this assumption where required (Draper & Smith 1998). When significant differences were detected using ANOVA, we conducted post-hoc analyses using Tukey Honestly Significant Difference tests ($\alpha = 0.05$) (Zar 1999). We performed all univariate statistical analyses using SYSTAT® (ver. 10.2, SPSS).

All multivariate statistical analyses of eelgrass variables and lease characteristics were based on Euclidean distance matrices of normalized data (Clarke & Warwick 2001). To test the effects of overwintering practices on eelgrass variable data collected during the 2007 multi-bay productivity and BSS detail surveys, we used permutational multivariate analysis of variance (PERMANOVA; $p < 0.05$) by conducting 4999 random permutations for each dataset (Anderson et al. 2008). We determined the variance in eelgrass variables explained by lease characteristic predictor variables using distance-based multivariate multiple linear regression (DISTLM; Anderson et al. 2008). Stepwise regressions (R^2 ; $p < 0.05$) were performed using 9999 permutations for each dataset (Anderson et al. 2008). Stepwise DISTLM begins with a null model and first determines the single explanatory variable explaining the highest proportion of variation in the assemblage dataset. The test then proceeds to sequentially add and/or remove variables to improve the selection criterion, thus increasing the R^2 value (Anderson et al. 2008). The test is completed when no improvement in selection criterion is possible (i.e. the maximum R^2 value is achieved). We performed all multivariate statistical analyses using PRIMER with PERMANOVA+ (ver. 6.1.11, PRIMER-E).

RESULTS

Gradient survey (2006)

Above-ground biomass differed significantly among distances and bays sampled with no significant interaction (Table 2; Fig. 3a). A monotonic decrease was observed from the 500 m to the UND and BW samples, with the latter groups not differing from each other and having 79% lower biomass than that measured at 500 m (Fig. 3a). The lowest above-ground biomass values were observed at RICH while BSS had the greatest, with values 7.1 times greater than those at RICH.

Similar trends in shoot density and canopy height were observed among distances regardless of the bay sampled (Table 2). Shoot density was reduced at UND and BW locations although this trend was not statistically significant for 100 m or 500 m locations (Fig. 3b). In general, shoot density was lowest in RICH and greatest in BSS. Canopy height was significantly lower at UND and BW versus all other distances, with BW shoots being 59% shorter than those at 500 m (Fig. 3c).

Table 2. Results of 2-way ANOVA testing effects of bay and position within and distance from suspended bag oyster aquaculture lease (Distance) on above-ground biomass, shoot density, and canopy height of eelgrass sampled in August 2006. Bold values indicate statistical significance at $\alpha = 0.05$

Source	df	MS	F	p
Biomass				
Bay	2	3.222	9.659	<0.001
Distance	4	2.868	8.598	<0.001
Bay × Distance	8	0.577	1.731	0.105
Error	75	0.334		
Shoot density				
Bay	2	893200	22.42	<0.001
Distance	4	210400	5.281	0.001
Bay × Distance	8	75710	1.901	0.072
Error	75	39830		
Canopy height				
Bay	2	1.233	0.298	0.743
Distance	4	33.42	8.068	<0.001
Bay × Distance	8	4.816	1.163	0.333
Error	75	4.142		

Multi-bay productivity survey (2007)

Above-ground biomass was significantly different between leases and reference sites with leases reduced 53 % relative to reference areas 300 m away (Table 3; Fig. 4a). Shoot density did not differ significantly between leases and reference sites or among bays sampled; however effects due to each factor were tending towards statistical significance (Table 3; Fig. 4b). Responses were variable from bay to bay, but 3 of 5 bays showed lower mean shoot densities at leases and, overall, shoot density was reduced 37 % within leases. No significant differences in epiphyte biomass per shoot were noted between leases and reference sites or among bays (Table 3; Fig. 4c).

No significant lease or bay effects were observed for leaf growth rates (Table 3; Fig. 4d). Leaf growth rates were 22 % lower on leases overall. However, our sampling design had only moderate power (0.62) to detect a minimum effect size of 30 % between leases and reference sites. Neither shoot density ($r^2 = 0.07$, $p = 0.248$, $n = 10$) nor above-ground biomass ($r^2 = 0.04$, $p = 0.378$, $n = 10$) were significant predictors of leaf growth rate.

The percentage of eelgrass shoots in reproductive condition varied as a function of the interaction between Lease and Bay (Table 3; Fig 4e). Analysis of simple effects shows that reference locations at NEG had a significantly greater percentage of reproduc-

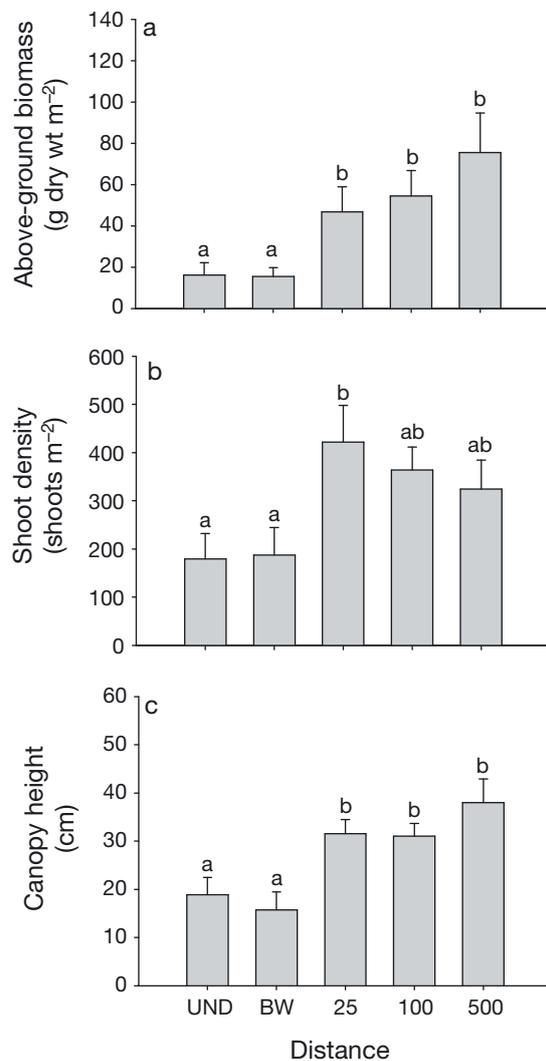


Fig. 3. Mean (+ SE) (a) above-ground biomass, (b) shoot density, and (c) canopy height of eelgrass sampled along a distance gradient extending from suspended bag oyster aquaculture leases in August 2006 (values averaged across all 3 bays, $n = 18$ quadrats per distance). Letters denote statistical significance, $\alpha = 0.05$. UND = within each lease directly under oyster lines; BW = between oyster lines; 25, 100, and 500 = subsequent distances of 25, 100, and 500 m away from the edge of the lease

tive shoots compared with lease locations (paired- $t_{0.05,1} = -14.059$, $p = 0.023$). Reproductive shoots were not present in samples from BSN or RICH (Fig. 4e).

Areal leaf productivity was significantly lower at lease sites (Table 3; Fig. 4f). Leaf productivity was 37.9 % greater at reference areas than leases within bays, with values ranging from 1.2 to 79.6 % greater productivity at BSN and NEG reference sites, respectively. The reference sites in the remaining 3 bays (BSS, RICH, and TAB) showed an average of 36.1 %

Table 3. Results of 2-way ANOVA testing effects of bay and lease presence on above-ground biomass, shoot density, percent reproductive shoots, epiphyte biomass, leaf growth rate, and leaf productivity of eelgrass sampled in July 2007 (5 bays; $n = 2$ per lease-reference area combination). **Bold** values indicate statistical significance at $\alpha = 0.05$

Source	df	MS	F	p
Above-ground biomass (g dry wt m ⁻²)				
Lease	1	29.38	10.99	0.021
Bay	4	2.291	0.59	0.686
Lease × Bay	4	8.769	3.282	0.112
Plot(Bay)	5	3.885	1.454	0.346
Lease × Plot(Bay)	5	2.671	–	–
Shoot density (shoots m ⁻²)				
Lease	1	107200	5.982	0.058
Bay	4	96710	4.648	0.061
Lease × Bay	4	50440	2.816	0.143
Plot(Bay)	5	20810	1.161	0.437
Lease × Plot(Bay)	5	17910	–	–
Percent reproductive shoots (%)				
Lease	1	1.87	35.66	0.002
Bay	4	5.121	0.979	0.494
Lease × Bay	4	0.509	9.71	0.014
Plot(Bay)	5	5.233	99.821	<0.001
Lease × Plot(Bay)	5	0.052	–	–
Epiphyte biomass (g dry wt m ⁻²)				
Lease	1	11.85	4.573	0.085
Bay	4	30.47	2.078	0.221
Lease × Bay	4	2.273	0.877	0.538
Plot(Bay)	5	14.66	5.659	0.04
Lease × Plot(Bay)	5	2.591	–	–
Leaf growth rate (mg shoot ⁻¹ d ⁻¹)				
Lease	1	0.143	3.734	0.111
Bay	4	0.351	2.252	0.198
Lease × Bay	4	0.146	3.811	0.087
Plot(Bay)	5	0.156	4.075	0.075
Lease × Plot(Bay)	5	0.038	–	–
Leaf productivity (mg m ⁻² d ⁻¹)				
Lease	1	0.291	9.714	0.026
Bay	4	0.05	5.839	0.04
Lease × Bay	4	0.062	2.08	0.221
Plot(Bay)	5	0.009	0.286	0.902
Lease × Plot(Bay)	5	0.03	–	–

greater productivity. In comparing average values across lease and reference sites for each bay, leaf productivity varied significantly among bays (Table 3; Fig. 4f) with mean values ranging from 497.3 to 1327 mg m⁻² d⁻¹ at BSS and NEG, respectively.

DISTLM demonstrated that both oyster stocking density (DISTLM pseudo- $F = 4.424$, $p = 0.024$, $df = 8$) and lease age (DISTLM pseudo- $F = 3.7091$, $p = 0.040$, $df = 8$) significantly influenced eelgrass metrics. When assessed individually, no significant influences of water depth, surface area of culture, or distance from shore were observed. Collective assessment of all potential lease characteristic predictor variables

using stepwise DISTLM indicated only oyster stocking density significantly explained variation in eelgrass metrics (stepwise DISTLM pseudo- $F = 4.424$, $p = 0.024$, $df = 8$, $R^2 = 0.346$) with no improvement when lease age (stepwise DISTLM pseudo- $F = 0.240$, $p = 0.783$, $df = 4$) or any other lease characteristics were included in sequential testing. No significant effect of overwintering practice was found among leases (PERMANOVA pseudo- $F_{2,7} = 1.867$, $p = 0.146$).

Univariate linear regressions demonstrated above-ground biomass was inversely related to estimated oyster stocking density ($r^2 = 0.654$, $p = 0.005$, $n = 10$; Fig. 5a) and lease age ($r^2 = 0.728$, $p = 0.002$, $n = 10$; Fig. 5b). Neither estimated oyster stocking density nor lease age were significant predictors of shoot density or areal leaf productivity. Interestingly, a significant positive relationship was found between lease age and estimated oyster stocking density ($r^2 = 0.542$, $p = 0.015$, $n = 10$).

Bay St. Simon detailed survey (2007)

An increased sampling effort at leases and reference areas within BSS provided trends comparable to results from the 2006 and 2007 multi-bay growth surveys with above-ground biomass and shoot density being significantly reduced at culture leases by 38.7 and 57.7%, respectively (Table 4). Additionally, as expected, leaf width and leaf surface area per shoot were significantly greater at leases versus reference areas (Table 4). Epiphyte biomass per unit of leaf surface area did not differ significantly between leases and reference sites, despite culture leases having 25% greater leaf surface area, and leaf tissue nitrogen content did not differ between leases and reference sites (Table 4). None of the other distribution, sedimentary, or nutrient response variables differed between leases and reference sites (Table 4). No significant relationships were found between lease characteristics and eelgrass metrics using individual or sequential DISTLM. No significant effect of overwintering practice was found among leases (PERMANOVA pseudo- $F_{2,6} = 1.769$, $p = 0.170$).

Fluorometry survey (2009)

Effective quantum yield of PSII (ϕ_{PSII}) was significantly greater at lease versus reference areas by 19.8% (paired- $t_{0.05,4} = 5.328$, $p = 0.003$; Fig. 6a). In contrast, maximum quantum yield of PSII (F_v/F_m) did not differ between leases and reference sites, (paired-

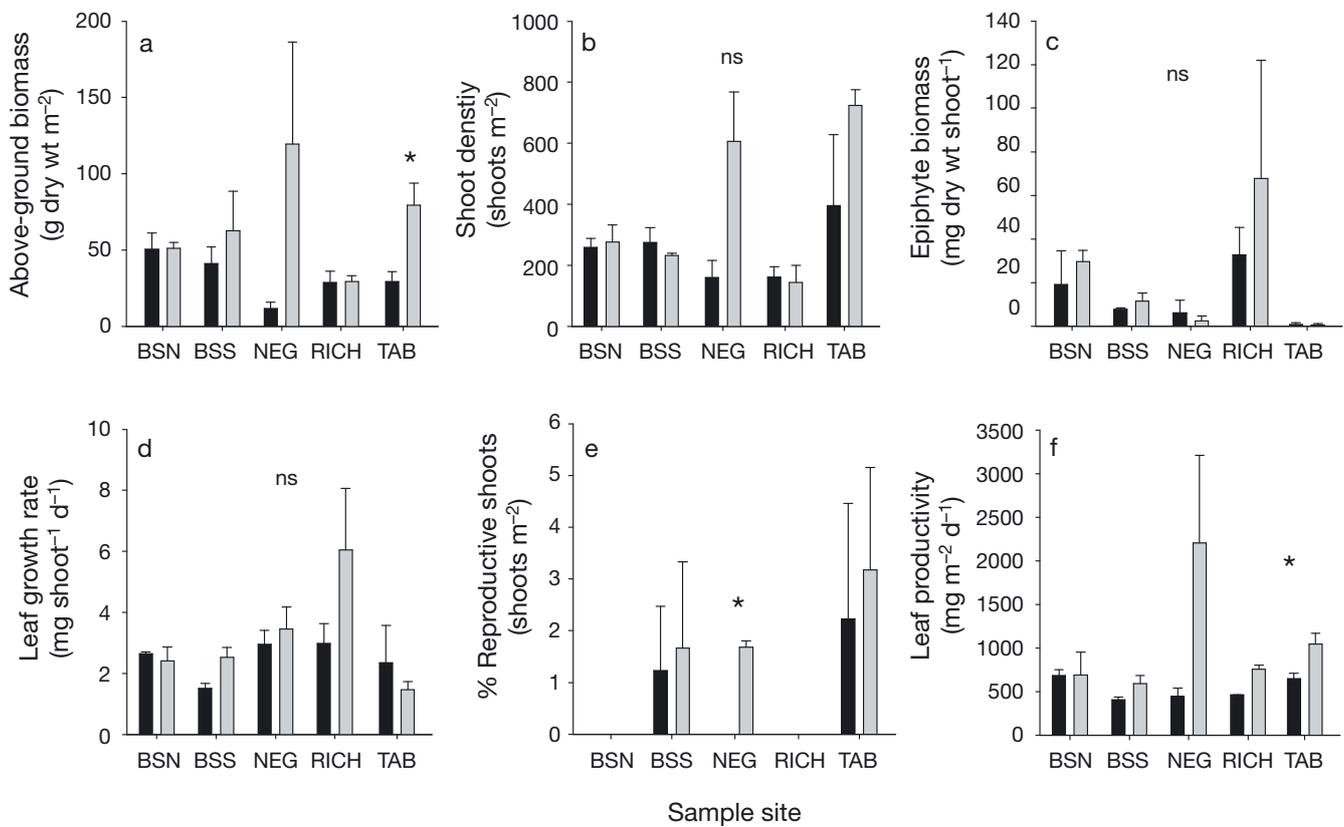


Fig. 4. Mean (+ SE) (a) above-ground biomass, (b) shoot density, (c) epiphyte biomass, (d) leaf growth rate of eelgrass, (e) percent reproductive shoots of eelgrass, and (f) leaf productivity rate sampled from suspended bag oyster aquaculture sites (lease, black bars) and reference areas (300 m, gray bars) in July 2007 (n = 2 sites per lease-reference area combination). Symbols denote statistical significance ($\alpha = 0.05$; ns = no significant difference). BSN = Bay St. Simon north; BSS = Bay St. Simon south; NEG = Neguac Bay; RICH = Richibucto Bay; TAB = Tabusintac Bay

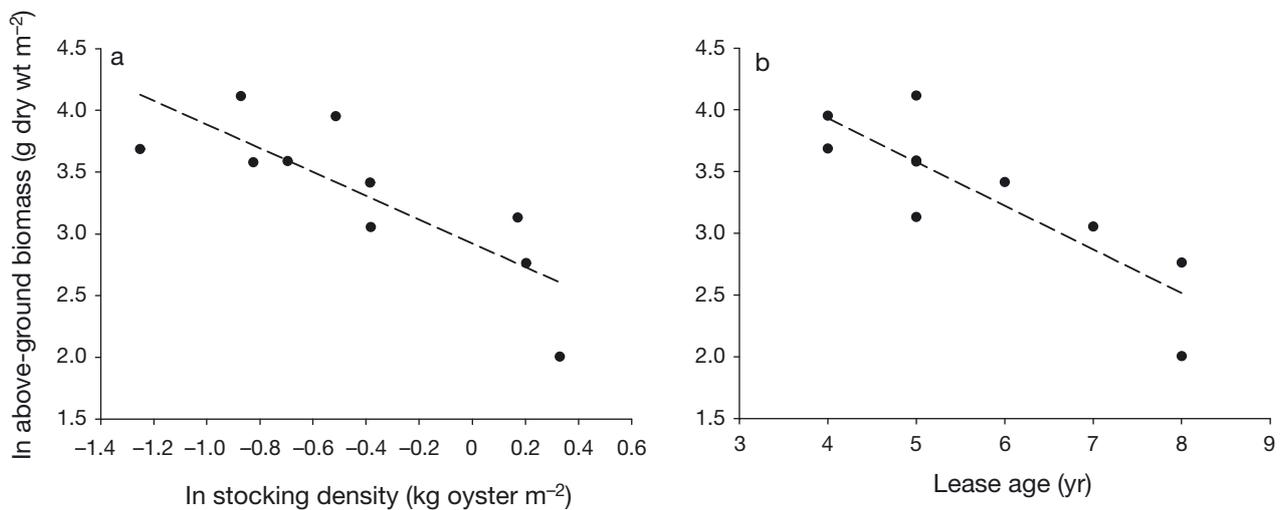


Fig. 5. Above-ground biomass of eelgrass versus (a) estimated stocking density and (b) lease age for leases of suspended bag oyster aquaculture sites sampled during the 2007 multi-bay productivity survey (n = 10). Dashed lines show the results of simple linear regressions. Only 9 data points are visible in (b) due to 2 nearly identical data points for leases THI and SG (see Table 1)

Table 4. Results of one-tailed paired-*t* tests comparing multiple eelgrass response variables between suspended bag oyster aquaculture leases and reference areas (300 m) sampled in Bay St. Simon south during August 2007. Bold values indicate statistical significance at $\alpha = 0.05$

Variable	df	Lease mean \pm SE	300 m mean \pm SE	Paired- <i>t</i> statistic	p
Distribution					
Above-ground biomass (g dry wt m ⁻²)	8	47.0 \pm 14.7	77.8 \pm 12.4	-2.06	0.037
Shoot density (shoots m ⁻²)	8	220 \pm 65.8	521 \pm 135	-2.18	0.030
Shoot morphometrics^a					
Canopy height (cm)	4	48.1 \pm 7.83	41.6 \pm 7.56	1.21	0.853
Sheath length (mm)	4	92.8 \pm 15.5	79.7 \pm 11.56	1.99	0.942
Leaves per shoot	4	3.40 \pm 0.206	3.60 \pm 0.039	-0.903	0.209
Leaf width (mm)	4	2.94 \pm 0.197	2.66 \pm 0.202	10.5	<0.001
Leaf length (mm)	4	392 \pm 63.5	336 \pm 64.3	1.13	0.161
Leaf surface area per shoot (cm ² leaf shoot ⁻¹)	4	63.0 \pm 14.7	50.4 \pm 11.5	2.75	0.026
Leaf area index (m ² leaf m ⁻² bottom)	4	0.464 \pm 0.088	0.696 \pm 0.170	-0.980	0.191
Epiphyte biomass (g dry wt epiphyte leaf cm ⁻²)	4	0.038 \pm 0.014	0.085 \pm 0.045	-0.935	0.799
Sediment					
Organic content (%)	7	0.068 \pm 0.009	0.061 \pm 0.009	0.741	0.241
Water content (%)	7	0.476 \pm 0.031	0.496 \pm 0.033	-0.530	0.306
Silt and clay content (%)	7	11.8 \pm 0.902	12.0 \pm 1.48	-0.111	0.543
Nutrients					
Leaf tissue N (% dry wt)	5	1.30 \pm 0.061	1.17 \pm 0.062	1.24	0.134
$\delta^{13}\text{C}$	5	-8.60 \pm 0.223	-8.91 \pm 0.145	1.19	0.856
$\delta^{15}\text{N}$	5	4.24 \pm 0.247	4.75 \pm 0.629	-1.18	0.146
Leaf tissue C/N	5	30.1 \pm 1.55	32.1 \pm 1.21	-0.831	0.222

^aOne sample accidentally processed for nutrient content before shoot morphometrics recorded

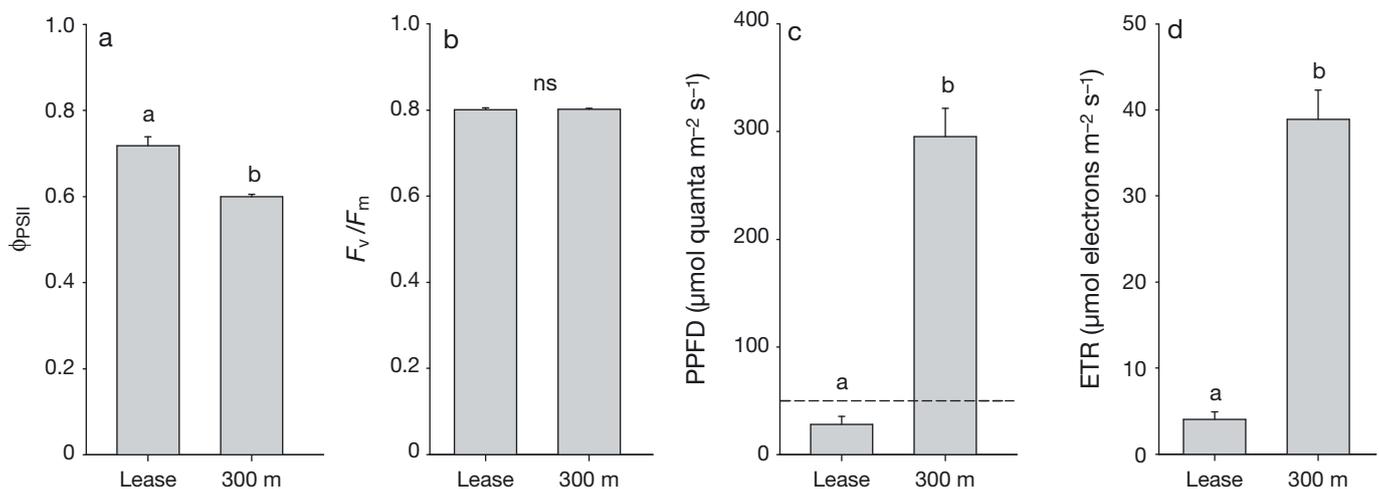


Fig. 6. Mean values (\pm SE) for (a) effective quantum yield (ϕ_{PSII}), (b) maximum quantum yield (F_v/F_m), (c) photosynthetic photon flux density (PPFD), and (d) electron transport rate (ETR) of eelgrass sampled from suspended bag oyster aquaculture sites (lease) and reference areas (300 m) in September 2009 ($n = 5$ sites per lease-reference area combination). The dashed reference line in (c) represents the minimum instantaneous midday PPFD requirement for eelgrass during spring and summer months from Thom et al. (2008). Letters denote statistical significance ($\alpha = 0.05$; ns = no significant difference)

$t_{0.05,4} = -0.337$, $p = 0.623$; Fig. 6b). As expected, the instantaneous light levels available to shoots were significantly lower within leases, with values being reduced to 9.4% of reference areas (paired- $t_{0.05,4} =$

-10.760 , $p < 0.001$; Fig. 6c). PPFD in leases averaged $27.818 \pm 7.391 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ compared with $295.145 \pm 26.441 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ in reference areas. These light reductions, in turn, resulted in

electron transport rate values which were 89.6% lower in lease versus reference areas (paired- $t_{0.05,4} = -11.187$, $p < 0.001$; Fig. 6d), following predicted trends.

DISCUSSION

These 4 surveys conducted during 3 different years at 3 different spatial scales provide the first quantitative measures of the impact of SBOA on *Zostera marina*, the dominant coastal macrophyte in the sGSL. We demonstrated consistent declines in above-ground biomass, on average 57% and by as much as 79% at multiple locations in bays and estuaries in the sGSL separated by as much as 125 km. Although the grow-out method used in this study differs from those evaluated in previous studies, these declines are in agreement with aquaculture-related seagrass declines previously observed in other regions (Carleton et al. 1991, Pregnall 1993, Everett et al. 1995, Simenstad & Fresh 1995, Rumrill & Poulton 2004, Wisehart et al. 2007, Tallis et al. 2009). Similar to Tallis et al. (2009), we noted a negative relationship between eelgrass above-ground biomass and culture density; however, this is the first known study to document a negative relationship between eelgrass and lease age. Finally, the capacity of eelgrass photochemistry was impaired and accompanied by reduced primary production at culture leases.

We demonstrated that impacts were limited to the lease footprint by sampling eelgrass along a gradient at multiple leases in 2006 to delineate near-field effects. Regardless of the response variable examined, lease samples were significantly lower than the nearest reference stations at 25 m from the lease boundary, as was predicted. Owing to the consistent lack of significant differences among the 25, 100, and 500 m 'reference' stations, we are confident in our conclusion that impacts to eelgrass distribution are largely limited to the lease footprint or some distance less than 25 m from lease boundaries.

Water depths at the vast majority of oyster leases in the region, and those sampled during this study, are more than sufficient to ensure there is little or no direct contact between suspended oyster bags and eelgrass shoots. During SCUBA sampling, very few shoots with broken leaves were observed. Further, the reductions in canopy height at culture leases are based on measures of whole shoots, which indicates shoots at leases are simply smaller and not broken or damaged. Thus, damage to shoots resulting from foot and boat traffic during lease maintenance or harvest-

ing does not appear to be a factor in the observed declines in eelgrass distribution at the sGSL oyster leases.

We found no difference between samples taken directly under the oyster bags and those taken between lines of bags, suggesting that the mechanism causing the reduction was diffuse in nature, and supporting our hypothesis that the declines observed were related to shading, organic enrichment, nutrient alteration, or some combination thereof. As oyster culturists intentionally leave some slack in the lines of bags to allow for movement with tides to reduce strain in the grow-out infrastructure (Transport Canada 2007), this result is not surprising as lines would potentially be able to travel over the majority of the lease area containing oysters, despite the appearance of evenly spaced lines.

Overall, eelgrass variables responded according to predicted trends when replicate leases in multiple bays across the study range were examined in 2007. Once again, above-ground biomass was much lower at culture leases, with the magnitude of difference from reference sites comparable to those observed in 2006. Additionally, above-ground biomass was negatively related to both oyster stocking density and lease age. Previous work by Tallis et al. (2009) has also documented negative relationships between various intertidal oyster culture methods and eelgrass structure and productivity. The strength of the relationships noted between oyster stocking density and eelgrass above-ground biomass in our study are comparable to the results of Rumrill & Poulton (2004) and Tallis et al. (2009). While the negative relationship between lease age and above-ground biomass was not expected, it is not surprising. Rumrill & Poulton (2004) noted significant differences between high density, intertidal longline oyster plots and control plots after 20 mo. The newest leases in our study were in operation for at least 48 mo and would be expected to have exhibited negative responses in eelgrass by that point given the results of Rumrill & Poulton (2004). Further to this, preliminary results from an experiment examining causal mechanisms of eelgrass declines at suspended oyster leases indicate that significant reductions in eelgrass density occur in as little as 6 wk (Skinner et al. unpubl. data).

Separating the relative influence of oyster stocking density and lease age in our study is complicated by the fact that both of these predictor variables were correlated, with older leases having higher stocking densities. While the current study was not designed to tease out these effects, evidence for the importance of stocking density over lease age is found in

the DISTLM results. This analysis demonstrated that oyster stocking density explained 35% of the variation in eelgrass structure and productivity while lease age did not significantly contribute to the explained variation in the model.

The differences observed for above-ground biomass during the multi-bay survey in 2007, however, were not detected for shoot density or leaf growth rates. The lack of differences in shoot density is most likely due to bias introduced during sample collection. These samples were collected primarily for determination of leaf growth rates. During collection, it was difficult to locate areas within leases with sufficient eelgrass density to permit shoot marking and, as such, we were forced to search out areas with higher shoot density. Therefore, these growth samples may not be considered as being 'random' estimates of shoot densities that adequately represent general conditions within leases. These shoot density data, although biased at culture leases, are still accurate for reference locations and also provide insight into estimates of primary production, as discussed below.

An explanation for the lack of predicted significant differences in leaf growth rates between lease and reference locations is not entirely evident. Growth rates of seagrass exposed to many types of disturbances have shown responses varying from increases to no effect and decreases, sometimes within the same study (Ralph et al. 2007). Tallis et al. (2009) reported increased growth rates for eelgrass exposed to bottom culture and no difference from controls for intertidal suspended culture. The 2 most probable explanations for a lack of difference in growth rates in our study are ecological or physiological processes, either alone or in combination. Ecologically, as shoot density declines, there would be a decrease in inter-shoot light competition, so shaded shoots would then be able to maintain a growth rate comparable to unshaded plants (Carlson et al. 2003, Enríquez & Pantoja-Reyes 2005, Collier et al. 2007, Ralph et al. 2007). From a physiological standpoint, eelgrass growth rate is positively correlated with light intensity up to a saturating critical light maximum after which growth is reduced (Dennison & Alberte 1985, Fourqurean & Zieman 1991, Olesen 1993). That said, evidence exists that *Zostera marina* is capable of maintaining positive leaf growth even at light levels too low to support net gains in biomass (Olesen 1993). Such responses illustrate that large-bodied seagrasses such as *Z. marina* are able to decouple growth from light availability by relying on carbohydrate reserves in the rhizomes to sustain leaf

growth in an effort to photoacclimate and by maximizing production of photosynthetic tissues while decreasing the respiratory burden of rhizome biomass (Ralph et al. 2007). As we did not measure below-ground biomass or carbohydrate reserves, we are only able to speculate on the role of physiological versus ecological mechanisms that account for variation in leaf growth rates.

Reliance on growth rates alone may not provide reliable conclusions on the existence of impacts on eelgrass, but in conjunction with density data, knowledge of growth rates allows for estimation of productivity rates. Eelgrass primary production estimates from our multi-bay survey, expressed as the rate of growth per unit area per day, clearly demonstrate the influence of suspended oyster culture on eelgrass at meadow to regional scales of impact. The 37.9% overall decrease in productivity reported here is much lower than the 70% reduction in productivity reported from the Pacific coast of North America by Tallis et al. (2009). In addition to the sampling bias previously discussed, our sampling did not account for below-ground biomass, which could explain why our values are lower than those of Tallis et al. (2009). Reproductive shoot density at leases was also decreased relative to that in reference locations, albeit only statistically significantly so for NEG. This outcome was expected as the initiation and duration of sexual reproduction in *Zostera marina* is light mediated, and reduced irradiance inhibits flowering (Backman & Barilotti 1976). Given the high variability within sampling locations, and the fact that mean values were consistently lowest within leases in the 3 bays where reproductive shoots were found, the lack of detectable differences is likely a function of low statistical power. That said, this reduction in reproductive viability deserves further study.

Concentrated sampling of many leases and reference areas within a single system (BSS detailed survey) provides further confirmation of reduced eelgrass distribution at oyster leases. Further, the results of detailed morphological, sedimentary, and leaf tissue nutrient analyses lend weight to the hypothesis that light reduction is the mechanism driving eelgrass declines in oyster aquaculture leases. First, organic matter concentration or grain size of sediment did not differ between leases and reference areas, essentially negating any arguments for bivalves fertilizing sediments and stimulating eelgrass growth at the culture sites (Peterson & Heck 2001, Carroll et al. 2008, Wall et al. 2008, 2011). Second, epiphyte biomass, C:N ratios, and leaf tissue nitrogen concentrations were indistinguishable be-

tween lease and reference areas, and leaf tissue nitrogen values at each sampling location were below the critical median 1.8% N value for temperate seagrasses (Duarte 1990), indicating that eelgrass at these sites were nutrient limited and therefore excessive nutrients were not a factor. These points, together with increased leaf surface area and width, both of which are photoacclimatory responses of *Zostera marina* to decreased irradiance (Dennison & Alberte 1985, Olesen 1993, Bintz et al. 2003, Ralph et al. 2007), point to light limitation as the main factor responsible for reduced eelgrass distribution. In contrast to the results of the multi-bay survey, eelgrass variables were not found to be significantly influenced by either oyster stocking density or lease age during the BSS detailed survey. This lack of differences was most likely due to the lower variation in these factors among leases within BSS as opposed to the variation found in stocking density and age when examined across multiple bays, particularly given the older leases in RICH and NEG as well as higher stocking density at NEG leases.

Surveying the photosynthetic status of *Zostera marina* at suspended oyster aquaculture leases relative to reference sites provided additional evidence that light limitation is the main causative factor reducing *Z. marina* cover at oyster culture leases. The amount of light available to eelgrass for photosynthesis was well below the published minimum light level required for survival (Thom et al. 2008). Elevated levels of effective quantum yield (Genty et al. 1989), an instantaneous measure of the efficiency of PSII photochemistry, showed that shoots at culture leases were much more efficient at light harvesting under ambient light levels (Maxwell & Johnson 2000). ETR, a measure of the instantaneous flow of electrons through the photosynthetic chain and a proxy for photosynthetic rate, was also greatly reduced at oyster culture leases. These results indicate that *Z. marina* at oyster culture leases is only capable of photosynthesizing at rates approximately 10-fold lower than that at reference locations as a result of substantial light reductions, and are in agreement with previous studies examining the effects of light limitation on eelgrass (Ralph & Gademann 2005, Sasil-Orbita & Mukai 2006, Ochieng et al. 2010). While continuous light logging data were not collected during these surveys, a concurrent experiment conducted in Bay St. Simon during 2009 demonstrated PPFD under suspended oyster lines were 2.4 times lower than reference sites with daily integrated PPFD values of 5.37 and 13.1 $\mu\text{mol m}^{-2} \text{d}^{-1}$, respectively (Skinner et al. unpubl. data).

These findings of decreased *Zostera marina* photosynthetic output in conjunction with reductions in distribution and productivity described for surveys ranging from near-field (footprint) to regional (bays separated by up to 125 km) scales, allow for a pathway of effects to be traced via molecular- (photosynthetic and chemical) through individual- (shoot) to population- (meadow) level responses that provide a weight of evidence pointing to light limitation as the primary mechanism driving declines of *Z. marina* at SBOA leases in the sGSL.

CONCLUSIONS

We demonstrated that the most common method of culturing oysters in Atlantic Canada has a negative impact on eelgrass. Above-ground biomass was the endpoint that demonstrated the least variability and was the most consistently detectable difference when comparing aquaculture leases to reference areas during the various surveys. If a regional eelgrass monitoring program, as per Neckles et al. (2012), were to be developed for Atlantic Canada, above-ground biomass or a proxy measure would be the most logical endpoint as it integrates density and individual size. The major drawback to relying solely on above-ground biomass, however, is that detectable declines may not be apparent until weeks to months after a disturbance and may not provide any insight into the mechanism responsible for the decline. Incorporation of morphological measures, such as leaf surface area, allows for changes to be detected on the time scale of days to weeks while allowing for the development of hypotheses to test potential causal mechanisms (e.g. light limitation due to direct shading, suspended sediment events, or eutrophication). Also, if differences are detected, the same sample collected for above-ground biomass and surface area can be further processed for nutrient content with little extra commitment of time or resources. Further, the current study has shown that the low intra-site variability of above-ground biomass also allows for differences to be detected with as few as 5 sites sampled. That said, any monitoring sampling should attempt to sample over larger areas with multiple quadrat samples either nested within transects or allocated among strata as per Neckles et al. (2012). Such sampling would better capture the heterogeneity in eelgrass distribution related to aquaculture activity observed during the surveys conducted for this study. Another important consideration is the temporal variability in above-ground

biomass during the growing season. Any monitoring initiatives would have to factor this variability into sampling designs either through repeated sampling throughout the growing season or restricting sampling to periods of maximal biomass before the end of summer declines. Another element required for an effective monitoring program is a simple sediment core for determination of grain size and organic matter content, each of which provide explanatory power if sedimentation or biodeposition were potential causal mechanisms. Finally, the addition of continuous light loggers would also be beneficial for providing insight into potential water clarity issues due to sedimentation, eutrophication, and/or anthropogenic structures.

We hypothesize that shading from aquaculture equipment and products impairs eelgrass photosynthesis, resulting in reduced productivity, reproductive viability, and survival. We are conducting experiments to test this hypothesis as well as hypotheses related to potential interactive effects of organic matter loading to the benthos.

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LITERATURE CITED

- Anderson M, Gorley R, Clarke K (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth
- Backman TW, Barilotti DC (1976) Irradiance reduction: effects on standing crops of the eelgrass *Zostera marina* in a coastal lagoon. *Mar Biol* 34:33–40
- Barranguet C (1997) The role of microphytobenthic primary production in a Mediterranean mussel culture area. *Estuar Coast Shelf Sci* 44:753–765
- Bastien-Daigle S, Hardy M, Robichaud G (2007) Habitat management qualitative risk assessment: water column oyster aquaculture in New Brunswick. *Can Tech Rep Fish Aquat Sci* 2728:vii
- Bier S, Vilenkin B, Weil A, Veste M, Susel L, Eshel A (1998) Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry. *Mar Ecol Prog Ser* 174:293–300
- Bier S, Bjork M, Gademann R, Ralph RJ (2001) Measurement of photosynthesis in seagrasses. In: Short FT, Coles RG (eds) *Global seagrass research methods*. Elsevier Science Publishers, Amsterdam, p 183–198
- Bintz J, Nixon S, Buckley B, Granger S (2003) Impacts of temperature and nutrients on coastal lagoon plant communities. *Estuaries Coasts* 26:765–776
- Cabaço S, Alexandre A, Santos R (2005) Population-level effects of clam harvesting on the seagrass *Zostera noltii*. *Mar Ecol Prog Ser* 298:123–129
- Carleton JT, Ruiz GM, Everett RA (1991) The structure of benthic estuarine communities associated with dense suspended populations of the introduced Japanese oyster *Crassostrea gigas*: years 1 and 2. Final Report to South Slough National Estuarine Reserve
- Carlson PR Jr, Yarbro LA, Madley K, Arnold H and others (2003) Effect of El Niño on demographic, morphological, and chemical parameters in turtle-grass (*Thalassia testudinum*): an unexpected test of indicators. *Environ Monit Assess* 81:393–408
- Carroll J, Gobler C, Peterson B (2008) Resource-restricted growth of eelgrass in New York estuaries: light limitation, and alleviation of nutrient stress by hard clams. *Mar Ecol Prog Ser* 369:51–62
- Clarke K, Warwick R (2001) *Changes in marine communities: an approach to statistical analysis and interpretation*, 2nd edn. PRIMER-E, Plymouth
- Collier C, Lavery P, Masini R, Ralph P (2007) Morphological, growth and meadow characteristics of the seagrass *Posidonia sinuosa* along a depth-related gradient of light availability. *Mar Ecol Prog Ser* 337:103–115
- De Casabianca ML, Laugier T, Collart D (1997) Impact of shellfish farming eutrophication on benthic macrophyte communities in the Thau lagoon, France. *Aquacult Int* 5: 301–314
- Dennison WC, Alberte RS (1985) Role of daily light period in the depth distribution of *Zostera marina* (eelgrass). *Mar Ecol Prog Ser* 25:51–61
- Doiron S (2008) Reference manual for oyster aquaculturists. New Brunswick Department of Agriculture, Fisheries and Aquaculture Report. Available at www.gnb.ca/0168/oyster.pdf
- Draper NR, Smith H (1998) *Applied regression analysis*. Wiley, New York, NY
- Duarte CM (1990) Seagrass nutrient content. *Mar Ecol Prog Ser* 67:201–207
- Duarte CM, Kirkman H (2001) Methods for the measurement and seagrass abundance and depth distribution. In: Short FT, Coles RG (eds) *Global seagrass research methods*. Elsevier Science Publishers, Amsterdam, p 141–154
- Dumbauld BR, Ruesink JL, Rumrill SS (2009) The ecological role of bivalve shellfish aquaculture in the estuarine environment: a review with application to oyster and clam culture in West Coast (USA) estuaries. *Aquaculture* 290:196–223
- Durako MJ, Kunzelman JI (2002) Photosynthetic characteristics of *Thalassia testudinum* measured in situ by pulse-amplitude modulated (PAM) fluorometry: methodological and scale-based considerations. *Aquat Bot* 73: 173–185
- Enríquez S, Pantoja-Reyes NI (2005) Form-function analysis of the effect of canopy morphology on leaf self-shading in the seagrass *Thalassia testudinum*. *Oecologia* 145: 234–242
- Everett RA, Ruiz GM, Carlton JT (1995) Effect of oyster mariculture on submerged aquatic vegetation: an experimental test in a Pacific-Northwest estuary. *Mar Ecol Prog Ser* 125:205–217

- Fisheries and Oceans Canada (2010) Facts and figures: aquaculture in Canada. www.dfo-mpo.gc.ca/aquaculture/ref/stats/aqua-ff-fc-2009-eng.htm
- Fourqurean JW, Zieman JC (1991) Photosynthesis, respiration and whole plant carbon budget of the seagrass *Thalassia testudinum*. *Mar Ecol Prog Ser* 69:161–170
- Gaeckle J, Short FT (2002) A plastochrone method for measuring leaf growth in eelgrass, *Zostera marina* L. *Bull Mar Sci* 71:1237–1246
- Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta* 990:87–92
- Giles H, Pilditch C (2006) Effects of mussel (*Perna canaliculus*) biodeposit decomposition on benthic respiration and nutrient fluxes. *Mar Biol* 150:261–271
- Grant J, Cranford P, Hargrave B, Carreau M and others (2005) A model of aquaculture biodeposition for multiple estuaries and field validation at blue mussel (*Mytilus edulis*) culture sites in eastern Canada. *Can J Fish Aquat Sci* 62:1271–1285
- Green R (2005) Marine coastal monitoring: designing an effective offshore oil and gas environmental monitoring program. In: Armsworthy S, Cranford P, Lee K (eds) Offshore oil and gas environmental effects monitoring: approaches and technologies. Battelle Press, Columbus, OH, p 373–397
- Hatcher A, Grant J, Schofield B (1994) Effects of suspended mussel culture (*Mytilus* spp.) on sedimentation, benthic respiration and sediment nutrient dynamics in a coastal bay. *Mar Ecol Prog Ser* 115:219–235
- Jamieson G, Chew L, Gillespie G, Robinson A and others (2001) Phase 0 review of the environmental impacts of intertidal shellfish aquaculture in Baynes Sound. Canadian Science Advisory Secretariat 2001/125
- Kirkman H (1996) Baseline and monitoring methods for seagrass meadows. *J Environ Manage* 47:191–201
- Mallet al. Carver CE, Hardy M (2009) The effect of floating bag management strategies on biofouling, oyster growth and biodeposition levels. *Aquaculture* 287:315–323
- Maxwell K, Johnson GN (2000) Chlorophyll fluorescence: a practical guide. *J Exp Bot* 51:659–668
- Neckles H, Kopp B, Peterson B (2012) Integrating scales of seagrass monitoring to meet conservation needs. *Estuaries Coasts* 35:23–46
- Ochieng CA, Short FT, Walker DI (2010) Photosynthetic and morphological responses of eelgrass (*Zostera marina* L.) to a gradient of light conditions. *J Exp Mar Biol Ecol* 382:117–124
- Olesen B (1993) Seasonal acclimatization of eelgrass *Zostera marina* growth to light. *Mar Ecol Prog Ser* 94:91–99
- Peterson BJ, Heck KL (2001) Positive interactions between suspension-feeding bivalves and seagrass: a facultative mechanism. *Mar Ecol Prog Ser* 213:143–155
- Peterson CH, Summerson HC, Fegley SR (1987) Ecological consequences of mechanical harvesting of clams. *Fish Bull* 85:281–298
- Pregnall M (1993) Regrowth and recovery of eelgrass (*Zostera marina*) and recovery of benthic community structure in areas disturbed by commercial oyster culture in the South Slough National Estuarine Research Reserve, Oregon. MS thesis, Bard College, Annandale-On-Hudson, New York
- Ralph PJ, Gademann R (2005) Rapid light curves: a powerful tool to assess photosynthetic activity. *Aquat Bot* 82:222–237
- Ralph PJ, Durako MJ, Enríquez S, Collier CJ, Doblin MA (2007) Impact of light limitation on seagrasses. *J Exp Mar Biol Ecol* 350:176–193
- Rumrill S, Poulton V (2004) Ecological role and potential impacts of molluscan shellfish culture in the estuarine environment of Humboldt Bay, CA: annual report. Oregon Department of State Lands, South Slough National Estuarine Research Reserve, Estuarine and Coastal Science Laboratory, Charleston, SC
- Sasil-Orbita ML, Mukai H (2006) A comparative study of the photosynthetic activity among three temperate seagrass species in Northern Japan. *PSZN I: Mar Ecol* 27:388–396
- Short F, Duarte C (2001) Methods for the measurement of seagrass growth and production. In: Short FT, Coles RG (eds) Global seagrass research methods. Elsevier Science, Amsterdam, p 155–182
- Simenstad CA, Fresh KL (1995) Influence of intertidal aquaculture on benthic communities in Pacific Northwest estuaries: scales of disturbance. *Estuaries* 18:43–70
- Tallis HM, Ruesink JL, Dumbauld B, Hacker S, Wisheart LM (2009) Oysters and aquaculture practices affect eelgrass density and productivity in a Pacific Northwest estuary. *J Shellfish Res* 28:251–261
- Thom RM, Parkwell TL, Niyogi DK, Shreffler DK (1994) Effects of graveling on the primary productivity, respiration and nutrient flux of two estuarine tidal flats. *Mar Biol* 118:329–341
- Thom RM, Southard SL, Borde AB, Stoltz P (2008) Light requirements for growth and survival of eelgrass (*Zostera marina* L.) in Pacific Northwest (USA) estuaries. *Estuaries Coasts* 31:969–980
- Transport Canada (2007) Replacement class screen report: water column oyster aquaculture in New Brunswick. Report of the Canadian Environmental Assessment Agency, Moncton, New Brunswick
- Wall CC, Peterson BJ, Gobler CJ (2008) Facilitation of seagrass *Zostera marina* productivity by suspension-feeding bivalves. *Mar Ecol Prog Ser* 357:165–174
- Wall CC, Peterson BJ, Gobler CJ (2011) The growth of estuarine resources (*Zostera marina*, *Mercenaria mercenaria*, *Crassostrea virginica*, *Argopecten irradians*, *Cyprinodon variegatus*) in response to nutrient loading and enhanced suspension feeding by adult shellfish. *Estuaries Coasts* 34:1262–1277
- Wisheart LM, Dumbauld BR, Ruesink JL, Hacker SD (2007) Importance of eelgrass early life history stages in response to oyster aquaculture disturbance. *Mar Ecol Prog Ser* 344:71–80
- Zar JH (1999) Biostatistical analysis, 4th edn. Prentice Hall, Upper Saddle River, NJ

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