Journal of Experimental Marine Biology and Ecology 472 (2015) 41–53

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Light indirectly mediates bivalve habitat modification and impacts on seagrass

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ARTICLE INFO

Article history:
Received 6 January 2015
Received in revised form 2 July 2015
Accepted 3 July 2015
Available online xxxx

Keywords:
Blue mussel *Mytilus edulis*
Estuarine eutrophication
Eelgrass *Zostera marina*
Light stress gradient
Oxygen
Sulfide

ABSTRACT

Environmental context may influence the sign, strength, and mechanisms of species interactions but few studies have experimentally tested the potential for abiotic conditions to mediate interactions through multiple co-occurring stress pathways. Abiotic conditions may mediate species interactions by directly or indirectly influencing the effects of habitat-modifying organisms that are capable of simultaneously ameliorating and exacerbating multiple stressors. It was hypothesized that light availability changes seagrass metabolism and thereby indirectly regulates bivalve habitat modification and subsequent impacts on seagrasses by shifting net effects between alleviation of nutrient stress and intensification of sulfide stress. To test this hypothesis, manipulations of light availability and blue mussel (*Mytilus edulis*) abundance were made in eelgrass (*Zostera marina*) mesocosms and biogeochemical and plant responses were measured. Light modified the effect of mussels on porewater ammonium, but eelgrass was not nutrient limited and, therefore, mussels did not enhance growth. Mussels increased sediment sulfides irrespective of light availability and, by reducing net oxygen flux (production and respiration), mussels and low light availability exacerbated sulfide intrusion of eelgrass tissues. Surprisingly, sulfide stress did not affect plant growth, survival, or energy stores. Thus, habitat modification by mussels may represent a risk to eelgrass, especially during low productivity conditions, but eelgrass can resist harm from short-term stress, even during light limitation. These findings suggest that while small-scale bivalve impacts on seagrasses may be variable in oligotrophic estuaries, they have the potential to be negative in eutrophic systems, which are increasing globally.

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1. Introduction

The sign, strength, and mechanisms of interactions among species can depend on environmental context (Menge and Sutherland, 1987; Connolly and Roughgarden, 1999). Abiotic stress is an important class of environmental conditions that can shift the direction of species interactions, as predicted by the stress gradient hypothesis (Bertness and Callaway, 1994; Bertness and Hacker, 1994). This hypothesis has been especially applicable to species that physically or chemically ameliorate a single environmental stressor or increase the availability of a limiting resource (e.g., Norkko et al., 2006). Habitat-modifying organisms often simultaneously alleviate and exacerbate several stressors, creating the potential for highly complex species interactions (Kawai and Tokeshi, 2007). However, few studies have experimentally assessed the potential for environmental context to mediate the sign or strength of species interactions through multiple co-occurring stress pathways.

Estuaries are excellent ecosystems for testing the effects of environmental conditions on habitat modification and species interactions by virtue of their dynamic abiotic gradients (e.g., salinity, nutrients, light) and numerous species that modify physical and biogeochemical conditions of the seafloor, such as suspension-feeding bivalves (e.g., Haven and Morales-Alamo, 1966; Bertness, 1984; Kautsky and Evans, 1987) and seagrasses (e.g., Frederiksen and Glud, 2006; Holmer, 2009; Castorani et al., 2014). Bivalves are common, often abundant inhabitants of temperate seagrass meadows (e.g., Peterson et al., 1984; Peterson, 1986) and may have positive, negative, or no effect on these plants. For instance, clams and mussels may facilitate seagrass growth by increasing the availability of sediment nutrients through biodeposition of feces and pseudofeces (e.g., Reusch et al., 1994; Carroll et al., 2008). However, other studies have shown that sediment enrichment by mussels can inhibit the growth of seagrasses by increasing concentrations of toxic sulfides

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http://dx.doi.org/10.1016/j.jembe.2015.07.001
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(Vinther and Holmer, 2008; Vinther et al., 2012). In other instances, bivalves may have mixed (Reusch and Williams, 1998) or no (e.g., Worm and Reusch, 2000; Wagner et al., 2012) effects on seagrass growth. These variable and inconsistent effects cannot be satisfactorily explained by nutrient availability, suggesting more complex interactions. Variation in light availability—the primary determinant of seagrass productivity (Dennison and Alberte, 1985; Zimmerman et al., 1995)—might help reconcile these disparate findings.

Here, the hypothesis is tested that bivalve modification of benthic biogeochemical conditions and the sign and strength of subsequent impacts on seagrasses are indirectly mediated by light availability through changes to seagrass metabolism. It was predicted that bivalve biodeposition relieves seagrass nutrient stress by increasing sediment nitrogen, but only when light is not limiting. It was also predicted that bivalve enhancement of sediment sulfides inhibits seagrasses under light limitation, when low productivity diminishes the ability of seagrasses to maintain an oxygenated rhizosphere and resist sulfide intrusion (Pedersen et al., 2004; Holmer et al., 2005; Frederiksen and Glud, 2006). Thus, it was hypothesized that light availability mediates bivalve impacts on seagrasses by shifting the net effect between alleviation of nutrient stress and exacerbation of sulfide stress. To test these predictions, bivalve abundance and light availability were manipulated in seagrass mesocosms and biogeochemical and seagrass responses were measured.

2. Materials and methods

2.1. Study system

The blue mussel, *Mytilus edulis* L., is a suspension-feeding epibenthic bivalve that commonly co-occurs with eelgrass, *Zostera marina* L., in intertidal and shallow-subtidal zones of the temperate North Atlantic Ocean, North Sea, and Baltic Sea (e.g., Reusch et al., 1994; Reusch and Chapman, 1995; Reusch, 1998; Bologna et al., 2005). In the Baltic Sea, studies of the effects of *M. edulis* on *Z. marina* have had particularly conflicting results (e.g., Reusch et al., 1994; Worm and Reusch, 2000; Vinther et al., 2012). This study was conducted with seawater, sediments, and organisms collected from the Danish Straits, which connect the North Sea and Baltic Sea. Eelgrass and blue mussels are widely distributed within many Danish fjords and along most Danish coastlines, co-occurring in both mixed and patchy spatial distributions (Reusch et al., 1994; Worm and Reusch, 2000; Kristensen, 2002; Frederiksen et al., 2004; Vinther et al., 2012). In this region of Denmark, coastal waters are often eutrophic (median total nitrogen = 550 μg N L^{-1}; median chlorophyll *a* = 5.1 μg L^{-1}) and turbid (median turbidity = 10.0 mg dry L^{-1}), resulting in highly-variable benthic light availability (Secchi depth range = 0.3–17.0 m) (Nielsen et al., 2002).

2.2. Experimental design

To determine the role of light availability in mediating blue mussel habitat modification and impacts on eelgrass, mussel abundance (present vs. absent) and light availability (high vs. low) were manipulated in a factorial design for 37 days in an indoor mesocosm experiment at the University of Southern Denmark (Odense, Denmark; 55° 22′ 9″ N, 10° 25′ 40″ E). Mesocosms consisted of transplanted sediments and eelgrass in 5.4 L plastic buckets (20 cm diameter × 17 cm tall; n = 6 buckets per treatment). Eelgrass mesocosms were housed in two aquaria (each 1.1 m length × 0.7 m width × 0.6 m height) that were respectively illuminated at high and low light availability. Aquaria shared flowing seawater (total vol. ≈ 1200 L; connected through a sump with plastic filtration media) that was collected unfiltered from the Marine Biological Research Centre (Kerteminde, Denmark; 55° 27′ 11″ N, 10° 39′ 54″ E) and partially (15% = 175 L) replaced weekly to prevent nutrient accumulation. To maintain 100% air-saturation of the water, 14 air stones were distributed evenly across each aquarium. Both aquaria were illuminated on a 12 h:12 h day:night cycle using Philips SGR 140/400 W lamps (three HQT-BT/D bulbs in high-light aquarium vs. one bulb in low-light aquarium; Royal Philips, Amsterdam, The Netherlands). To further reduce light in the low-light aquarium, the top of this aquarium was shaded with neutral-density black plastic netting.

To characterize abiotic conditions, temperature and light availability were measured every 15 min using a data-logging pendant sensor (HOBO UA-002-64, Onset Computer Company, Bourne, Massachusetts, United States of America (USA)) in each aquarium, attached to stands at eelgrass canopy height (30 cm above the sediment (see below) and 13 cm below the air-water interface). Illuminance readings were calibrated to photosynthetically-active radiation (PAR; 400–700 nm) measured with a PAR sensor (LI-COR LI-250A light meter with LI-193 Underwater Spherical Quantum Sensor, LI-COR Biosciences, Lincoln, Nebraska, USA). During daytime, mean canopy-height irradiance in high-light and low-light aquaria was 574 ± 289 (SD) and 97 ± 70 μmol photons s^{-1} m^{-2}, respectively (high variability in measured irradiance was due to air bubbles periodically crossing the sensor and occasional fouling of the sensor surface). These irradiance levels were chosen to represent light conditions within the range experienced by natural eelgrass communities and at which light availability typically does or does not limit eelgrass growth (i.e., above and slightly below the light-saturation point, P_{max}), respectively (Dennison and Alberte, 1985). At night, both aquaria were completely dark. Aquaria had similar flow (1200 L h^{-1}), temperature (14.4 °C), salinity (13.4 ± 0.8), water-column oxygen air-saturation (100%), and water-column nutrients (18.5 ± 1.7 μg NO_3 L^{-1}; 11.5 ± 7.7 μmol NH_4^+ L^{-1}).

2.3. Field collections

In February and March 2013, sediments and eelgrass were collected from haphazardly-selected patches at a shallow (0–2 m depth) subtidal site at Svenstrup Beach in western Funen, Denmark (55° 28′ 7″ N, 9° 45′ 17″ E). Sediments were excavated from the upper 15 cm by shovel, sieved to remove fauna and detritus >1 mm, homogenized by hand, and stored in the recirculating seawater system until planting (3 days). Sediments were sandy (porosity: 30 ± 3%) and low in organic matter (0.42 ± 0.12%). Eelgrass was carefully uprooted, rinsed of sediments, and transported in coolers to the laboratory (<2 h). To reduce thermal shock, eelgrass was kept in a temperature-controlled room, with aerated seawater and intermediate light (150–250 μmol photons s^{-1} m^{-2}), in which the temperature was increased gradually (+1 °C d^{-1}) from 1 °C to 14 °C, the typical seawater temperature in coastal Denmark in the spring and early summer, when eelgrass productivity is high (Olesen and Sand-Jensen, 1994a,b; due to time constraints, plants could not be collected in the spring or summer). Next, undamaged terminal shoots (leaf length > 10 cm and rhizome length ≥ 2 cm, with 3–5 internodes, intact roots, and no lateral shoots) were selected and carefully removed of senescent tissues. Then, each mesocosm was filled with sediment (10 cm layer) and 28 shoots were transplanted in haphazard arrangement (=891 leaf shoots m^{-2}, corresponding to eelgrass densities typically observed in mixed eelgrass–mussel patches (Reusch et al., 1994; Vinther et al., 2012)). Transplanted eelgrass was allowed two weeks to establish under intermediate light (150–250 μmol photons s^{-1} m^{-2}) and then each mesocosm was randomly assigned a light and mussel treatment.

In March 2013, mussels were gathered by hand from haphazardly-selected floating docks at the Marine Biological Research Centre and medium-sized mussels (51.9 ± 5.1 mm length, a typical size for mussels from local mixed eelgrass–mussel beds; HF Vinther, unpublished data) were retained. Mussels were transported to the laboratory and acclimated using the same procedure as for eelgrass. To half of the mesocosms, 28 mussels (=891 mussels m^{-2} or 27.6 ± 3.6 g dry soft tissue per mesocosm) were carefully added in their natural orientation,

creating an unhummocked epibenthic layer of approximately 100% mussel cover. This mussel density corresponds to densities commonly found in mixed eelgrass–mussel patches in the Baltic Sea (Reusch et al., 1994; Reusch and Chapman, 1995; Vinther et al., 2012). Mussels that died during the experiment (<5%) were replaced. Mussels were fed a microalgal diet of resuspended dried *Spirulina* sp. (2 g dry $d^{-1} \approx 5$ μg chl. *a* L^{-1} recirculated through the integrated seawater system), which supports the growth of *M. edulis* (Alunno-Bruscia et al., 2000, 2001).

After applying light and mussel treatments, the arrangement of mesocosms was randomized within each aquarium and, to control for within-aquarium heterogeneity in water flow or light availability, re-randomized twice per week. Control mesocosms, filled with sediments but not eelgrass or mussels, were also created in both high-light and low-light aquaria (n = 4 per light treatment) to quantify initial benthic biogeochemical conditions without disturbing the sediment in experimental mesocosms.

2.4. Benthic biogeochemical measurements

Porewaters and sediments were collected to characterize treatment effects on benthic biogeochemical conditions. To determine sediment nutrient pools at both the start and end of the experiment, porewaters (0–5 cm depth) were sampled from all mesocosms using porous "sippers" (0.15 μm pore size, 5 cm length; Rhizon SMS, Rhizosphere Research Products, Wageningen, The Netherlands; Seeberg-Elverfeldt et al., 2005) oriented vertically and arranged haphazardly (3 per mesocosm). Porewater samples were filtered (0.45 μm), frozen, and later analyzed colorimetrically for the concentration of ammonium—the preferred nitrogen source for eelgrass (Short and McRoy, 1984)—by the salicylate–hypochlorite method (Bower and Holm-Hansen, 1980) on a flow-injection autoanalyzer (Lachat Quick Chem 8500, Lachat Instruments, Loveland, Colorado, USA). From these same porewater samples, concentrations of dissolved organic carbon (DOC) were also measured via high-temperature catalytic oxidation (Suzuki et al., 1992) using a total organic carbon analyzer (Shimadzu TOC-5000, Shimadzu Corporation, Kyoto, Japan).

To determine how light and mussels affected sediment sulfur pools, sediments were collected at both the start of the experiment (from control mesocosms only) and end of the experiment (from all mesocosms) by coring haphazardly within each mesocosm (18 mm diameter × 3 cm depth; 3 homogenized cores per mesocosm). In experimental mesocosms, care was taken to avoid coring roots and rhizomes. All sediments were preserved in 20% zinc acetate, frozen, and later distilled (Fossing and Jørgensen, 1989) to quantify acid-volatile sulfides (AVS = porewater H_2S + iron-monosulfides) by colorimetric concentration analysis (Cline, 1969). Under short-term organic enrichment (e.g., biodeposition), AVS is the main form of sediment sulfide accumulation (Holmer and Frederiksen, 2007).

To aid in determining sulfide intrusion of eelgrass tissues (see below), separate sediment cores were collected (at both the start and end of the experiment, as above) and distilled to measure sulfur isotopic composition ($\delta^{34}S$). Seawater (sulfate) $\delta^{34}S$ was quantified by collecting water-column samples (n = 3) and precipitating sulfate using hot barium chloride (Frederiksen et al., 2006). Subsequently, sediment and seawater $\delta^{34}S$ were determined using a continuous-flow isotope ratio mass spectrometer plus elemental analyzer (Frederiksen et al., 2008; Thermo Scientific Delta V Advantage plus Flash EA 1112, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Stable isotope signatures are reported in standard delta notation (units per mil, ‰):

$$\delta^{34}S = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \times 1000$$

where $R = {}^{34}S/{}^{32}S$.

2.5. Production and respiration measurements

To estimate community-scale production and respiration, every week for the first four weeks fluxes of oxygen were measured in submerged mesocosm enclosures for a subset of experimental mesocosms (n = 3 randomly-selected mesocosms per treatment per week; it was not possible to concurrently measure all mesocosms). The enclosures were clear cylindrical plastic bags (6 L) that were sealed over each bucket and contained a small pump (Ehiem AS1000, Eheim GmbH & Co. KG, Deizisau, Germany) that circulated water (5 L min^{-1}). Affixed to each enclosure was an oxygen optode patch that was used to measure the oxygen concentration every 10–15 min using a fiber-optic oxygen optode setup (PreSens Fibox 3, PreSens Precision Sensing GmbH, Regensburg, Germany). Incubations were conducted for 3–4 h across the end of the light cycle and beginning of the subsequent dark cycle (Fenchel and Glud, 2000). Hourly rates of oxygen change were calculated using linear regressions. Then, net community production (NCP) was estimated as oxygen flux during the light incubation (more positive values indicate greater production), community respiration (CR) as oxygen flux during the dark period (more negative values indicate greater respiration), and gross primary production (GPP) as NCP + |CR| (Glud et al., 2009).

2.6. Eelgrass survival, growth, and condition measurements

To quantify changes in eelgrass survival over time, shoots were counted weekly during the first 3 weeks and twice per week during the final 2.5 weeks (changes to eelgrass density accelerated during this time). The needle-punch method (Dennison, 1987) was used to measure leaf growth by puncturing all shoots 10 days before the experiment concluded and, at the end of the experiment, haphazardly-selecting 10 terminal shoots from each mesocosm and measuring the width and linear elongation of all leaves. Rates of leaf areal production (mm^2 $shoot^{-1}$ d^{-1}) were converted to leaf mass production (g dry $shoot^{-1}$ d^{-1}) by linear regression of leaf area (estimated as leaf length × width) and leaf dry mass ($P < 0.0001$, $R^2 = 93.8\%$).

At the end of the experiment (37 days after applying treatments), all eelgrass was collected, separated by tissue type (shoots + leaves, rhizomes, and roots), freeze-dried for 48 h, and weighed. Eelgrass leaves were colonized by very few epiphytes and did not require scraping. Young leaves (youngest leaf pair), young rhizomes (youngest two internodes), and young roots (attached to young rhizomes) were homogenized and analyzed separately for total carbon and nitrogen content by elemental analysis (Kristensen and Anderson, 1987; Carlo Erba EA1108 CHN analyzer, Carlo Erba Reagents, Milan, Italy). To determine eelgrass energy stores (i.e., non-structural carbohydrates), soluble sugars and starches were sequentially extracted from young rhizomes using hot ethanol and potassium hydroxide, respectively (Zimmerman et al., 1995). Sugar and starch concentrations were measured colorimetrically using an anthrone assay standardized to sucrose (Yemm and Folkes, 1954). Young tissues were the focus of these analyses because of confidence that they grew under the experimental manipulations (as opposed to older tissues, which might reflect responses to prior field conditions).

To assess sulfide intrusion of young eelgrass tissues (Frederiksen et al., 2006), the fraction of tissue total sulfur (TS) derived from sediment sulfides ($F_{sulfide}$) was determined as:

$$F_{sulfide} = \frac{\delta^{34}S_{tissue} - \delta^{34}S_{sulfate}}{\delta^{34}S_{sulfide} - \delta^{34}S_{sulfate}}$$

where $\delta^{34}S_{tissue}$ is the value measured in the leaves, rhizomes, or roots, $\delta^{34}S_{sulfate}$ is the value measured in seawater, and $\delta^{34}S_{sulfide}$ is the value measured in sediment AVS pools (Frederiksen et al., 2006, 2008). Eelgrass tissue was measured for TS and $\delta^{34}S$ using methods identical to sediments (see above). If sulfides invade eelgrass roots, the delivery of

oxygen via aerenchyma causes sulfides to precipitate as elemental sulfur (S^0) and other oxidized compounds (Holmer et al., 2005). Thus, to provide ancillary evidence of sulfide intrusion, S^0 concentrations were measured in young roots by high-performance liquid chromatography (Mascaró et al., 2009; Agilent 1100 Series HPLC with UV detector (265 nm), Agilent Technologies Inc., Santa Clara, California, USA) following methanol extraction (Zopfi et al., 2001).

2.7. Statistical analyses

The effects of mussels, light, and the interaction between these factors on benthic biogeochemical and eelgrass conditions were tested using separate fully-factorial analyses of variance (ANOVAs) in R (version 3.0.2; R Core Team, 2013). The effects of light and mussel treatments, time (i.e., week of sampling), and interactions between all factors on NCP and CR were tested using repeated-measures ANOVAs in JMP (version 10.0; SAS Institute Inc., Cary, North Carolina, USA). It was hypothesized that porewater ammonium might explain variation in leaf C:N. It was also predicted that porewater DOC might explain variation in sediment sulfides (AVS). Lastly, it was hypothesized that sediment AVS or oxygen flux during day (NCP) or night (CR) might explain variation in sulfide invasion of plant tissues ($F_{sulfide}$ or root S^0). Therefore, relationships between these a priori selected response variables were tested using linear regressions in R.

Before performing ANOVAs or linear regressions, the normality of the residuals was determined using normal probability plots and the homoscedasticity of the residuals were assessed by visual examination of the relationship between residuals and fitted values, and by Cochran's test for equality of variances. For linear regressions, data linearity was evaluated by visual examination of the relationship between response and predictor variables. Before performing repeated-measures ANOVAs, sphericity was determined using Mauchly's test. When necessary, response variables were log-transformed [$\ln(y+1)$] to meet the assumptions of these parametric analyses.

3. Results

3.1. Benthic biogeochemical conditions

Mussel and light treatments had an interactive effect on porewater ammonium concentrations (Table 1). At high light availability, mussels increased porewater ammonium concentrations relative to initial levels (819 ± 59 μmol L^{-1}) and to eelgrass mesocosms lacking mussels (Fig. 1A). At low light availability, however, ammonium concentrations were high regardless of mussel presence (Fig. 1A). In contrast, neither mussels nor light affected porewater DOC (Table 1). AVS pools increased in all mesocosms relative to initial levels (0.39 ± 0.04 μmol cm^{-3} sediment) and mussels enhanced sediment AVS pools irrespective of light treatment (Table 1; Fig. 1B). Porewater DOC was positively correlated with sediment AVS pools ($P = 0.0099$, $R^2 = 27.7\%$).

3.2. Production and respiration

Mussel and light treatments had an interactive effect on net community production (Table 2), as both low light and mussels suppressed NCP, but mussels had a relatively greater effect in high light than in low light (Fig. 2A). High light and mussels enhanced community respiration without interaction (Fig. 2B). Neither NCP nor CR varied through time. NCP data revealed that high-light mesocosms were net autotrophic (NCP > 0) and low-light mesocosms were net heterotrophic (NCP < 0; Fig. 2A). On occasion, measurement inaccuracies caused GPP values to be slightly negative for mesocosms under low light with mussels (Fig. 2C).

3.3. Eelgrass condition: nutrient content and sulfide intrusion

High light reduced leaf nitrogen (Table 1; Fig. 3A), increased leaf carbon (but this effect was slight: 36.05 ± 0.36 vs. 35.39 ± 0.74% by weight for high light and low light, respectively), and increased leaf C:N

Table 1
Results of fully-factorial ANOVAs testing for the effects of light treatment, mussel treatment, and the interaction of light and mussel treatments on benthic biogeochemical conditions, eelgrass nutrient condition, sulfide intrusion of eelgrass, and eelgrass survival, growth, and energy stores. *P* values < 0.05 are shown in bold.

Source of variation	Light			Mussels			Light × mussels		
Response variable	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Benthic biogeochemical conditions									
Porewater ammonium	1, 20	8.542	**0.008**	1, 20	12.231	**0.002**	1, 20	6.48	**0.019**
Porewater dissolved organic carbon	1,19	2.469	0.133	1,19	0.822	0.376	1,19	0.096	0.761
Sediment acid-volatile sulfides	1,19	1.097	0.308	1,19	10.234	**0.005**	1,19	1.419	0.248
Eelgrass nutrient condition									
Leaf percent carbon	1, 20	7.693	**0.012**	1, 20	1.196	0.287	1, 20	0.574	0.458
Leaf percent nitrogen	1, 20	119.71	<**0.001**	1, 20	1.513	0.233	1, 20	0.757	0.395
Leaf carbon:nitrogen	1, 20	201.834	<**0.001**	1, 20	1.210	0.284	1, 20	0.597	0.449
Sulfide intrusion of eelgrass									
Leaf $F_{sulfide}$	1, 19	5.290	**0.033**	1, 19	17.754	<**0.001**	1, 19	7.751	**0.012**
Rhizome $F_{sulfide}$	1, 19	14.182	**0.001**	1, 19	13.755	**0.002**	1, 19	0.674	0.422
Root $F_{sulfide}$	1, 19	6.528	**0.019**	1, 19	0.005	0.945	1, 19	0.984	0.334
Root elemental sulfur[a]	1, 20	18.853	<**0.001**	1, 20	6.444	**0.020**	1, 20	0.598	0.448
Leaf $\delta^{34}S$	1, 20	2.412	0.136	1, 20	22.329	<**0.001**	1, 20	15.882	<**0.001**
Rhizome $\delta^{34}S$	1, 20	18.629	<**0.001**	1, 20	16.665	<**0.001**	1, 20	2.433	0.134
Root $\delta^{34}S$	1, 20	5.536	**0.029**	1, 20	0.641	0.433	1, 20	1.329	0.263
Leaf total sulfur	1, 20	20.262	<**0.001**	1, 20	2.427	0.135	1, 20	0.153	0.700
Rhizome total sulfur	1, 20	11.595	**0.003**	1, 20	7.608	**0.012**	1, 20	1.249	0.277
Root total sulfur	1, 20	2.111	0.162	1, 20	0.076	0.786	1, 20	1.918	0.181
Eelgrass survival, growth, and energy stores									
Total shoot density	1, 20	139.401	<**0.001**	1, 20	1.024	0.324	1, 20	0.000	1.000
Terminal shoot density	1, 20	99.011	<**0.001**	1, 20	0.309	0.584	1, 20	1.104	0.306
Lateral shoot density[a]	1, 20	198.416	<**0.001**	1, 20	0.218	0.646	1, 20	1.422	0.247
Leaf growth rate	1, 20	211.888	<**0.001**	1, 20	2.235	0.151	1, 20	0.553	0.466
Rhizome soluble sugars	1, 20	218.559	<**0.001**	1, 20	0.059	0.810	1, 20	0.383	0.543
Rhizome starches	1, 20	0.391	0.539	1, 20	0.018	0.895	1, 20	<0.001	0.999

[a] Variables log-transformed [$\ln(y+1)$] prior to analysis.

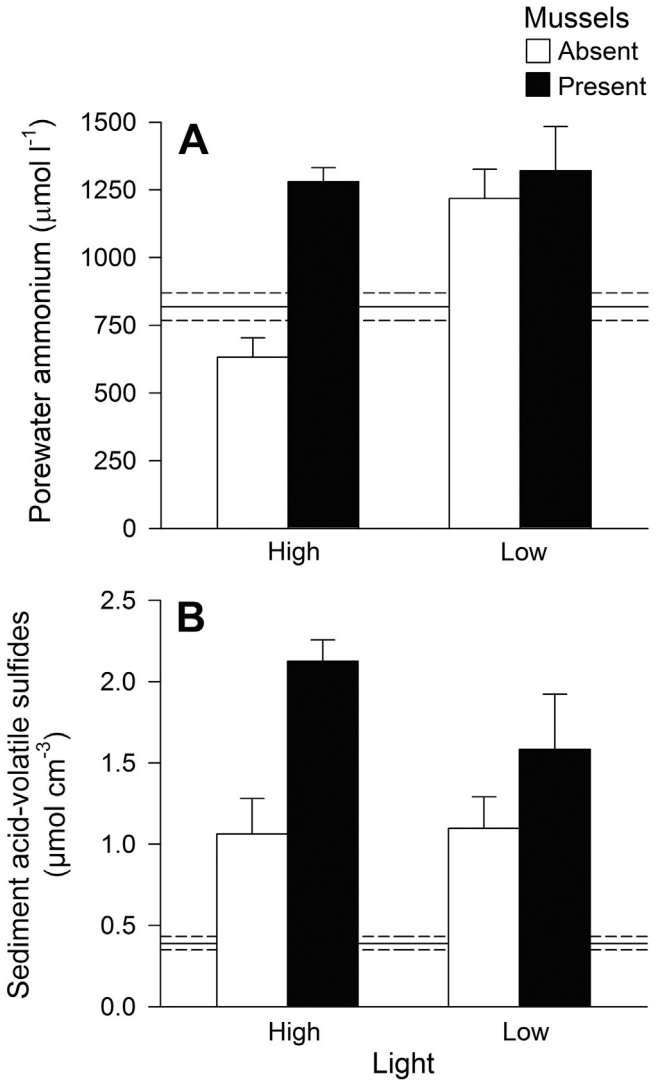

Fig. 1. Effects of light and mussels on benthic biogeochemical conditions. Mean (+1 SE) concentrations of (A) porewater ammonium (μmol L^{-1}) and (B) sediment acid-volatile sulfides (μmol cm^{-3} sediment). Horizontal solid and dashed lines indicate mean ± SE, respectively, for sediments and porewaters collected at the beginning of the experiment from control mesocosms.

(Fig. 3B), while mussels had no effects on these variables. Porewater ammonium was positively correlated with leaf nitrogen ($P = 0.0205$, $R^2 = 22.1\%$) but not leaf growth, regardless of light treatment ($P = 0.4996$ and $P = 0.3453$ for high light and low light, respectively).

Table 2
Results of fully-factorial repeated-measures ANOVAs testing for the effects of light treatment, mussel treatment, time (i.e., week of sampling), and interactions between all factors on net community production (NCP) and community respiration (CR). *P* values < 0.05 are shown in bold.

Response variable	NCP			CR		
Source of variation	df	*F*	*P*	df	*F*	*P*
Light	1, 8	359.389	<**0.0001**	1, 8	304.132	<**0.0001**
Mussels	1, 8	158.986	<**0.0001**	1, 8	75.856	<**0.0001**
Light × mussels	1, 8	6.085	**0.0389**	1, 8	0.271	0.6167
Time	3, 6	2.343	0.1724	3, 6	0.129	0.9392
Time × light	3, 6	1.152	0.4019	3, 6	0.164	0.9169
Time × mussels	3, 6	0.749	0.5615	3, 6	4.388	0.0587
Time × light × mussels	3, 6	0.289	0.8320	3, 6	0.898	0.4948

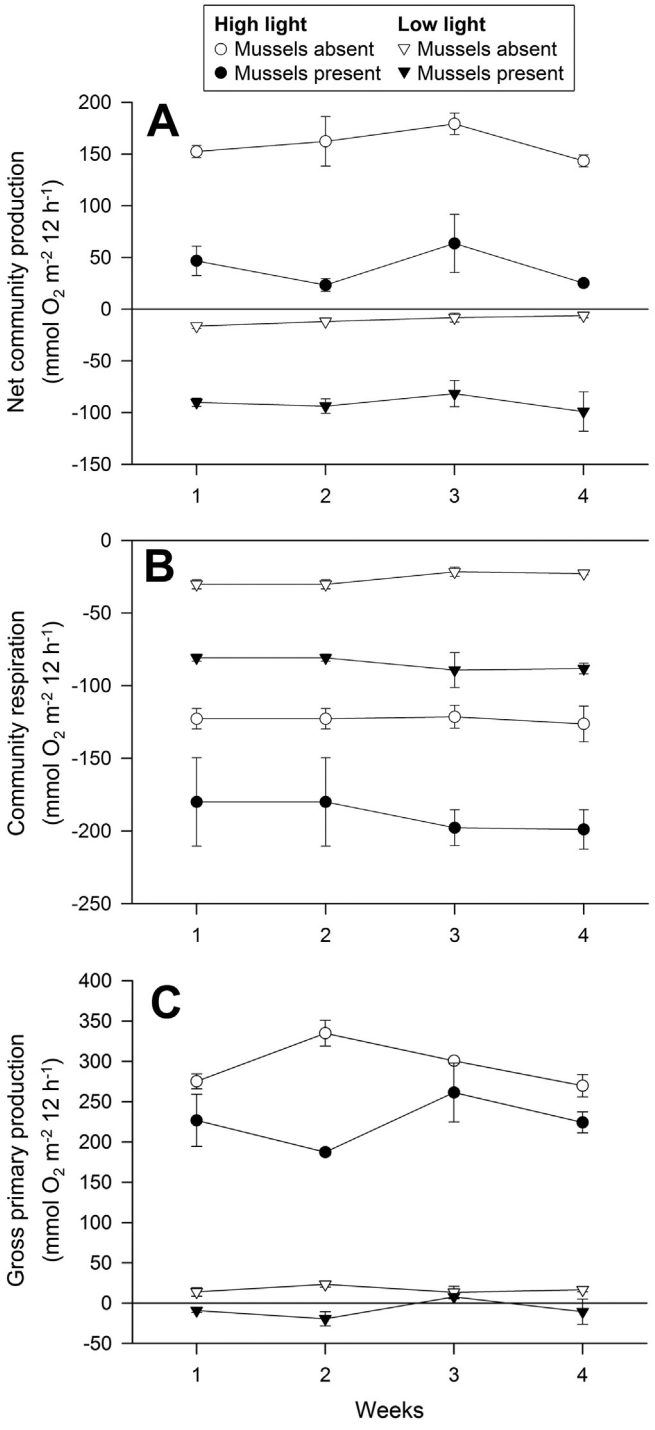

Fig. 2. Time series of (A) net community production (NCP), (B) community respiration (CR), and (C) gross primary production (GPP = NCP + |CR|), expressed as mean oxygen flux ± SE. Note that more positive NCP values indicate greater production, whereas more negative CR values indicate greater respiration. Small negative GPP values in mesocosms under low light with mussels are likely caused by measurement inaccuracies. Symbols indicate separate treatments. Note differences in the scale of *y*-axes.

Mussels and light both caused changes in sulfide intrusion of eelgrass (evidenced by $F_{sulfide}$, root S^0, $\delta^{34}S$, and TS (Tables 1 and 3)). $F_{sulfide}$ was greatest for roots (range = 23–85%), followed by rhizomes (13–45%) and leaves (4–23%). In high light, mussels increased sulfide intrusion of leaves ($F_{sulfide}$ (Fig. 4A) and $\delta^{34}S$), while in low light leaf sulfide intrusion was high regardless of mussel treatment (low light increased leaf TS irrespective of mussel treatment). Low light and mussels increased sulfide intrusion of rhizomes ($F_{sulfide}$ (Fig. 4C), $\delta^{34}S$,

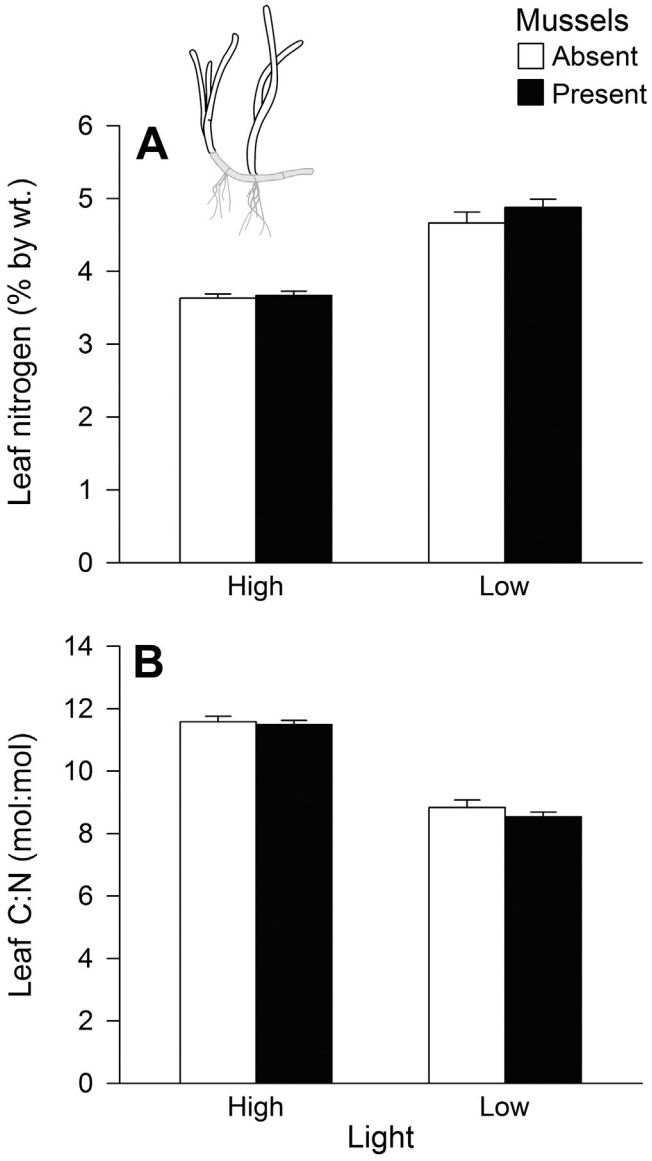

Fig. 3. Effects of light and mussels on eelgrass nutrient condition. Mean (+1 SE) leaf tissue (A) nitrogen content (percent by weight) and (B) carbon-to-nitrogen ratio (mol:mol).

and TS). Root $F_{sulfide}$ (Fig. 4E) and $\delta^{34}S$ were highly variable, with effects of light but not mussels (neither affected root TS). However, low light and mussels both increased root S^0 concentrations (Fig. 5A).

Sulfide intrusion of eelgrass tissues was only partly driven by sediment sulfides. AVS was positively correlated with leaf $F_{sulfide}$ ($P = 0.0019$, $R^2 = 37.6\%$) but not rhizome $F_{sulfide}$ ($P = 0.0619$) or root $F_{sulfide}$ ($P = 0.0837$). Instead, sulfide intrusion was primarily influenced by oxygen flux during day (i.e., NCP), but not night (i.e., CR). NCP explained substantial $F_{sulfide}$ variation for all tissue types (leaves: $P = 0.0004$, $R^2 = 45.8\%$, Fig. 4B; rhizomes: $P < 0.0001$, $R^2 = 56.4\%$, Fig. 4D; roots: $P = 0.0418$, $R^2 = 18.3\%$, Fig. 4F). In contrast, CR was not correlated with leaf $F_{sulfide}$ ($P = 0.9967$) or rhizome $F_{sulfide}$ ($P = 0.2797$), and only modestly correlated with root $F_{sulfide}$ ($P = 0.0443$, $R^2 = 17.9\%$). Root S^0 was not correlated with sediment AVS ($P = 0.9088$), but strongly negatively correlated with NCP ($P < 0.0001$, $R^2 = 59.6\%$, Fig. 5B).

3.4. Eelgrass survival, growth, and energy stores

Despite mussel impacts on benthic biogeochemical conditions, oxygen consumption, and sulfide intrusion of eelgrass tissues, light drove all differences in eelgrass survival, growth, and energy stores (Table 1). Shoot density had a stable-to-increasing trend under high light, while eelgrass deteriorated under low light, particularly after about 3 weeks (Fig. 6A). Declines in shoot density in low light were driven by mortality of (transplanted) terminal shoots (Fig. 6B) and near-absence of (new) lateral shoot propagation (Fig. 6C). By contrast, nearly all eelgrass survived in high light and propagated an average of 10 ± 4.3 new shoots per mesocosm. Similarly, leaf growth (Fig. 6D) and rhizome soluble sugars (Fig. 6E) were over six times greater in high light than low light, but were unaffected by mussels. Rhizome starch concentrations were unaffected by treatments (Table 1).

4. Discussion

Environmental context can alter the degree of habitat modification and the sign and strength of subsequent species interactions in plant and animal communities (e.g., Menge and Sutherland, 1987; Bertness and Hacker, 1994; Connolly and Roughgarden, 1999; Norkko et al., 2006), but complex interactions can arise when abiotic conditions simultaneously affect multiple stress pathways (Kawai and Tokeshi, 2007). In this study, light availability indirectly mediated blue mussel habitat modification by altering plant metabolism, and these biogeochemical changes (i.e., sulfide and oxygen concentrations) fed back to influence eelgrass vulnerability to sulfide stress but not eelgrass performance. Light modified the effect of mussels on porewater ammonium (Fig. 1A), but eelgrass was not nutrient limited (Fig. 3) and, therefore, mussels did not enhance growth (Fig. 6). Mussels increased sediment sulfides irrespective of light availability (Fig. 1B). Light, mussels, and their interaction strongly affected oxygen fluxes (Fig. 2) and these in turn regulated sulfide intrusion of eelgrass tissues (Figs. 4 and 5; Table 1). Light mediated the effect of mussels on sulfide intrusion of leaves, but not rhizomes or roots, which were governed by light and

Table 3
Summary of $\delta^{34}S$ (‰) for sediment acid-volatile sulfides (AVS) and leaf, rhizome, and root tissues, and total sulfur (TS; μmol [g dry tissue]$^{-1}$) for leaf, rhizome, and root tissues as a function of light and mussel treatments. Values are reported as mean ± SD.

Light treatment	High light		Low light	
Mussel treatment	Absent	Present	Absent	Present
Response variable				
$\delta^{34}S$ (‰)				
Sediment AVS	−24.41 ± 3.97	−20.56 ± 3.28	−22.94 ± 4.59	−19.43 ± 4.17
Leaf	16.59 ± 0.63	12.18 ± 2.03	13.79 ± 0.95	13.41 ± 0.84
Rhizome	13.08 ± 0.48	8.79 ± 2.30	8.61 ± 2.28	6.69 ± 1.79
Root	1.93 ± 5.86	0.90 ± 5.04	−8.27 ± 8.62	−2.59 ± 8.32
TS (μmol [g dry tissue]$^{-1}$)				
Leaf	121.90 ± 7.05	137.06 ± 18.04	159.97 ± 24.65	169.05 ± 21.69
Rhizome	84.06 ± 11.06	123.41 ± 35.53	129.98 ± 22.48	146.63 ± 24.14
Root	148.51 ± 22.01	196.61 ± 51.17	230.72 ± 102.41	198.58 ± 80.89

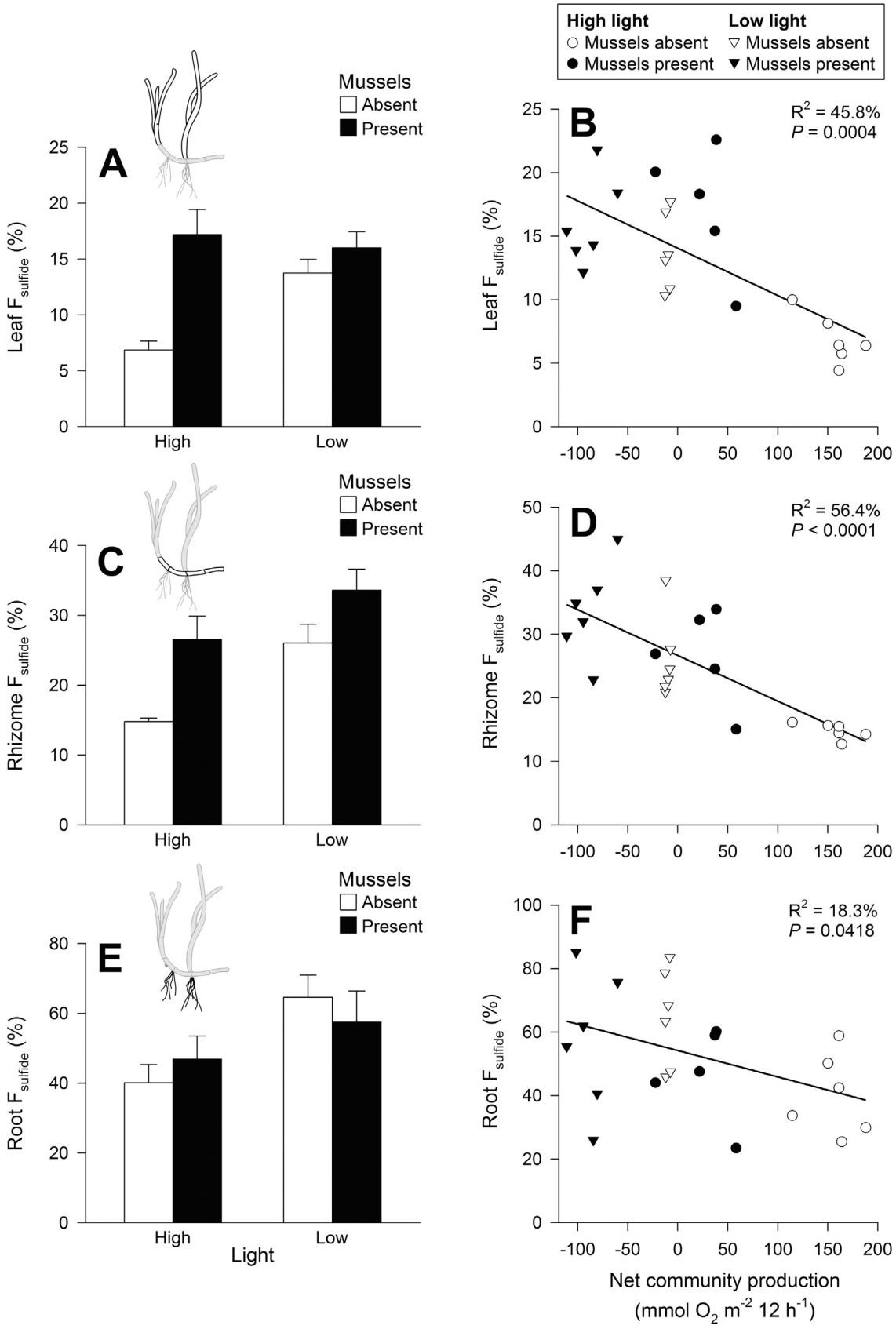

Fig. 4. Effects of light and mussels on sulfide intrusion of eelgrass tissues, measured as $F_{sulfide}$. Panels A, C, and E show mean (+1 SE) $F_{sulfide}$ (%) in leaves, rhizomes, and roots, respectively. Panels B, D, and F show linear regressions between net community production (mmol O_2 m^{-2} 12 h^{-1}) and $F_{sulfide}$ in leaves, rhizomes, and roots, respectively. Symbols indicate separate treatments. Note differences in the scale of *y*-axes.

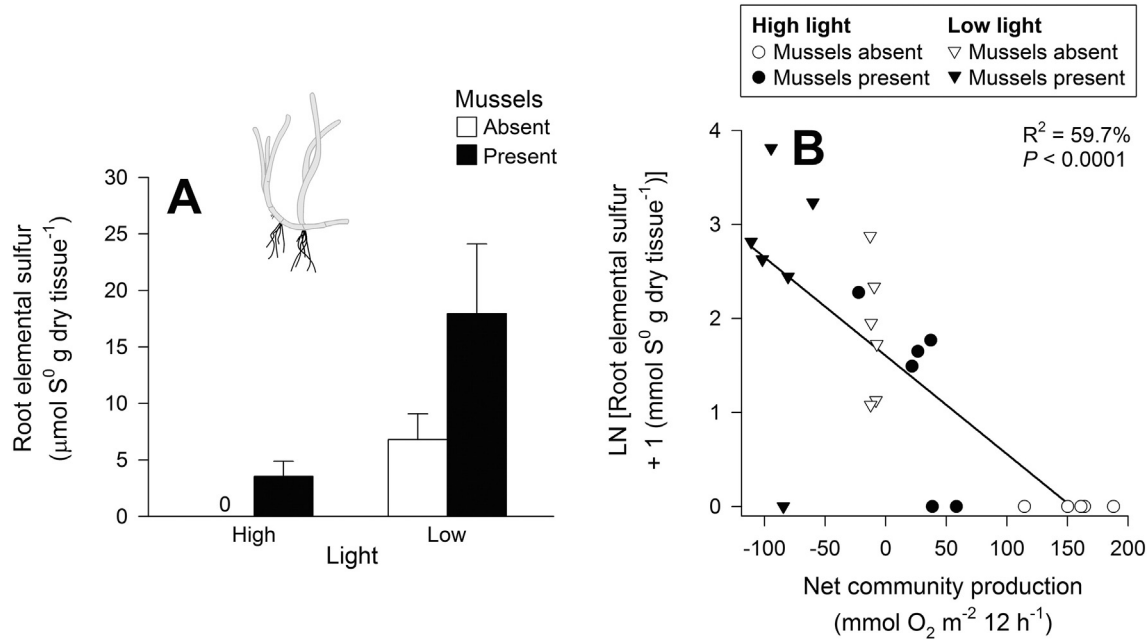

Fig. 5. Effects of light and mussels on sulfide intrusion of eelgrass roots, measured as root elemental sulfur (S^0). Panel A shows mean (+1 SE) root S^0 (µmol [g dry tissue]$^{-1}$). Panel B shows linear regression between net community production (mmol O_2 m^{-2} 12 h^{-1}) and root S^0 (log-transformed, ln(y + 1)). Symbols indicate separate treatments.

mussels without an interaction of these factors (Figs. 4 and 5; Table 1). Surprisingly, sulfide stress did not affect plant survival, growth, or energy stores, which were categorically impaired by low light but unaffected by mussels (Fig. 6). Thus, although low light and mussels exacerbate sulfide stress, eelgrass is capable of resisting harm, at least over short time scales (e.g., weeks).

4.1. Nutrient dynamics in sediments and eelgrass

Results from this study indicate that the potential for blue mussels to enhance sediment nutrients depends on light availability. Suspension-feeding bivalves are capable of enhancing porewater nutrients by capturing phytoplankton and suspended particulates, and transferring this organic material to the sediment via biodeposition (Haven and Morales-Alamo, 1966; Bertness, 1984; Kautsky and Evans, 1987). Severe reductions in light availability have been associated with increases in blue mussel feeding and biodeposition (Nielsen and Strömgren, 1985). Here, however, mussel biodeposition increased porewater ammonium in high light, whereas concentrations were high regardless of mussel presence in low light (Fig. 1A). Thus, the interaction between mussels and light availability on porewater nutrients was likely due to reduced root uptake of ammonium by eelgrass (Dennison et al., 1987) and/or eelgrass mortality and decomposition of leaf litter (Pedersen et al., 1999) under low light availability, irrespective of mussel presence.

As expected, leaf nitrogen was positively correlated with porewater ammonium (Pedersen and Borum, 1993), but changes to nutrient availability among treatments did not translate into differences in eelgrass nutrient condition (Fig. 3), probably because nitrogen supply exceeded plant demand (Dennison et al., 1987). Instead, light availability primarily drove patterns of leaf nitrogen. Nitrogen content may have been diluted by greater growth in high-light plants, whereas low-light plants with impaired photosynthesis and reduced carbon demand may have stored nitrogen in their tissues instead of assimilating it into new growth, resulting in leaves with greater nitrogen and lower C:N (Moore and Wetzel, 2000). Furthermore, porewater nutrients were not correlated with leaf growth, regardless of light treatment. Mussels probably did not enhance eelgrass growth because for all treatments ammonium concentrations were above levels at which eelgrass growth saturates (~100 µmol NH_4^+ [L porewater]$^{-1}$; Dennison et al., 1987; Williams and Ruckelshaus, 1993). Water-column and porewater nutrient concentrations in this experiment were similar to those found in many eelgrass beds of eutrophic regions, such as in the Baltic and North Seas (Pedersen and Borum, 1993; Nielsen et al., 2002; Govers et al., 2014), suggesting that similar dynamics may occur in the field.

4.2. Sulfur dynamics, oxygen fluxes, and sulfide intrusion of eelgrass

Blue mussels enhanced sediment sulfide concentrations irrespective of light conditions (Fig. 1B). Mussels likely stimulated bacterially-mediated sulfate reduction by depositing organic matter (Kautsky and Evans, 1987), consuming oxygen (Fig. 2; Carlsson et al., 2010), and/or reducing oxygen flux across the sediment–water interface with the physical structure of their shells (Jørgensen, 1982). Interestingly, however, sediment sulfide concentrations were not the primary driver of sulfide intrusion into eelgrass tissues. AVS pools explained $F_{sulfide}$ patterns in leaves, but not rhizomes or roots. Instead, NCP (i.e., daytime oxygen flux; Fig. 2A) was the best predictor of sulfide intrusion (Figs. 4B, D, F, and 5B). Because estuarine sediments are mostly anoxic and reduced, seagrass roots and rhizomes experience periods of oxygen deprivation and rely on oxygen supplied internally by leaves via photosynthesis or externally by passive diffusion from the water column via aerenchyma (Borum et al., 2006). In the absence of sufficient oxygenation of belowground tissues, the oxidative barrier surrounding eelgrass roots deteriorates (Pedersen et al., 2004; Frederiksen and Glud, 2006), allowing for the invasion of toxic sulfides through root tips and eventually leading to meristem necrosis. In this experiment, eelgrass grown under low light produced less oxygen (Fig. 2A) and mussel respiration enhanced near-bottom oxygen consumption (Fig. 2B). Therefore, it is likely that sulfide intrusion was primarily driven by a combination of reduced eelgrass photosynthesis and mussel respiration.

Corroborating previous studies (e.g., Frederiksen et al., 2006), results here demonstrate that the risk of tissue intrusion by sediment sulfides is greatest for roots, intermediate for rhizomes, and lowest for leaves (Fig. 4). Mussels increased sulfide intrusion of leaves in high light, whereas intrusion was relatively high regardless of mussel presence in low light (Fig. 4A). For rhizomes, both low light and mussels exacerbated sulfide stress (Fig. 4B). For roots, it was expected that the fraction of eelgrass tissue sulfur derived from sediment sulfides ($F_{sulfide}$) might be

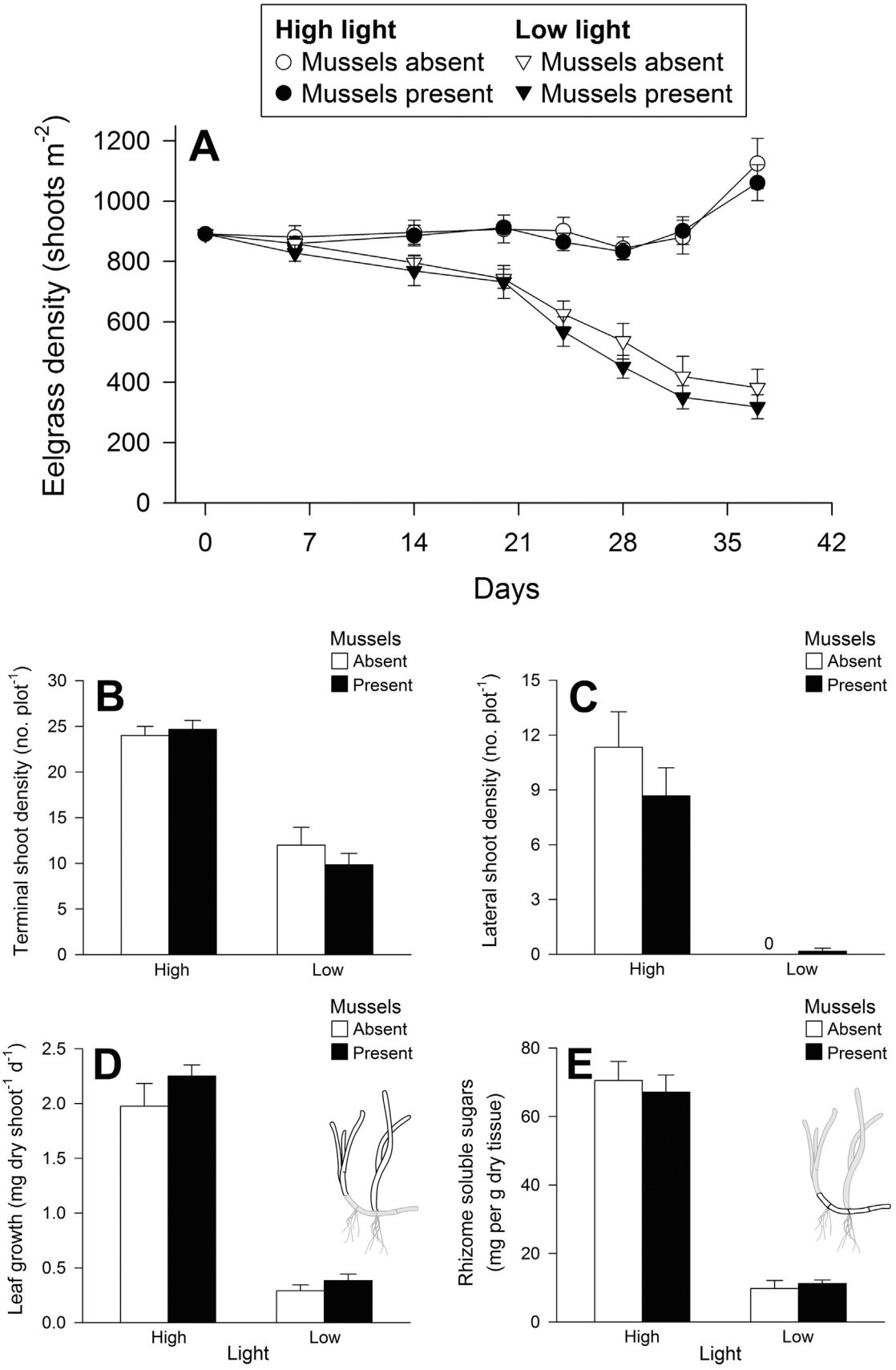

Fig. 6. Effects of light and mussels on eelgrass density, growth, and energy stores. (A) Time series of mean (±SE) eelgrass density (shoots m^{-2}), where symbols indicate separate treatments. Panels B–E show mean (+1 SE) terminal (B) and lateral (C) shoot density (no. of shoots $mesocosm^{-1}$), (D) leaf growth rate (mg dry $shoot^{-1}$ d^{-1}), and (E) soluble sugar concentration in rhizome tissues (mg per g dry tissue).

highly variable (Fig. 4E); eelgrass roots often have highly dynamic $\delta^{34}S$ due to the heterogeneous nature of porewater dissolved sulfide pools (Oakes and Connolly, 2004). However, despite highly variable root $F_{sulfide}$, the concentrations of elemental sulfur (S^0) in root tissues clearly indicate both low light and mussels enhanced sulfide intrusion (Fig. 5A). Both indicators of sulfide intrusion that were measured in this mesocosm study ($F_{sulfide}$ and root S^0) are similar to values observed in situ for *Zostera marina* (Frederiksen et al., 2006; Borum et al., 2013; Holmer and Hasler-Sheetal, 2014), suggesting that similar sulfur dynamics may occur in coastal regions of the North and Baltic Seas.

These findings suggest that mussel impacts on eelgrass depend on environmental context. Mussel-driven enhancement of sediment sulfides may enhance the risk of sulfide intrusion only when eelgrass productivity is reduced (e.g., under low light availability) or water-column dissolved oxygen concentrations are diminished (e.g., during hypoxic events). Eelgrass living among mussels may be at greatest risk of sulfide stress in low light environments, such as in turbid estuaries or near the lower depth range for eelgrass, where mussels might limit colonization. Low water clarity and benthic hypoxia commonly accompany eutrophication (Cloern, 2001; Howarth et al., 2011), suggesting that coastal nutrient pollution may mediate mussel effects on eelgrass sulfide stress. This possibility is particularly concerning because eutrophication has increased in temperate estuaries worldwide, including regions where eelgrass and mussels frequently co-occur in the North Atlantic Ocean, North Sea, and Baltic Sea (Burkholder et al., 2007; Howarth et al., 2011, and references therein).

4.3. Eelgrass survival, growth, and energy stores

Sulfide intrusion of eelgrass is typically accompanied by a reduction in photosynthetic activity (Goodman et al., 1995), growth (Holmer et al., 2005), and rhizome sugar reserves (Holmer and Bondgaard, 2001). While estimates of sulfide toxicity vary widely (Lamers et al., 2013; Hasler-Sheetal and Holmer, 2015), the levels of sulfide intrusion measured in this study are indicative of harm (Frederiksen et al., 2008; Borum et al., 2013). Nevertheless, while light and mussels both influenced sulfide intrusion of eelgrass, mussels did not affect eelgrass nutrient content, growth, rhizome energy stores, or survival (Figs. 3 and 6). Instead, light availability drove all changes in these responses irrespective of mussel presence. Not surprisingly, eelgrass thrived under high light, growing quickly, propagating new lateral shoots, and accumulating excess sugars in their rhizomes. Conversely, plants under low light grew slowly, suffered high shoot mortality, propagated few lateral shoots, and nearly exhausted their energy stores.

The findings presented here suggest that during short periods (i.e., days to weeks), eelgrass is resistant to sulfide stress caused by mussels, even under low-light conditions. However, this conclusion may be contingent on the temporal scale of study. While sulfide intrusion was relatively substantial, even the highest sediment sulfide concentrations in mesocosms in this study were modest when compared to eelgrass sediments in many field settings (e.g., Frederiksen et al., 2006; Holmer and Nielsen, 2007; Holmer, 2009; Borum et al., 2013). Therefore, had this experiment been longer in duration or higher in temperature (which reduces oxygen solubility and enhances the rate of sulfate reduction; Robador et al., 2009), it is possible that mussel biodeposition could have caused an accumulation of sediment sulfides and cumulative harm to eelgrass. In the field, mussels may also have stronger negative effects on eelgrass seedlings, which are more sensitive to sulfide stress than adults (Dooley et al., 2013; Jovanovic et al., 2015). Lastly, mesocosms in this study contained sandy, low-organic sediments typical of exposed Danish coastlines. The effects of mussels on sediment biogeochemical fluxes and feedbacks to eelgrass are perhaps more subtle in systems with high-organic silts and muds, such as low-energy fjords, which are typically higher in porewater nutrients and sulfides, and lower in dissolved oxygen (Frederiksen et al., 2006; Carstensen et al., 2013).

4.4. Context-dependent effects of bivalves on seagrasses

Despite intensive research efforts, generalizations about the effects of suspension-feeding bivalves on seagrasses remain elusive. Findings from this and prior studies indicate that although bivalve habitat modification and impacts on seagrasses appear to vary based on environmental context, they are not deterministically governed by light or ambient nutrient concentrations. The novel results described here helps to resolve this puzzle, but further experiments are needed to disentangle the complex physical and biogeochemical mechanisms that mediate bivalve–seagrass interactions.

In oligotrophic waters (<~150 μmol NH_4^+ [L porewater]$^{-1}$), where seagrasses can be nutrient limited (Dennison et al., 1987; Williams and Ruckelshaus, 1993), bivalves are capable of facilitating seagrass growth. For example, blue mussels (Reusch et al., 1994) and hard clams (*Mercenaria mercenaria*; Carroll et al., 2008) can relieve nutrient limitation and enhance growth in eelgrass, even under moderate shading (Carroll et al., 2008). Similarly, by fertilizing porewaters, tulip mussels (*Modiolus americanus*) can improve turtlegrass (*Thalassia testudinum*) leaf nutrient condition (Peterson and Heck, 1999) and growth (Peterson and Heck, 2001a,b). However, other studies have shown that enhancement of porewater ammonium by blue mussels, geoduck clams (*Panopea generosa*), and cultured oysters (*Crassostrea gigas*) does not affect eelgrass leaf nitrogen or growth (Worm and Reusch, 2000; Ruesink and Rowell, 2012; Wagner et al., 2012), despite low ambient nutrient concentrations (<30, <100, and <20 μmol NH_4^+ [L porewater]$^{-1}$, respectively). Impacts on eelgrass by non-native Asian nest mussels (*Arcuatula senhousia*) in an oligotrophic bay (<100 μmol NH_4^+ [L porewater]$^{-1}$) vary from facilitative to inhibitive depending on the physiological response considered (i.e., leaf vs. rhizome growth; Reusch and Williams, 1998). In fact, at least two studies have documented negative impacts of oysters (*Crassostrea virginica* and *C. gigas*) on seagrasses (shoal grass, *Halodule wrightii*, and eelgrass) in low nutrient (<~150 μmol NH_4^+ [L porewater]$^{-1}$) estuaries, possibly by stimulating sediment sulfides or physically competing for space (Dumbauld et al., 2009; Booth and Heck, 2009). Some of these apparent inconsistencies may be explained by variation in water-column nutrient availability, particularly in coastal upwelling regions such as in the eastern North Pacific, but further studies are needed to disentangle the interaction between benthic and pelagic nutrient fluxes, bivalve fertilization, and seagrass growth.

In contrast to studies in oligotrophic systems, experiments under eutrophic conditions (>~500 μmol NH_4^+ [L porewater]$^{-1}$) have found exclusively that bivalves negatively impact seagrasses by enhancing sulfate reduction and inducing sulfide stress (Vinther and Holmer, 2008; Vinther et al., 2008, 2012). This mechanism may help explain why, in some eutrophic regions of the Baltic Sea, blue mussels and eelgrass have an inverse spatial relationship in otherwise similar habitats (Vinther et al., 2012). The results from this experiment, which mimicked eutrophic conditions, generally support these earlier findings, improve the understanding of the biogeochemical mechanisms underpinning bivalve–seagrass interactions, and illustrate the potential role of light in governing some of these dynamics.

Based on comparisons between this and other studies, the effects of bivalves on seagrasses over small spatial and temporal scales may be variable in oligotrophic ecosystems, where light availability is usually high and nutrients can limit productivity, but are likely to be negative in high-nutrient estuaries, where the potential for fertilization is minimal but the risk for sulfide stress is high due to reduced light availability and oxygen concentration that typically accompany eutrophication (Cloern, 2001; Howarth et al., 2011). Seagrasses generally facilitate bivalves by fostering settlement (Bologna and Heck, 2000), improving recruitment (Peterson, 1986; Reusch, 1998), enhancing growth (Peterson et al., 1984), and offering refuges from predation (Peterson, 1982; Irlandi, 1994) and storm disturbance (Reusch and Chapman, 1995). In

eutrophic systems, however, the results presented here suggest that this facilitation could ultimately tip the balance away from seagrass dominance via increased sulfide stress.

4.5. Caveats

It is important to consider a few caveats to these conclusions. First, mesocosm experiments are, by design, controlled approximations of real ecosystems. Several factors excluded in this study, such as tides, strong hydrodynamic mixing, continuous diel changes in light availability, bioturbation by infauna, and predator effects on bivalve populations and feeding behavior, may play important roles in mediating bivalve habitat modification and impacts on seagrasses.

Second, if bivalve structure plays an important role in driving sulfate reduction by altering porewater advection and diffusion (Jørgensen, 1982), surface-dwelling (e.g., *Crassostrea* spp., *Mytilus* spp.) and burrowing (e.g., *Cerastoderma* spp., *Macoma* spp., *Mercenaria* spp.) bivalves may have very different impacts on seagrasses under eutrophication. Furthermore, relative to surface-dwelling bivalves, burrowing bivalves may have fundamentally different impacts on sediment biogeochemistry by enhancing sediment turnover and increasing porewater irrigation, thus improving remineralization (Aller, 1994) and sulfide oxidation (Jørgensen, 1982).

Third, this experiment tested seagrass–bivalve interactions on very small spatial and temporal scales. Generalizing from small, short-term experiments to scales of space and time with greater relevance is a central challenge in ecology and ecosystem management (Levin, 1992; Thrush et al., 1997). Bivalves may inhibit seagrasses in eutrophic systems on small scales (e.g., cm^2 to 10 s of m^2) by causing sulfide stress, but at landscape to ecosystem scales (e.g., hectares to 1000 s of km^2) bivalves may facilitate seagrasses by improving light penetration through filtration of the water-column (Wall et al., 2008). In fact, suspension-feeding bivalves are capable of reducing phytoplankton populations on the scales of entire estuaries (Officer et al., 1982; Newell and Koch, 2004; Ruesink et al., 2005), although there may be limits to this top-down control (Pomeroy et al., 2006). Due to the increasing eutrophy of temperate estuaries worldwide (Cloern, 2001; Howarth et al., 2011), it is possible that bivalve–seagrass interactions may be commonly represented by simultaneous small-scale inhibition (via increased sulfide stress) and large-scale facilitation (via decreased light stress).

5. Conclusions

Seagrass populations, including eelgrass, are rapidly declining worldwide due in large part to accelerating coastal eutrophication (Orth et al., 2006; Burkholder et al., 2007; Waycott et al., 2009; Howarth et al., 2011). Concurrently, human activities have dramatically changed the abundance and composition of bivalve assemblages in and near seagrass meadows through commercial aquaculture (Pawiro, 2010), overharvest of wild populations (Newell, 1998; Cloern, 2001), restoration or mitigation of water quality using bivalves (Petersen et al., 2014), and introduction of non-native bivalves, which sometimes compete directly or indirectly with native species (Ruesink et al., 2005; Trimble et al., 2009; Castorani and Hovel, 2015). Thus, understanding how bivalves and eutrophication interactively impact seagrasses is important to ecosystem-based management and conservation of estuaries.

Results from this and earlier studies indicate that blue mussels enhance sulfate reduction (Vinther and Holmer, 2008; Vinther et al., 2008, 2012) and induce sulfide stress in eelgrass by depositing organic matter (Kautsky and Evans, 1987), increasing community respiration (Carlsson et al., 2010), and/or reducing benthic oxygen flux (Jørgensen, 1982). By changing eelgrass metabolism, light mediates the impacts of these biogeochemical changes on eelgrass growth, survival, and energy storage. While reductions in light availability may increase sulfide intrusion of tissues, eelgrass appears to be capable of resisting harm from short-term stress, even during light limitation.

Experimental studies of bivalve–seagrass interactions under eutrophic conditions are largely limited to a single pair of species (*Z. marina* and *M. edulis*) within one region (the western Baltic Sea). Therefore, to determine the degree to which the hypotheses explored here can be generalized, future studies should assess bivalve–seagrass interactions in other eutrophic estuaries and across a broader taxonomic range. Future work should also aim to disentangle the conflated physical and chemical effects of eutrophication (e.g., light availability, nutrient concentrations in the porewater and water column, and dissolved oxygen saturation) in mediating bivalve impacts on seagrasses, as well as the potential for dependency on the spatial or temporal scales of bivalve-mediated changes.

Acknowledgments

The authors are indebted to B. Christensen, M. Flindt, A. Glud, K. Hancke, R.O. Holm, K.C. Kirkegaard, A.K.H. Lauesen, P. Martensen, H.U. Riisgård, and S.W. Thorsen for research assistance. K.A. Hovel and S.L. Williams provided critical advice on research design. J.P. Allem and K.J. Rice advised statistical analyses. Comments from K.A. Hovel, C.H. Peterson, M.A. Whalen, S.L. Williams, and two anonymous reviewers improved the manuscript. This project was funded by a U.S. National Science Foundation (NSF) Graduate Research Fellowship and a NSF–Danish National Research Foundation (DNRF) Nordic Research Opportunity Fellowship to M.C.N. Castorani. This is a contribution from the Nordic Center for Earth Evolution (Grant DNRF53). **[SS]**

References

Aller, R.C., 1994. Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation. Chem. Geol. 114, 331–345.
Alunno-Bruscia, M., Petraitis, P.S., Bourget, E., Fréchette, M., 2000. Body size–density relationship for *Mytilus edulis* in an experimental food regulated situation. Oikos 90, 28–42.
Alunno-Bruscia, M., Bourget, E., Fréchette, M., 2001. Shell allometry and length-mass-density relationship for *Mytilus edulis* in an experimental food-regulated situation. Mar. Ecol. Prog. Ser. 219, 177–188.
Bertness, M.D., 1984. Ribbed mussels and *Spartina alterniflora* production in a New England salt marsh. Ecology 65, 1794–1807.
Bertness, M.D., Callaway, R., 1994. Positive interactions in communities. Trends Ecol. Evol. 9, 191–193.
Bertness, M.D., Hacker, S.D., 1994. Physical stress and positive associations among marsh plants. Am. Nat. 144, 363–372.
Bologna, P.A.X., Heck, K.L., 2000. Impacts of seagrass habitat architecture on bivalve settlement. Estuar. Coasts 23, 449–457.
Bologna, P.A.X., Fetzer, M.L., McDonnel, S., Moody, E.M., 2005. Assessing the potential benthic-pelagic coupling in episodic blue mussel (*Mytilus edulis*) settlement events within eelgrass (*Zostera marina*) communities. J. Exp. Mar. Biol. Ecol. 316, 117–131.
Booth, D.M., Heck, K.L., 2009. Effects of the American oyster *Crassostrea virginica* on growth rates of the seagrass *Halodule wrightii*. Mar. Ecol. Prog. Ser. 389, 117–126. http://dx.doi.org/10.3354/meps08163.
Borum, J., Sand-Jensen, K., Binzer, T., Pedersen, O., Greve, T.M., 2006. Oxygen movement in seagrasses. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.), Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, The Netherlands, pp. 255–270.
Borum, J., Raun, A.L., Hasler-Sheetal, H., Pedersen, M.Ø., Pedersen, O., Holmer, M., 2013. Eelgrass fairy rings: sulfide as inhibiting agent. Mar. Biol. 161, 351–358. http://dx.doi.org/10.1007/s00227-013-2340-3.
Bower, C.E., Holm-Hansen, T., 1980. A salicylate–hypochlorite method for determining ammonia in seawater. Can. J. Fish. Aquat. Sci. 37, 794–798.
Burkholder, J.M., Tomasko, D.A., Touchette, B.W., 2007. Seagrasses and eutrophication. J. Exp. Mar. Biol. Ecol. 350, 46–72. http://dx.doi.org/10.1016/j.jembe.2007.06.024.
Carlsson, M.S., Glud, R.N., Petersen, J.K., 2010. Degradation of mussel (*Mytilus edulis*) fecal pellets released from hanging long-lines upon sinking and after settling at the sediment. Can. J. Fish. Aquat. Sci. 67, 1376–1387. http://dx.doi.org/10.1139/F10-067.
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. http://dx.doi.org/10.3354/meps07593.
Carstensen, J., Krause-Jensen, D., Markager, S., Timmermann, K., Windolf, J., 2013. Water clarity and eelgrass responses to nitrogen reductions in the eutrophic Skive Fjord, Denmark. Hydrobiologia 704, 293–309. http://dx.doi.org/10.1007/s10750-012-1266-y.
Castorani, M.C.N., Hovel, K.A., 2015. Invasive prey indirectly increase predation on their native competitors. Ecology 96, 1911–1922. http://dx.doi.org/10.1890/14-1538.1.

Castorani, M.C.N., Hovel, K.A., Williams, S.L., Baskett, M.L., 2014. Disturbance facilitates the coexistence of antagonistic ecosystem engineers in California estuaries. Ecology 95, 2277–2288. http://dx.doi.org/10.1890/13-1846.1.
Cline, J.D., 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14, 454–458.
Cloern, J., 2001. Our evolving conceptual model of the coastal eutrophication problem. Mar. Ecol. Prog. Ser. 210, 223–253.
Connolly, S.R., Roughgarden, J., 1999. Theory of marine communities: competition, predation, and recruitment-dependent interaction strength. Ecol. Monogr. 69, 277–296.
Dennison, W.C., 1987. Effects of light on seagrass photosynthesis, growth and depth distribution. Aquat. Bot. 27, 15–26. http://dx.doi.org/10.1016/0304-3770(87)90083-0.
Dennison, W.C., Alberte, R.S., 1985. Role of daily light period in the depth distribution of *Zostera marina* (eelgrass). Mar. Ecol. Prog. Ser. 25, 51–61. http://dx.doi.org/10.3354/meps025051.
Dennison, W.C., Aller, R.C., Alberte, R.S., 1987. Sediment ammonium availability and eelgrass (*Zostera marina*) growth. Mar. Biol. 94, 469–477.
Dooley, F.D., Wyllie-Echeverria, S., Roth, M.B., Ward, P.D., 2013. Tolerance and response of *Zostera marina* seedlings to hydrogen sulfide. Aquat. Bot. 105, 7–10. http://dx.doi.org/10.1016/j.aquabot.2012.10.007.
Dumbauld, B.R., Ruesink, J.L., Rumrill, S.S., 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.
Fenchel, T., Glud, R.N., 2000. Benthic primary production and O_2–CO_2 dynamics in a shallow water sediment: spatial and temporal heterogeneity. Ophelia 53, 159–171.
Fossing, H., Jørgensen, B.B., 1989. Measurement of bacterial sulfate reduction in sediments: evaluation of a single-step chromium reduction method. Biogeochemistry 8, 205–222.
Frederiksen, M.S., Glud, R.N., 2006. Oxygen dynamics in the rhizosphere of *Zostera marina*: a two-dimensional planar optode study. Limnol. Oceanogr. 51, 1072–1083.
Frederiksen, M., Krause-Jensen, D., Holmer, M., Laursen, J.S., 2004. Long-term changes in area distribution of eelgrass (*Zostera marina*) in Danish coastal waters. Aquat. Bot. 78, 167–181. http://dx.doi.org/10.1016/j.aquabot.2003.10.002.
Frederiksen, M.S., Holmer, M., Borum, J., Kennedy, H., 2006. Temporal and spatial variation of sulfide invasion in eelgrass (*Zostera marina*) as reflected by its sulfur isotopic composition. Limnol. Oceanogr. 51, 2308–2318.
Frederiksen, M.S., Holmer, M., Pérez, M., Invers, O., Ruiz, J.M., Knudsen, B.B., 2008. Effect of increased sediment sulfide concentrations on the composition of stable sulfur isotopes ($\delta^{34}S$) and sulfur accumulation in the seagrasses *Zostera marina* and *Posidonia oceanica*. J. Exp. Mar. Biol. Ecol. 358, 98–109. http://dx.doi.org/10.1016/j.jembe.2008.01.021.
Glud, R.N., Woelfel, J., Karsten, U., Kühl, M., Rysgaard, S., 2009. Benthic microalgal production in the Arctic: applied methods and status of the current database. Bot. Mar. 52, 559–571. http://dx.doi.org/10.1515/BOT.2009.074.
Goodman, J.L., Moore, K.A., Dennison, W.C., 1995. Photosynthetic responses of eelgrass (*Zostera marina* L.) to light and sediment sulfide in a shallow barrier island lagoon. Aquat. Bot. 50, 37–47.
Govers, L.L., de Brouwer, J.H., Suykerbuyk, W., Bouma, T.J., Lamers, L.P., Smolders, A.J., van Katwijk, M.M., 2014. Toxic effects of increased sediment nutrient and organic matter loading on the seagrass *Zostera noltii*. Aquat. Toxicol. 155, 253–260. http://dx.doi.org/10.1016/j.aquatox.2014.07.005.
Hasler-Sheetal, H., Holmer, M., 2015. Sulfide intrusion and detoxification in the seagrass *Zostera marina*. PLoS One 10 (6), e0129136.
Haven, D., Morales-Alamo, R., 1966. Aspects of biodeposition by oysters and other invertebrate filter feeders. Limnol. Oceanogr. 11, 487–498.
Holmer, M., 2009. Productivity and biogeochemical cycling in seagrass ecosystems. In: Perillo, G.M.E., Wolanski, E., Cahoon, D.R., Brinson, M.M. (Eds.), Coastal Wetlands: An Integrated Ecosystem Approach. Elsevier, Amsterdam, The Netherlands, pp. 377–401 http://dx.doi.org/10.1016/B978-0-444-53103-2.00013-2.
Holmer, M., Bondgaard, E.J., 2001. Photosynthetic and growth response of eelgrass to low oxygen and high sulfide concentrations during hypoxic events. Aquat. Bot. 70, 29–38.
Holmer, M., Frederiksen, M.S., 2007. Stimulation of sulfate reduction rates in Mediterranean fish farm sediments inhabited by the seagrass *Posidonia oceanica*. Biogeochemistry 85, 169–184. http://dx.doi.org/10.1007/s10533-007-9127-x.
Holmer, M., Hasler-Sheetal, H., 2014. Sulfide intrusion in seagrasses assessed by stable sulfur isotopes — a synthesis of current results. Front. Mar. Sci. 1, 64.
Holmer, M., Nielsen, R.M., 2007. Effects of filamentous algal mats on sulfide invasion in eelgrass (*Zostera marina*). J. Exp. Mar. Biol. Ecol. 353, 245–252. http://dx.doi.org/10.1016/j.jembe.2007.09.010.
Holmer, M., Frederiksen, M.S., Møllegaard, H., 2005. Sulfur accumulation in eelgrass (*Zostera marina*) and effect of sulfur on eelgrass growth. Aquat. Bot. 81, 367–379. http://dx.doi.org/10.1016/j.aquabot.2004.12.006.
Howarth, R., Chan, F., Conley, D.J., Garnier, J., Doney, S.C., Marino, R., Billen, G., 2011. Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. Front. Ecol. Environ. 9, 18–26.
Irlandi, E.A., 1994. Large- and small-scale effects of habitat structure on rates of predation: how percent coverage of seagrass affects rates of predation and siphon nipping on an infaunal bivalve. Oecologia 98, 176–183.
Jørgensen, B.B., 1982. Mineralization of organic matter in the sea bed—the role of sulphate reduction. Nature 296, 643–645.
Jovanovic, Z., Pedersen, M.Ø., Larsen, M., Kristensen, E., Glud, R.N., 2015. Rhizosphere O_2 dynamics in young *Zostera marina* and *Ruppia maritima*. Mar. Ecol. Prog. Ser. 518, 95–105. http://dx.doi.org/10.3354/meps11041.
Kautsky, N., Evans, S., 1987. Role of biodeposition by *Mytilus edulis* in the circulation of matter and nutrients in a Baltic coastal ecosystem. Mar. Ecol. Prog. Ser. 38, 201–212.
Kawai, T., Tokeshi, M., 2007. Testing the facilitation–competition paradigm under the stress-gradient hypothesis: decoupling multiple stress factors. Proc. R. Soc. B Biol. Sci. 274, 2503–2508. http://dx.doi.org/10.1098/rspb.2007.0871.
Kristensen, P.S., 2002. Blue mussels (*Mytilus edulis* L.) in Little Belt in 1995 (fishing zones 40–44). Biomass and Fishery. DFU-Rapport Nr. 101-01p. 74 (in Danish).
Kristensen, E., Anderson, F.Ø., 1987. Determination of organic carbon in marine sediments: a comparison of two CHN-analyzer methods. J. Exp. Mar. Biol. Ecol. 109, 15–23. http://dx.doi.org/10.1016/0022-0981(87)90182-1.
Lamers, L.P.M., Govers, L.L., Janssen, I.C.J.M., Geurts, J.J.M., Van der Welle, M.E.W., Van Katwijk, M.M., Van der Heide, T., Roelofs, J.G.M., Smolders, A.J.P., 2013. Sulfide as a soil phytotoxin—a review. Front. Plant Sci. 4, 268. http://dx.doi.org/10.3389/fpls.2013.00268.
Levin, S.A., 1992. The problem of pattern and scale in ecology. Ecology 73, 1943–1967.
Mascaró, O., Valdemarsen, T., Holmer, M., Pérez, M., Romero, J., 2009. Experimental manipulation of sediment organic content and water column aeration reduces *Zostera marina* (eelgrass) growth and survival. J. Exp. Mar. Biol. Ecol. 373, 26–34. http://dx.doi.org/10.1016/j.jembe.2009.03.001.
Menge, B.A., Sutherland, J.P., 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. Am. Nat. 130, 730–757. http://dx.doi.org/10.2307/2461716.
Moore, K.A., Wetzel, R.L., 2000. Seasonal variations in eelgrass (*Zostera marina* L.) responses to nutrient enrichment and reduced light availability in experimental ecosystems. J. Exp. Mar. Biol. Ecol. 244, 1–28.
Newell, R.I.E., 1998. Ecological changes in Chesapeake Bay: are they the result of overharvesting the American oyster, *Crassostrea virginica*? Understanding the Estuary. Advances in Chesapeake Bay Research. US EPA CBP/TRS 24/88. CRC Publication 129. Chesapeake Bay Consortium. Solomons, Maryland, USA, pp. 536–546
Newell, R.I.E., Koch, E.W., 2004. Modeling seagrass density and distribution in response to changes in turbidity stemming from bivalve filtration and seagrass sediment stabilization. Estuaries 27, 793–806.
Nielsen, M.V., Strömgren, T., 1985. The effect of light on the shell length growth and defaecation rate of *Mytilus edulis* (L.). Aquaculture 47, 205–211. http://dx.doi.org/10.1016/0044-8486(85)90066-3.
Nielsen, S.L., Sand-Jensen, K., Borum, J., Geertz-Hansen, O., 2002. Phytoplankton, nutrients, and transparency in Danish coastal waters. Estuar. Coasts 25, 930–937.
Norkko, A., Hewitt, J.E., Thrush, S.F., Funnell, G.A., 2006. Conditional outcomes of facilitation by a habitat-modifying subtidal bivalve. Ecology 87, 226–234. http://dx.doi.org/10.1890/05-0176.
Oakes, J.M., Connolly, R.M., 2004. Causes of sulfur isotope variability in the seagrass, *Zostera capricorni*. J. Exp. Mar. Biol. Ecol. 302, 153–164. http://dx.doi.org/10.1016/j.jembe.2003.10.011.
Officer, C.B., Smayda, T.J., Mann, R., 1982. Benthic filter feeding: a natural eutrophication control. Mar. Ecol. Prog. Ser. 9, 203–210.
Olesen, B., Sand-Jensen, K., 1994a. Biomass-density patterns in the temperate seagrass *Zostera marina*. Mar. Ecol. Prog. Ser. 109, 283–291. http://dx.doi.org/10.3354/meps109283.
Olesen, B., Sand-Jensen, K., 1994b. Demography of shallow eelgrass (*Zostera marina*)—shoot dynamics and biomass development populations. J. Ecol. 82, 379–390.
Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L.J., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006. A global crisis for seagrass ecosystems. Bioscience 56, 987–996. http://dx.doi.org/10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2.
Pawiro, S., 2010. Bivalves: global production and trade trends. In: Rees, G., Pond, K., Kay, D., Bartram, J., Santo Domingo, J. (Eds.), Safe Management of Shellfish and Harvest Waters. IWA Publishing on behalf of the World Health Organization, London, U.K., pp. 11–19.
Pedersen, M.F., Borum, J., 1993. An annual nitrogen budget for a seagrass *Zostera marina* population. Mar. Ecol. Prog. Ser. 101, 169–177.
Pedersen, A.-G.U., Berntsen, J., Lomstein, B.A., 1999. The effect of eelgrass decomposition on sediment carbon and nitrogen cycling: a controlled laboratory experiment. Limnol. Oceanogr. 44, 1978–1992. http://dx.doi.org/10.4319/lo.1999.44.8.1978.
Pedersen, O., Binzer, T., Borum, J., 2004. Sulphide intrusion in eelgrass (*Zostera marina* L.). Plant Cell Environ. 27, 595–602.
Petersen, J.K., Hasler, B., Timmermann, K., Nielsen, P., Tørring, D.B., Larsen, M.M., Holmer, M., 2014. Mussels as a tool for mitigation of nutrients in the marine environment. Mar. Pollut. Bull. 82, 137–143. http://dx.doi.org/10.1016/j.marpolbul.2014.03.006.
Peterson, C.H., 1982. Clam predation by whelks (*Busycon* spp.): experimental tests of the importance of prey size, prey density, and seagrass cover. Mar. Biol. 66, 159–170. http://dx.doi.org/10.1007/BF00397189.
Peterson, C.H., 1986. Enhancement of *Mercenaria mercenaria* densities in seagrass beds: is pattern fixed during settlement season or altered by subsequent differential survival? Limnol. Oceanogr. 31, 200–205.
Peterson, B.J., Heck, K.L.J., 1999. The potential for suspension feeding bivalves to increase seagrass productivity. J. Exp. Mar. Biol. Ecol. 240, 37–52. http://dx.doi.org/10.1016/S0022-0981(99)00040-4.
Peterson, B.J., Heck, K.L.J., 2001a. An experimental test of the mechanism by which suspension feeding bivalves elevate seagrass productivity. Mar. Ecol. Prog. Ser. 218, 115–125.
Peterson, B.J., Heck, K.L.J., 2001b. Positive interactions between suspension-feeding bivalves and seagrass — a facultative mutualism. Mar. Ecol. Prog. Ser. 213, 143–155.
Peterson, C.H., Summerson, H.C., Duncan, P.B., 1984. The influence of seagrass cover on population structure and individual growth rate of a suspension-feeding bivalve, *Mercenaria mercenaria*. J. Mar. Res. 42, 123–138.
Pomeroy, L.R., D'Elia, C.F., Schaffner, L.C., 2006. Limits to top-down control of phytoplankton by oysters in Chesapeake Bay. Mar. Ecol. Prog. Ser. 325, 301–309.

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria http://www.R-project.org/.
Reusch, T.B.H., 1998. Differing effects of eelgrass *Zostera marina* on recruitment and growth of associated blue mussels *Mytilus edulis*. Mar. Ecol. Prog. Ser. 167, 149–153. http://dx.doi.org/10.3354/meps167149.
Reusch, T.B.H., Chapman, A.R.O., 1995. Storm effects on eelgrass (*Zostera marina* L.) and blue mussel (*Mytilus edulis* L.) beds. J. Exp. Mar. Biol. Ecol. 192, 257–271. http://dx.doi.org/10.1016/0022-0981(95)00074-2.
Reusch, T.B.H., Williams, S.L., 1998. Variable responses of native eelgrass *Zostera marina* to a non-indigenous bivalve *Musculista senhousia*. Oecologia 113, 428–441.
Reusch, T.B.H., Chapman, A.R.O., Groeger, J.P., 1994. Blue mussels *Mytilus edulis* do not interfere with eelgrass *Zostera marina* but fertilize shoot growth through biodeposition. Mar. Ecol. Prog. Ser. 108, 265–282. http://dx.doi.org/10.3354/meps108265.
Robador, A., Brüchert, V., Jørgensen, B.B., 2009. The impact of temperature change on the activity and community composition of sulfate-reducing bacteria in arctic versus temperate marine sediments. Environ. Microbiol. 11, 1692–1703.
Ruesink, J.L., Rowell, K., 2012. Seasonal effects of clams (*Panopea generosa*) on eelgrass (*Zostera marina*) density but not recovery dynamics at an intertidal site. Aquat. Conserv. Mar. Freshw. Ecosyst. 22, 712–720. http://dx.doi.org/10.1002/aqc.2269.
Ruesink, J.L., Lenihan, H.S., Trimble, A.C., Heiman, K.W., Micheli, F., Byers, J.E., Kay, M.C., 2005. Introduction of non-native oysters: ecosystem effects and restoration implications. Annu. Rev. Ecol. Evol. Syst. 36, 643–689. http://dx.doi.org/10.1146/annurev.ecolsys.36.102003.152638.
Seeberg-Elverfeldt, J., Schlüter, Feseker, T., Kölling, M., 2005. Rhizon sampling of porewaters near the sediment-water interface of aquatic systems. Limnol. Oceanogr. Methods 3, 361–371.
Short, F.T., McRoy, C.P., 1984. Nitrogen uptake by leaves and roots of the seagrass *Zostera marina* L. Bot. Mar. 27, 547–555.
Suzuki, Y., Tanoue, E., Ito, H., 1992. A high-temperature catalytic oxidation method for the determination of dissolved organic carbon in seawater: analysis and improvement. Deep Sea Res. Part A, Oceanogr. Res. Pap. 39, 185–198.
Thrush, S.F., Cummings, V.J., Dayton, P.K., Ford, R., Grant, J., Hewitt, J.E., Hines, A.H., Lawrie, S.M., Pridmore, R.D., Legendre, P., 1997. Matching the outcome of small-scale density manipulation experiments with larger scale patterns: an example of bivalve adult/juvenile interactions. J. Exp. Mar. Biol. Ecol. 216, 153–169.
Trimble, A.C., Ruesink, J.L., Dumbauld, B.R., 2009. Factors preventing the recovery of a historically overexploited shellfish species, *Ostrea lurida* Carpenter 1864. J. Shellfish Res. 28, 97–106.
Vinther, H.F., Holmer, M., 2008. Experimental test of biodeposition and ammonium excretion from blue mussels (*Mytilus edulis*) on eelgrass (*Zostera marina*) performance. J. Exp. Mar. Biol. Ecol. 364, 72–79. http://dx.doi.org/10.1016/j.jembe.2008.07.003.
Vinther, H.F., Laursen, J.S., Holmer, M., 2008. Negative effects of blue mussel (*Mytilus edulis*) presence in eelgrass (*Zostera marina*) beds in Flensborg fjord, Denmark. Estuar. Coast. Shelf Sci. 77, 91–103. http://dx.doi.org/10.1016/j.ecss.2007.09.007.
Vinther, H.F., Norling, P., Kristensen, P.S., Dolmer, P., Holmer, M., 2012. Effects of coexistence between the blue mussel and eelgrass on sediment biogeochemistry and plant performance. Mar. Ecol. Prog. Ser. 447, 139–149. http://dx.doi.org/10.3354/meps09505.
Wagner, E., Dumbauld, B., Hacker, S., Trimble, A., Wisehart, L., Ruesink, J., 2012. Density-dependent effects of an introduced oyster, *Crassostrea gigas*, on a native intertidal seagrass, *Zostera marina*. Mar. Ecol. Prog. Ser. 468, 149–160. http://dx.doi.org/10.3354/meps09952.
Wall, C., Peterson, B., Gobler, C., 2008. Facilitation of seagrass *Zostera marina* productivity by suspension-feeding bivalves. Mar. Ecol. Prog. Ser. 357, 165–174. http://dx.doi.org/10.3354/meps07289.
Waycott, M., Duarte, C.M., Carruthers, T.J., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck Jr., K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Natl. Acad. Sci. U. S. A. 106, 12377–12381. http://dx.doi.org/10.1073/pnas.0905620106.
Williams, S.L., Ruckelshaus, M.H., 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. Ecology 74, 904–918.
Worm, B., Reusch, T.B.H., 2000. Do nutrient availability and plant density limit seagrass colonization in the Baltic Sea? Mar. Ecol. Prog. Ser. 200, 159–166.
Yemm, E.W., Folkes, B.F., 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57, 508–514.
Zimmerman, R.C., Reguzzoni, J.L., Alberte, R.S., 1995. Eelgrass (*Zostera marina* L.) transplants in San Francisco Bay: role of light availability on metabolism, growth and survival. Aquat. Bot. 51, 67–86.
Zopfi, J., Kjær, T., Nielsen, L.P., Jørgensen, B.B., 2001. Ecology of *Thioploca* spp.: nitrate and sulfur storage in relation to chemical microgradients and influence of *Thioploca* spp. on the sedimentary nitrogen cycle. Appl. Environ. Microbiol. 67, 5530–5537. http://dx.doi.org/10.1128/AEM.67.12.5530.