



Seasonal Response and Recovery of Eelgrass (*Zostera marina*) to Short-Term Reductions in Light Availability

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Received: 17 May 2019 / Revised: 7 October 2019 / Accepted: 25 October 2019 / Published online: 1 December 2019
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Abstract

Anthropogenic activities in the marine nearshore, such as dredging and construction, can significantly reduce light availability for seagrasses through increased turbidity. Seagrass resilience to such shading depends on the seasonal timing of the event, as water temperature and photoperiod strongly influence plant response to disturbance. We examined the response and recovery of *Zostera marina* to 9 weeks of low and moderate light reduction (35 and 64% reduction) implemented in situ during the spring/mid-summer growth and late summer/fall senescence periods. Fall shading had the most severe impacts, with shoot densities of both low and moderate shade plants decreasing faster to lower densities relative to controls (unshaded plants), than observed during spring shading. Moderate shade plants in the fall also had high leaf loss, reduced growth and chlorophyll content, and utilized stored rhizome water-soluble carbohydrates, whereas in the spring, they had lower leaf loss, increased growth and chlorophyll content, and did not utilize stored carbohydrates. Although most physiological aspects of both spring and fall shaded plants recovered rapidly with restoration of ambient light, shoot density did not, remaining lower than unshaded plants into the next growing season. The stronger impact of fall shading resulted from the decreased photoperiod and daily light saturation, which caused plants to drastically alter their morphology and density to maintain carbon balance. Our study shows that *Z. marina* resilience to light reduction depends on its seasonal timing, suggesting that nearshore activities affecting light availability should be conducted during periods of maximum plant resilience.

Keywords Carbohydrate reserves · H_{Sat} · Morphology · Overwintering · PAR · Sheath length

Introduction

Reduced light availability is one of the main factors underlying worldwide declines in seagrass coverage (Short and Wyllie-Echeverria 1996; Orth et al. 2006; Waycott et al. 2009). Anthropogenic activities such as dredging, marine construction, and agricultural practices can significantly reduce nearshore light availability for seagrasses through terrestrial runoff, eutrophication, and resuspension of bottom sediments (Giesen et al. 1990; Walker and McComb 1992; Erftemeijer and Robin Lewis 2006; Burkholder et al. 2007). Climate change effects predicted for some regions, such as increased storm intensity and frequency, can impact light conditions through increased turbidity, with

negative consequences for seagrasses (Moore et al. 1997; Longstaff and Dennison 1999; Bush and Lemmen 2019). Seagrasses typically respond to light reduction through morphological and physiological changes, reductions in their density to minimize self shading, or by mobilizing carbohydrate reserves, all in an effort to maintain carbon balance (Touchette and Burkholder 2000; Lee et al. 2007a). Despite these well-characterized responses, the effects of variations in light reduction have not been as closely examined. In particular, the effects of shading events that occur at different times of the year, and their interactions with seasonal light patterns and plant phenology have only received minimal attention, mainly in the tropical Indo-Pacific (Bulthuis 1983; Fitzpatrick and Kirkman 1995; Short et al. 2007; Lavery et al. 2009; Serrano et al. 2011; Kim et al. 2015; Chartrand et al. 2016). This knowledge could help optimize management of human activities that affect light availability for seagrasses, by adjusting the timing of the activity to when seagrasses are most resilient (Wu et al. 2017). Here, we examine the seasonal response and recovery of a temperate seagrass (eelgrass, *Zostera marina*) to light reductions that occur within and outside of the main growing season.

Communicated by Masahiro Nakaoka

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Seagrasses respond to reduced light availability across a range of spatial and temporal scales. Within the first minutes and days of light reduction, seagrasses will attempt to physiologically improve light capture by increasing chlorophyll concentration, and reducing chlorophyll a to b ratio, UV blocking pigments, and maximum electron transport rate (Abal et al. 1994; Ralph et al. 2007; Ochieng et al. 2010; McMahon et al. 2013). After several weeks in low light conditions, seagrasses may grow longer leaves to enhance photosynthetic area, but will eventually reduce growth rates to lower respiratory demand, resulting in shorter and fewer leaves (Ralph et al. 2007; McMahon et al. 2013). Energy used to produce secondary metabolites, such as phenolic acids that protect against disease and fouling, will be redirected to more fundamental metabolic processes (Vergeer et al. 1995). Long-term persistence of light reduction over several weeks to months can cause meadow-scale changes that reduce respiratory demand, such as decreased shoot density and biomass (Ralph et al. 2007; Lee et al. 2007a; McMahon et al. 2013). In addition to these changes, seagrasses will also utilize non-structural carbohydrates (sugars and starches) stored in the rhizomes (Alcoverro et al. 1999; Ralph et al. 2007; Vichkovitten et al. 2007). These internal carbohydrate reserves provide an important buffer for plant survival when light levels are inadequate to maintain positive carbon balance (Alcoverro et al. 1999). In temperate seagrasses, carbohydrate reserves are critical for overwintering when ambient light is naturally low (Vichkovitten et al. 2007), and concentrations at the end of the growing season can influence overwintering success (Govers et al. 2015).

The dependence of seagrasses on carbohydrate reserves during disturbances implies that their resilience to shading will depend not only on the duration of shading but also its timing relative to plant phenology and reserve availability. Temperate seagrasses typically display an annual cycle of growth and production that begins in early spring and continues until mid-summer when shoots reach their maximum density, height, and biomass (Duarte 1989; Wong et al. 2013; Clausen et al. 2014). Rhizome carbohydrate reserves are built during this period, with maximum stores obtained by the fall (Vichkovitten et al. 2007). Partial bed senescence begins in late summer with reductions in shoot density and leaf length that reduce self shading and carbon demand. Over the winter, naturally low light conditions restrict photosynthesis, and aboveground vegetation is often low. Stored carbohydrates are mobilized during this time to support plant survival and maintain carbon balance. Shading during the main growing season may prematurely utilize stored carbohydrates or restrict building of reserves, depending on the degree and length of shading, causing mortality or reducing carbohydrate availability for overwintering (Burke et al. 1996; Alcoverro et al. 1999). However, restoration of ambient light by mid-summer may allow seagrasses to recover and rebuild carbohydrate

reserves prior to the winter (McMahon et al. 2011). Shading during fall senescence may not strongly affect seagrass overwintering if carbohydrate stores were maximized during the summer and carbon demands remain low. On the other hand, fall shading may cause premature use of carbohydrate reserves that cannot be rebuilt before the winter, especially if shading is extreme (Alcoverro et al. 1999). Studies in the tropical Indo-Pacific indicate that summer shading has the most severe consequences for seagrasses, because high water temperatures increase light requirements needed to maintain a positive carbon balance (Bulthuis 1983; Fitzpatrick and Kirkman 1995; Lavery et al. 2009; Kim et al. 2015; Chartrand et al. 2016). In most of these studies, maximum shading was extreme ($\leq 10\%$ of surface irradiance) and/or implemented over extended periods of time (≥ 3 months). Impacts of more moderate shading across shorter time periods, which may allow recovery of seagrasses, has yet to be examined within the context of seasonal timing.

Against the backdrop of strong seasonal cycles in growth and light availability, we assess how seasonal timing of shading and degree of shading affects the response and resilience of a temperate seagrass to artificial light reductions. We conducted our study on the Atlantic coast of Nova Scotia, Canada, where extensive seagrass (eelgrass, *Zostera marina*) beds are often found in shallow waters (intertidal to 1.5 m deep) at the heads of bays in muddy/silty sediments (Wong et al. 2013; Wong 2018). These beds are susceptible to periodic light reduction from storm and wind events that re-suspend bottom sediments and increase terrestrial runoff, often reducing water clarity for weeks at a time (M. Wong, personal observation). Close proximity of these beds to dredging operations and nearshore construction can also influence light conditions periodically throughout the year. To our knowledge, this is the first study to examine impacts of seasonal shading in situ for northern *Z. marina* (see Kim et al. 2015 for a study with more southern *Z. marina* that used extreme shading). We used a field experiment where seagrass plants were exposed to low and moderate levels of shade in the spring/mid-summer growing season and the late summer/fall senescence period. Seagrass physiology (chlorophyll content, phenolic acids, water-soluble rhizome carbohydrates), morphology (sheath length, number of leaves), and meadow (shoot density) responses to shading were measured periodically during a nine week shading period, a recovery period where shades were removed, and in the spring after overwintering.

Materials and Methods

Field Site

We conducted the field experiment in an eelgrass (*Zostera marina*) bed located at the head of Port l'Hebert Bay, a semi-enclosed bay on the south shore of Nova Scotia,

Canada (Fig. 1a). The seagrass bed is situated on an extensive elevated mud flat that is ventilated by a series of narrow channels (Wong et al. 2013). The bed is continuous with little fragmentation and is $\sim 2 \text{ km}^2$ in size. The area where the experiment was conducted had little elevation change, with water depths ranging from 0.2 to 1.5 m during mean tides. The average canopy height is $\sim 55 \text{ cm}$ and shoot density ~ 700 shoots m^2 in July and August when plant biomass is at its maximum (Wong et al. 2013). The bed is predominantly comprised of vegetative shoots, with reproductive shoots being $< 1\%$ of total shoot density (Wong unpub. data). The sediment

type is mainly muddy/silty (52.8%) and has a high organic content (25%) (Wong 2018). The site is exposed to the prevailing southwest summer winds, resulting in frequent resuspension of bottom sediments and reductions in water clarity (Secchi depths often $< 5 \text{ cm}$ during wind events).

Field Experiment Design

We conducted two field experiments that manipulated light availability to *Z. marina* using shades. One experiment was conducted in the spring/early summer, where shading was implemented during the initial and peak growing season. A second experiment was conducted in the late summer/fall, where shading was implemented at the end of the main growing season. To simplify, we hereafter refer to these experiments as the spring and fall shading experiments, although it is important to note that both shading experiments also encompassed parts of the summer. In both experiments, three different levels of light reduction were used: 0, 34, and 64% reduction of ambient benthic light (1.2 m deep at mean high water), hereafter referred to as unshaded, low shade, and moderate shade treatments. Note that we call our highest shade level “moderate” because it is of moderate light reduction relative to other seagrass shading studies.

Plots with different shade treatments were deployed in an experimental area $\sim 50 \text{ m}$ wide by 175 m long. To account for potential spatial variability within this area, we allocated 3 different spatial blocks perpendicular to the shoreline (Fig. 1b). Within each block, six octagonal plots (2.94 m^2) were designated $\sim 10 \text{ m}$ apart. Each plot contained continuous seagrass comprised of mainly vegetative shoots; there were ~ 3 reproductive shoots per plot. Each plot was similar in water depth. One replicate of each shade level was randomly allocated to each plot within each block, for both the spring and fall experiments. The interspersed plots for both experiments allowed spatial effects to be reduced and meaningful comparisons to be made between experiments.

Plots were shaded using greenhouse shade cloth (sold as 30 and 50% shade cloth, ShelterLogic, Watertown, CT, USA) attached to an octagonal frame constructed from 1.27 cm diameter PVC strengthened by two cross bars (Fig. 1c). The shade cloth was brushed at each sampling period to clean off the minimal fouling that occurred. The shade cloth hung 30 cm below the frame edge to ensure that the plots were shaded during solar hours of 11:00 am–4:00 pm. Eight PVC legs were attached to each shade frame and were pushed into the sediment until the frame was 1 m above the bottom. Rhizomes at the perimeter of the plot were cut to isolate plants within the plot from plants outside the plots. Frames with no shade cloth were mounted over unshaded plots. All frames were exposed at low tide, potentially subjecting seagrass plants to higher light reductions compared to shading that results solely from reduced water clarity.

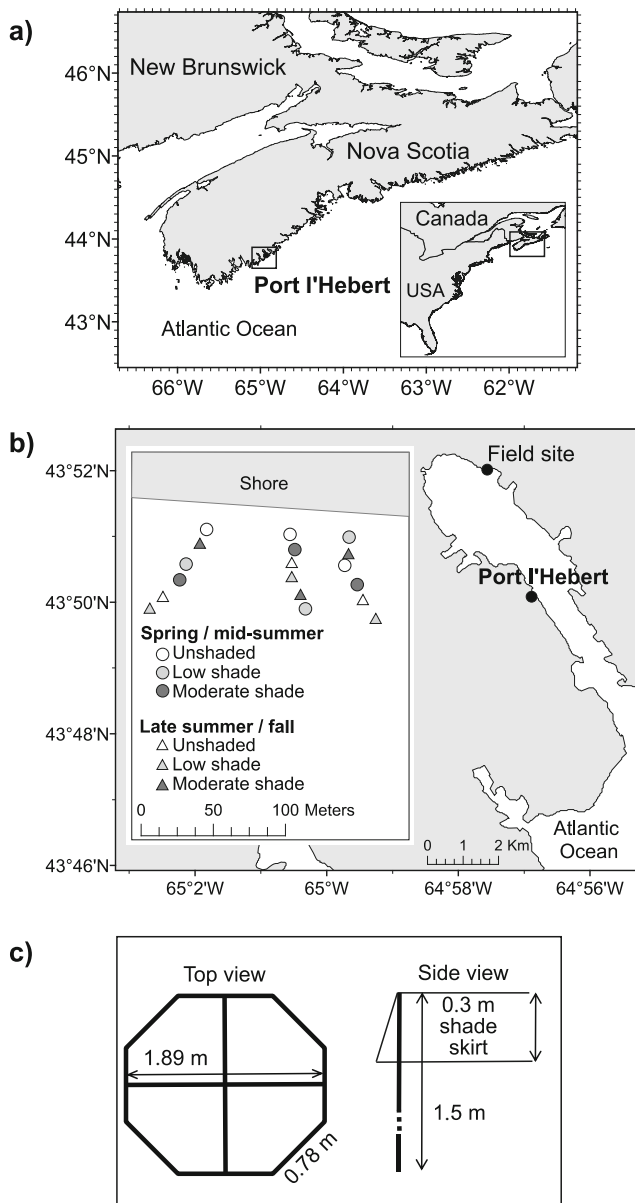


Fig. 1 **a** Maritimes Canada with box outlining the study site on the Atlantic coast of Nova Scotia. Inset shows Nova Scotia in relation to the east coast of North America. **b** Design of the field experiment, with symbols indicating the plots with different shade levels. **c** The octagonal shade frame (top view), with leg and overlying shade skirt (side view)

Plots were shaded for 9 weeks in both experiments (May 18 to July 20 and August 29 to November 1, 2017, in the spring and fall experiments, respectively), after which shades were removed and plants were allowed to recover. Sampling of plant shoot density, morphology, and physiology was conducted at the initiation of the experiment, and at 3, 6, and 9 weeks after shading began. After shades were removed at 9 weeks, plots were sampled at 13, 16, and 26 weeks from the start of the experiment (4, 7, 17 weeks after recovery began) in the spring experiment, and at 15 weeks (6 weeks after recovery began) in the fall experiment. Plots were also sampled after overwintering at the beginning of the next season's growth period for both experiments, for 2 years.

At each sampling period, shoot density in each plot was determined by counting the number of shoots in three 0.25×0.25 m haphazardly placed quadrats at least 0.5 m from the plot edge. Ten shoots with at least 5 cm of attached rhizome were then harvested randomly from each plot. This represents removal of ~ 2.8 and 2.2% of the initial density by the end of the spring and fall recovery periods, respectively. Shoots were kept on ice and refrigerated for up to 24 h prior to processing in the laboratory.

Light Conditions and Water Temperature

Water temperature at the field site was measured continuously in 10-min intervals using loggers (TidbiT v2; Onset Computer Corporation, Bourne, MA, USA) deployed over the duration of the field experiment. Photosynthetically active radiation (PAR, 400–700 nm) was measured at the water surface, above the canopy (~ 0.7 m deep) and at the bottom of the canopy (~ 1.2 m deep) during a mid-day mean high tide in April 2017 for all plots using an underwater quantum sensor (LI-192, LICOR). Three replicate measurements were taken at each depth both inside and outside the plots. The percent reduction in ambient PAR at the bottom of the canopy in shaded plots was determined as $100 - 100 \times (\text{PAR inside plot}/\text{PAR outside plot})$. The percent of surface irradiance available at the top and bottom of the canopy was calculated as $100 \times (\text{PAR at top or bottom of canopy})/(\text{unshaded surface irradiance})$.

We also modelled daily PAR reaching the water surface across the year, using the diurnal light curve equation of Vollenweider (1965) at 30-min intervals. This equation requires estimates of day length and daily maximum incident PAR. We determined day length based on latitude of the field site and solar declination as in Brock (1981) and Kirk (2011). We calculated daily maximum incident PAR using a polynomial relationship derived from annual surface light data obtained from a buoy (LOBO-0010) located in the Northwest Arm, Halifax, Canada (~ 200 km away). Weather patterns are generally similar between the two locations. Values were reduced by 10% to account for reflectance at the water surface (Madden and Kemp 1996). We used the estimates of daily

surface PAR to determine total cumulative incident PAR during the shade and recovery periods of the fall and spring experiments. We also used daily surface PAR and spring measurements of mean percent surface irradiance to determine total cumulative PAR at the canopy and bottom in shaded and unshaded plots for both experiments. Because growth and survival of *Z. marina* is more closely correlated with daily light period than absolute light intensity (Dennison and Alberte 1982, 1985), we also calculated the total duration above the daily light saturation point (H_{Sat}). The saturating irradiance (I_k) for *Z. marina* varies with temperature, and so we used a threshold of $90 \mu\text{mol m}^{-2} \text{s}^{-1}$ for the spring shading and recovery periods, and also for the fall shading period, while we used $36 \mu\text{mol m}^{-2} \text{s}^{-1}$ for the fall recovery period (Marsh et al. 1986). Furthermore, we also calculated the number of days where H_{Sat} was > 6 h, the minimum H_{Sat} required for *Z. marina* growth and survival (Dennison and Alberte 1985). We acknowledge that total cumulative PAR and H_{Sat} calculated for the fall experiment using percent surface irradiance measured in the spring may slightly overestimate light availability, as longer leaves may have provided additional shading.

Processing of Collected Plants

Seagrass shoots were processed by counting the number of leaves per shoot, measuring the sheath length, and measuring leaf length and width. The shoot was separated from the rhizome, and sections of leaves were harvested for processing for biochemical components.

Total Chlorophyll (a and b) Concentration A 20-mm mid-leaf section of leaf three was collected from 5 shoots per plot, its width measured, and then frozen at -80 °C. During processing, leaf sections were first ground in 0.6 mL of 90% acetone and then chlorophyll was extracted using a 2:1 solution of 90% acetone and 100% dimethylsulfoxide (Shoaf and Lium 1976). Extractions were conducted in the dark at -20 °C for 24 h. Chlorophyll a and b concentrations were determined in duplicate using a spectrophotometer read at 665, 652, and 750 nm (ThermoFisher Orion AquaMate 8000 UV-Vis) and absorbance equations from Ritchie (2006). The total chlorophyll concentrations (chlorophyll a + chlorophyll b) are reported in $\mu\text{g cm}^{-2}$.

Phenolic Acid Concentration The remaining third leaf from the chlorophyll analyses and the entire second leaf were collected from five shoots per plot and frozen at -80 °C. During processing, leaf sections were freeze-died overnight and ground using a mixer mill (Retsch MM 300). Phenolic acids were extracted from 5 mg dry weight (DW) tissue sample in duplicate by first heating the sample in 100 μl of 60% (v/v) methanol for 20 min at 40 °C. The solution was then centrifuged for

10 min at 4000g, the supernatants combined, and then frozen at $-20\text{ }^{\circ}\text{C}$ for ~ 10 days until further processing. Phenolic acid concentration was quantified using the Folin-Ciocalteu's reagent in a microplate assay modified from Ainsworth and Gillespie (2007) and S. MacKinnon (personal communication, National Research Council, Halifax, NS, Canada) and read at 760 nm (FLUOstar Optima, BMG Labtech). Gallic acid (Sigma F9252) was used as the standard as it is produced in more than 50% of all seagrass species (Zapata and McMillan 1979). Thus, all phenolic acid concentrations are reported as mg gallic acid equivalent (GAE) per g of dry weight (mg GAE per g DW^{-1}).

Water-Soluble Carbohydrates (Soluble Sugars) Preliminary data and other studies indicate that starch content is very low in *Z. marina* rhizomes relative to water-soluble carbohydrates ($\leq 1\%$ of total) (Burke et al. 1996; Alcoverro et al. 1999; P. Chow, personal communication, University of Calgary, Calgary, Alberta, Canada; B. Vercaemer, unpublished data), and so we focus only on the water-soluble sugar content in the rhizomes. From each of five rhizomes per plot, a 5–8-cm section was frozen at $-80\text{ }^{\circ}\text{C}$. During processing, samples were freeze-dried overnight and then ground using a mixer mill. Soluble sugars (mono- and oligo-saccharides) were hydrolyzed from duplicate 20–30 mg DW tissue samples using 80% ethanol at $80\text{ }^{\circ}\text{C}$ for 30 min in a closed 2-mL tube. The solution was then centrifuged at 13,000g for 15 min and supernatants were frozen at $-20\text{ }^{\circ}\text{C}$ until further processing. Soluble sugar concentration was determined using the phenol sulfuric acid method in a microplate assay as modified from Masuko et al. (2005) and read at 490 nm. The total soluble concentrations are reported as mg glucose equivalent per g of dry weight (mg g DW^{-1}).

Statistical Analyses

The effects of light reduction on *Z. marina* shoot density, morphology (sheath length, number of leaves per shoot), or physiology (concentrations of total chlorophyll, phenolic acids, rhizome water-soluble carbohydrates) were examined using linear mixed models with the fixed factors shade (3 levels: none, low, moderate) and time after shading (5–8 and 4–6 levels in the spring and fall experiments respectively; as explained further below), and the random factors spatial block (3 levels) and plot (9 levels), where plot was nested within the interaction of shade and block. Only random intercepts were included. Subsamples within each plot (i.e., the three quadrats, 10 plants, or 5 leaf sections) taken at each sampling time were averaged. Spring and fall experiments were analyzed separately. For the spring experiment, time after shading had 8 levels in total (0, 3, 6, 9, 13, 16, 26, 49 weeks after shading began), which incorporated the 9-week shading period (0–9 weeks after shading), the following recovery period (13–26 weeks

after shading), and first year of overwintering (49 weeks after shading). For the fall experiment, time after shading had 6 levels in total (0, 3, 6, 9, 15, 34 weeks after shading), which included the 9-week shading period (0–9 weeks after shading), the recovery period (15 weeks after shading), and first year of overwintering (34 weeks after shading). The second year of overwintering was excluded from the analyses because only shoot density was measured and inclusion of this time masked some treatment effects. Shoot density, sheath length, and number of leaves were analyzed over all times. Owing to logistical constraints, physiological metrics were analyzed at 0, 6, 9, 16, 26, and 49 (spring) or 0, 6, 9, 15, and 34 (fall) weeks after shading began, except for chlorophyll concentration, which was not analyzed after overwintering.

Contribution of random effects to the statistical models was evaluated by comparing AIC (Akaike information criterion) values for models that included only the fixed factors, both random factors, and only the random factor plot. Plot was always retained in the models with random effects because it accounted for the non-independence of the repeated measurements over time. In almost all cases, the random factor block did not improve model fit and was removed and plot was retained. The mixed model regressions were fit using maximum likelihood with the intercept (reference value) being no shading at the beginning of the experiment (week 0). Significance of the fixed effects was evaluated using ANOVA statistics with p-values approximated using Satterthwaite's method. Contributions of different factor levels to observed significance of the main effects or interactions were evaluated using regression parameter estimates and associated *p* values (from Satterthwaite's method), with relevant comparisons outside of the reference conditions examined from observations of the data. Residual plots were evaluated to assess the assumptions of homogeneity of variance and normality; in all cases assumptions were not violated. All statistical analyses were implemented in R (3.5.2 2018), using the packages "lme4" (mixed model) and "lmerTest" (Satterthwaite's method).

Time series analysis of the 2017 continuous water temperature record was conducted to characterize temperature variability on three different time scales: seasonal, subtidal, and tidal. The seasonal cycle was determined by computing monthly temperature means. The subtidal temperature variability for the summer period was isolated by first removing the seasonal cycle and then low-pass filtering, using a frequency cutoff of 36 h, to remove the higher-frequency tidal variations (Priestley 2004). These subtidal temperature variations mainly correspond to the upwelling events associated with wind-driven circulation in adjacent shelf waters (Platt et al. 1972). The higher frequency tidal variation in temperature was the signal obtained after removing the seasonal and subtidal variations from the original record; this isolates the summer temperature fluctuations associated with the diurnal

and semidiurnal tides independent of longer period fluctuations. The continuous water temperature data were also used to calculate the number of hours where water temperature exceeded 23 °C during the summer. The 23 °C value is the mean optimum temperature for *Z. marina* photosynthesis (Lee et al. 2007a), although this can vary according to light availability, with plants in low light conditions requiring lower optimum temperatures for photosynthesis. Because our experiment used different levels of shading, we elected to use 23 °C as a realistic threshold of temperature impact instead of higher values.

Results

Physical Measures

The percent reduction in ambient benthic PAR (1.2 m depth) at the beginning of the spring experiment was 34 and 64% for low and moderate shade plots, respectively (Table 1). Longer leaves in the fall experiment may have provided additional shading and further reduced light reaching the bottom. The percent surface irradiance above the canopy was 41, 21, and 12.5% in the unshaded, low, and moderate shade plots, respectively. At the bottom of the canopy, unshaded plots were 15% surface incident light, while low and moderate shade plots were far below (7.7 and 5.1% incident light, respectively).

The total incident PAR during spring shading was 1.5 times higher than during fall shading (4.1×10^4 vs 2.7×10^4 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Table 2). Total incident PAR at the canopy and bottom during spring shading was also higher than in the fall, with reductions evident across the shade levels. Total PAR during recovery after spring shading was an order of magnitude higher than after fall shading, partly because of the longer duration of the spring recovery period. More importantly, the total number of hours above daily photosynthetic light saturation (H_{Sat}) were much higher during spring shading than for fall shading for all shade levels at the canopy (683–878 and 481–684 h, respectively). Interestingly, for both shading periods, total hours above H_{Sat} at the canopy did not differ much between low shade and unshaded plots, while values for moderate shade plots were $\sim 75\%$ of unshaded plots. Stronger differences in hours above H_{Sat} among shade levels were evident at the bottom: during spring shading, hours above H_{Sat} in

low shade plots was 68% of that in unshaded plots, while for fall shading, it was 38%. For moderate shade plots, PAR levels at the bottom did not reach photosynthetic light saturation (I_k) in either experimental shading period. Furthermore, moderate shade plots in both shading periods had zero days where H_{Sat} was > 6 h at the bottom, which was also observed for low shade plots during fall shading. Lastly, total hours above H_{Sat} during recovery after fall shading was 33% of that during recovery after spring shading.

Water temperature showed an annual range in monthly means from 0.988 to 20.0 °C (Fig. 2a). A seasonal trend is evident with warming from February to August followed by cooling from September to January. Mean temperature during the spring and fall shading periods was 17.1 ± 3.0 and 16.0 ± 2.4 °C (mean \pm SD), respectively, while it was 16.5 ± 4.4 and 7.5 ± 2.8 °C during the recovery periods in the spring and fall experiment. The subtidal variability indicates the influence of offshore temperature events, with wind driven upwelling causing, for example, a 9° drop in temperature at the end of July (Fig. 2c). Water temperatures exceeded the optimal temperature for photosynthesis (23 °C) for a total of 152.8 h (Fig. 2b). Two extensive high temperature events were observed during the spring experiment: a 3-day event from July 19 to 21 (immediately prior to week 9 of the shading period) and a 5 day event from August 2 to 7 (after the shading period ended and 10 days prior to the first sampling of the recovery period). Furthermore, temperatures were sometimes > 30 °C, which can cause acute shoot mortality. Observations of detailed tidal variations across a 13-day period showed a daily signal dominated by spikes of high and low temperature (Fig. 2d), suggesting changes due to tidal advections and solar heating cycles in these very shallow waters.

Shoot Density

In the spring experiment, the effects of shading on shoot density were first evident in the moderate shade plots within six weeks (overall ANOVA week \times shade: $F_{14, 63} = 2.15$, $p = 0.021$). At this time, shoot density was significantly lower in moderate shade plots compared to low and unshaded plots (week 6 \times moderate shade: $t = -2.37$, $df = 63$, $p < 0.0001$; Fig. 3a), being 63% of shoot density in the unshaded plots. Low and unshaded plots maintained similar shoot density after 6 weeks of shading to initial conditions (Fig. 3a). After 9

Table 1 Light conditions in the shaded and unshaded plots measured at the beginning of the spring experiment

	Unshaded	Low shade	Moderate shade
Percent reduction in ambient benthic PAR	0	33.5 \pm 0.11	64.4 \pm 3.3
Percent surface irradiance above canopy	41.6 \pm 4.5	21.1 \pm 2.5	12.5 \pm 0.54
Percent surface irradiance at bottom	15.4 \pm 0.57	7.7 \pm 1.9	5.1 \pm 0.37

Depth above canopy was 0.7 m and depth at bottom was 1.2 m during mean high tide. Values are mean \pm standard deviation

Table 2 Light conditions during the shading and recovery periods of both the spring and fall experiments, based on modelled daily incident PAR and the mean light conditions in Table 1

Measure	Spring/mid-summer shading (day 138–202)			Recovery after spring/mid- summer shading (day 203–309)	Late summer/fall shading (day 240–305)			Recovery after late summer/fall shading (day 306–346)
	Shade level				Shade level			
	None	Low	High		None	None	Low	
Total incident PAR	4.1×10^4			4.9×10^4	2.7×10^4			9027
Total PAR at canopy	1.7×10^4	8697	5515	2.0×10^4	1.1×10^4	5669	3358	3755
Total PAR at bottom	6348	3174	2102	7590	4138	2069	1370	1390
H_{Sat} at canopy	878	813	683	1192	684	604	481	362
H_{Sat} at bottom	748	477	0	957	551	211	0	318
$H_{Sat} > 6$ h at canopy	65	65	65	107	66	66	57	41
$H_{Sat} > 6$ h at bottom	65	65	0	107	66	0	0	41

Total PAR = $\mu\text{mol m}^{-2} \text{s}^{-1}$, H_{Sat} = total number of hours of daily light saturation, $H_{Sat} > 6$ h = total number of days where H_{Sat} is greater than 6 h, Day = calendar day. See text for details

weeks of shading, shoot density in all plots decreased, likely from a high temperature event (see above), and shoot density in shaded plots did not differ from unshaded plots (week 9 \times low shade: $t = -1.44$, $df = 63$, $p = 0.155$; week 9 \times moderate shade: $t = -1.88$, $df = 63$, $p = 0.065$). However, after 4 weeks of recovery where shades were removed, shoot density in unshaded plots increased to levels prior to the high temperature event, while shoot density in low and moderate shade plots remained significantly lower (week 13 \times low shade: $t =$

-3.71 , $df = 63$, $p = 0.0004$; week 13 \times moderate shade: $t = -4.27$, $df = 63$, $p < 0.0001$). At this time, shoot densities in the low and moderate shade plots were 58 and 35% of control values, respectively. Although shoot density in the unshaded plots declined during the recovery period from reductions in natural ambient light, shoot density in the previously shaded plots remained lower for the remainder of the recovery period (week 26 \times low shade: $t = -2.07$, $df = 63$, $p = 0.042$; week 26 \times moderate shade: $t = -2.05$, $df = 63$, $p = 0.044$). Despite this,

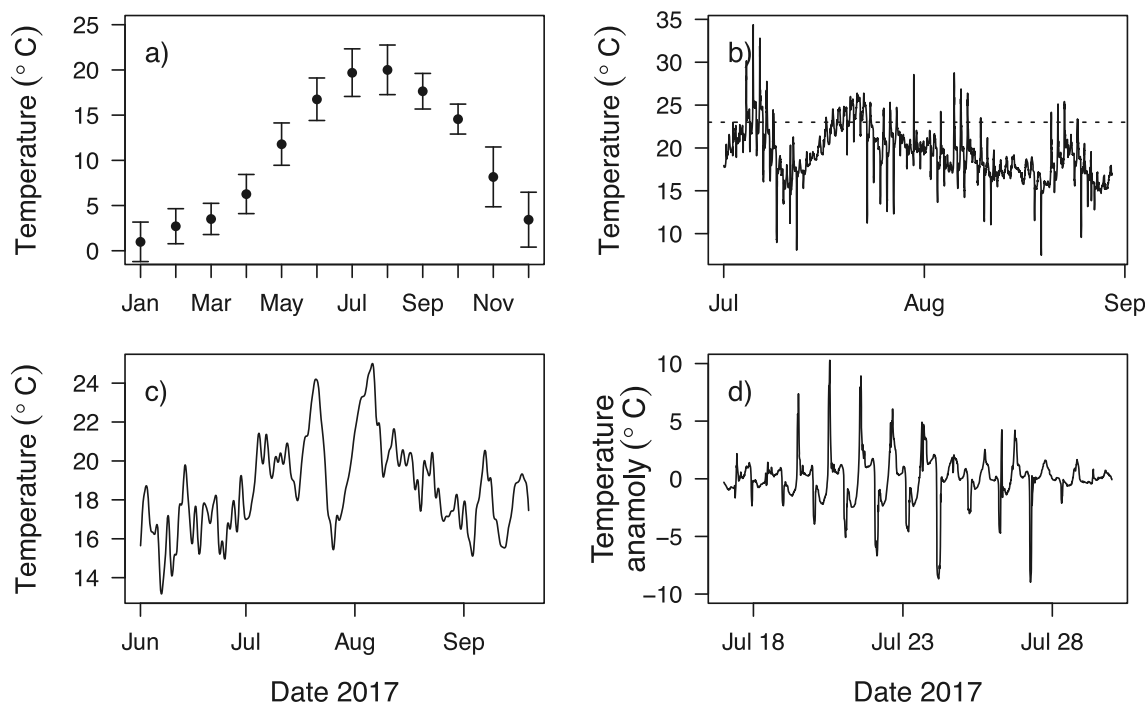


Fig. 2 **a** Mean (± 1 SE) monthly water temperature at the field site. **b** Water temperature between July and September, with dotted line indicating the temperature optimum for *Z. marina* photosynthesis (23

$^{\circ}\text{C}$). **c** Subtidal temperature variability. **d** Details of tidal variations in temperature anomalies for a 10-day period

some recovery was evident in moderate shade plots where shoot density tended to increase during the recovery period (Fig. 3a), while it remained mostly unchanged in low shade plots. After the first overwintering period (week 49), shoot density in all plots was lower relative to the end of the previous recovery period (week 26), and tended to be higher in unshaded plots than in shaded plots (Fig. 3a). This trend was still evident after the second overwintering period (week 99), although there was high variability in the low shade plots.

When shading was implemented in the fall, shoot density in shaded plots declined more quickly and with higher magnitude than observed in the spring (Fig. 3b). After only 3 weeks of shading, shoot density in both low and moderate shade plots was significantly lower than in unshaded plots (overall ANOVA week \times shade: $F_{10, 45} = 3.59$, $p = 0.001$; week 3 \times low shade: $t = -2.46$, $df = 45$, $p = 0.017$; week 3 \times moderate shade: $t = -3.51$, $df = 45$, $p = 0.001$), with a continued decline to the end of the shading period (week 9 \times low shade: $t = -3.31$, $df = 45$, $p = 0.001$; week 9 \times moderate shade: $t = -5.13$, $df = 45$, $p = 0.0067$). This occurred even with reduction in shoot density in unshaded plots due to natural light limitation. By the end of the shading period, shoot densities in low and moderate shade plots were 42 and 32% of density in unshaded plots, respectively. After removal of shades and six weeks recovery, shoot density in previously shaded plots remained lower than unshaded plots and showed no signs of recovery (week 15 \times low shade: $t = -2.14$, $df = 45$, $p = 0.038$; week 15 \times moderate shade: $t = -4.31$, $df = 45$, $p < 0.0001$; Fig. 3b), contrary to the spring experiment. At this time, shoot densities were 54 and 32% of control values in low and moderate shade plot, respectively. After the first year of overwintering (week 34), shoot densities in all plots were lower relative to those at the end of the previous recovery period (week 15; Fig. 3b). Shoot density in shaded plots were lower than in the unshaded plots, although this was only statistically significant for plots with moderate shade (week 34 \times low shade: $t = -1.15$, $df = 45$, $p = 0.255$; week 34 \times moderate

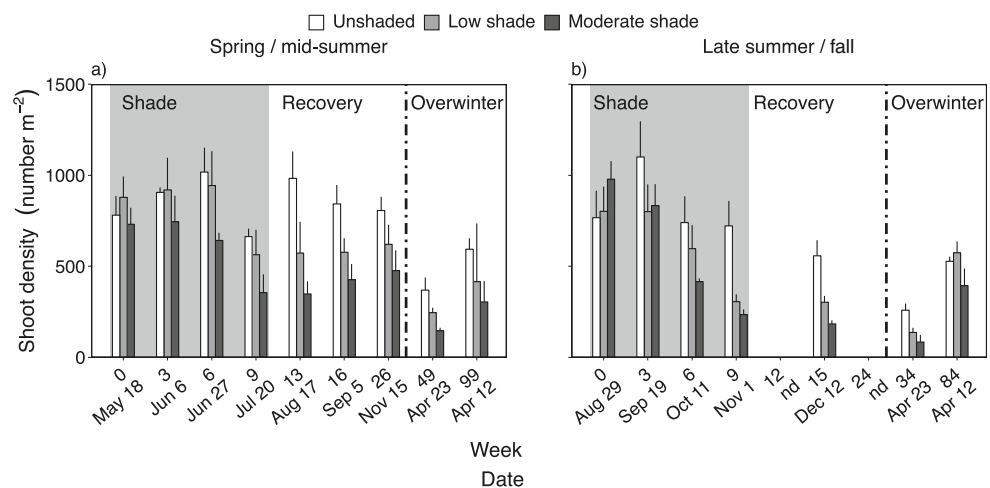
shade: $t = -2.84$, $df = 45$, $p = 0.007$). After the second overwintering period, shoot densities in all plots were higher than the previous year, but tended to be lowest in moderate shade plots.

Shoot Morphology

Sheath Length

Although sheath length increased steadily across the entire shading period in all plots during the spring experiment, it was significantly longer in moderate shade plots than low or unshaded plots by week 3 (overall ANOVA week \times shade: $F_{14,63} = 4.36$, $p < 0.0001$; comparisons: week 3 \times moderate shade: $t = 2.22$, $df = 63$, $p = 0.03$; Fig. 4a). Longer sheaths in the moderate shade plots persisted across the entire shading period, indicating higher growth in response to light limitation (week 9 \times moderate shade: $t = 1.23$, $df = 63$, $p = 0.222$). Sheath length in the low shade plots did not differ from those in unshaded plots at any time, even after 9 weeks of shading (week 9 \times low shade: $t = 0.440$, $df = 63$, $p = 0.661$). Four weeks after shades were removed, sheath length in all plots decreased, although sheaths in moderate shade plots remained significantly shorter than in low shade or unshaded plots (week 13 \times moderate shade: $t = -2.11$, $df = 63$, $p = 0.039$; Fig. 4a). This effect persisted until the end of the recovery period (week 26 \times moderate shade: $t = -1.91$, $df = 63$, $p = 0.061$), indicating lower growth and potentially low recovery from shading. Sheath length in low shade plots also tended to be shorter than in unshaded plots by the end of the recovery period (26 weeks), suggesting a carry-over effect from shading, despite no effect during the shading period. After overwintering, sheath lengths in all plots were shorter than values at the end of the recovery period, and did not differ among shading treatments (week 49 \times low shade: $t = 0.194$, $df = 63$, $p = 0.84$; week 49 \times moderate shade: $t = 0.076$, $df = 63$, $p = 0.939$; Fig. 4a).

Fig. 3 Mean shoot density (± 1 SE) during periods of shading, recovery, and after overwintering, when shading was implemented in the spring/mid-summer (a) and late summer/fall (b). Note that shading periods are referred to as spring and fall shading in the main text. nd = no data



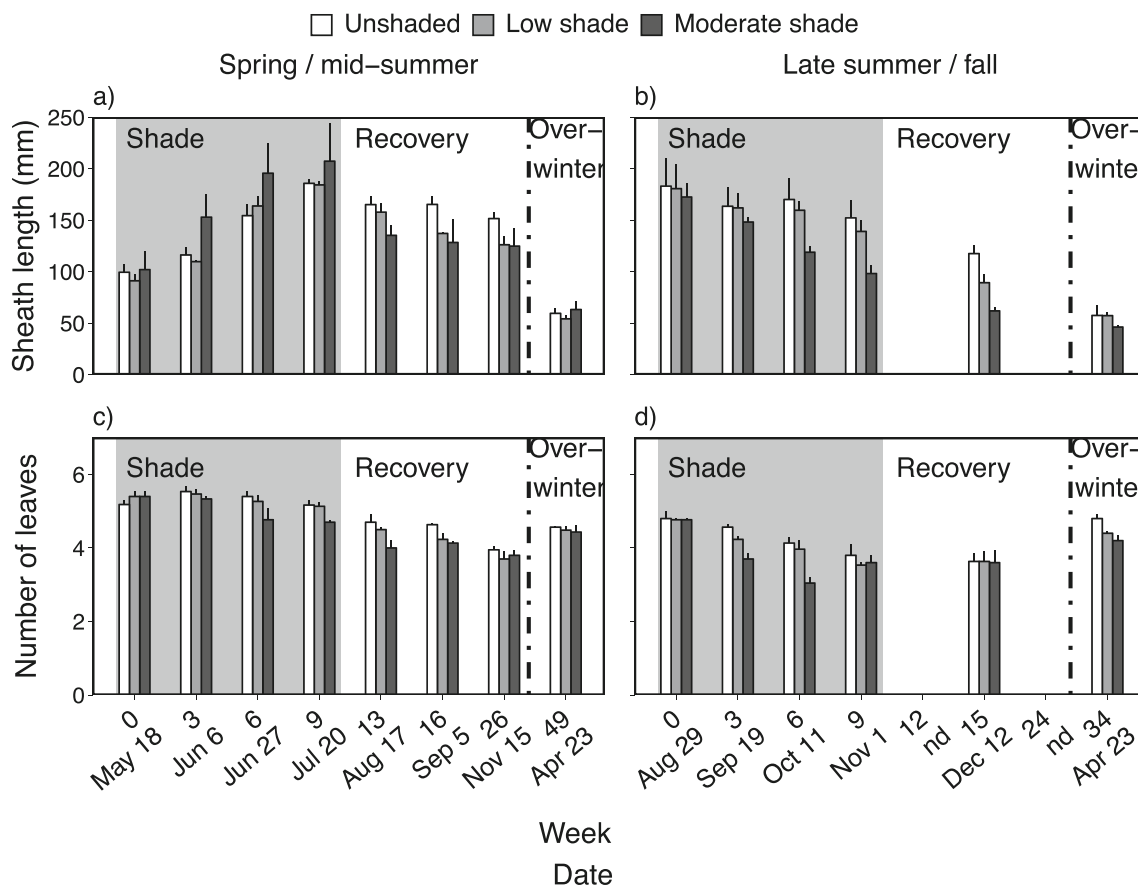


Fig. 4 Mean sheath length and number of leaves per shoot (+ 1 SE) during periods of shading, recovery and after overwintering, when shading was implemented in the spring/mid-summer (a, c) and late summer/fall (b, d). nd = no data

Contrary to the spring experiment, sheath length in the fall experiment decreased in all shaded and unshaded plots across the entire experimental period (Fig. 4b). However, sheaths were significantly shorter in moderate shade plots than unshaded or low shade plots by 6 weeks after shading began (overall ANOVA week \times shade: $F_{10,45} = 1.90$, $p = 0.070$; comparisons: week 6 \times moderate shade: $t = -2.36$, $df = 45$, $p = 0.0226$) and persisted until the end of the shading period (week 9 \times moderate shade: $t = -2.62$, $df = 45$, $p = 0.015$). After 6 weeks of recovery, sheath length in all shaded and unshaded plots decreased, with it being shorter in the moderate shade plots than low or no shade plots (week 15 \times moderate shade: $t = -2.62$, $df = 45$, $p = 0.012$; Fig. 4b). Similar to the spring experiment, sheath length in the low shade plots tended to also be lower than unshaded plots, perhaps exhibiting a carry-over effect from shading. After overwintering, sheath lengths were shorter in all plots than at the end of the fall recovery and did not differ from each other, as observed in the spring experiment (week 34 \times low shade: $t = 0.13$, $df = 45$, $p = 0.900$; week 34 \times moderate shade: $t = -0.046$, $df = 45$, $p = 0.964$; Fig. 4b). Patterns for leaf length were identical to sheath length for both experiments, and so are not presented here.

Number of Leaves per Shoot

In the spring experiment, the number of leaves per shoot declined over the shading period in both the low shaded and unshaded plots (Fig. 4c). However, leaf loss in moderate shade plots was highest throughout the shading period compared to the unshaded plots (overall ANOVA week \times shade: $F_{14,63} = 2.53$, $p = 0.006$; comparisons: week 3 \times moderate shade: $t = -2.14$, $df = 63$, $p = 0.036$; week 9 \times moderate shade: $t = -3.28$, $df = 63$, $p = 0.0017$). During the recovery period, moderate shade plots remained lower in number of leaves per shoot than in unshaded plots until the end of recovery (week 26), when the number of leaves in unshaded plots naturally declined. Interestingly, leaf loss in low shade plots only became evident 4 weeks into the recovery period (week 13), which persisted until mid-recovery (week 16 \times low shade: $t = -2.14$, $df = 63$, $p = 0.036$). After overwintering, the number of leaves per shoot was similar among all shaded and unshaded plots (Fig. 4c).

Higher leaf loss in all shaded and unshaded plots was evident during the shading period in the fall experiment compared to the spring experiment (Fig. 4d). In the moderate shade plots, leaf loss remained significantly higher than in low and unshaded plots until week 9 of the shading period,

where leaf loss became similar among all plots (overall ANOVA week \times shade: $F_{10,45} = 2.84$, $p = 0.007$; comparisons: week 9 \times low shade: $t = -0.785$, $df = 45$, $p = 0.437$; week 9 \times moderate shade: $t = -0.56$, $df = 45$, $p = 0.577$). The number of leaves per shoot remained similar in shaded and unshaded plots during the recovery period (week 15 \times low shade: $t = 0.112$, $df = 45$, $p = 0.911$; week 15 \times moderate shade: $t = 0.00$, $df = 45$, $p = 1.0$; Fig. 4d). After overwintering, shoots in moderate shade plots tended to have lower number of leaves than in the unshaded plots, although this trend was only marginally significant (week 36 \times moderate shade: $t = -1.91$, $df = 45$, $p = 0.063$; Fig. 4d).

Plant Physiology

Chlorophyll

In the spring experiment, chlorophyll concentration in seagrass leaves decreased in unshaded plots across the nine week shading period (Fig. 5a), likely because of the natural increase in light availability. This was also observed for seagrass leaves in the low shade plots, although levels were higher than in unshaded plots. In contrast, chlorophyll concentration in seagrass leaves was significantly higher in moderate shade plots than unshaded plots at both 6 and 9 weeks after shading (overall ANOVA week \times shade: $F_{8,36} = 2.86$, $p = 0.014$; comparisons: week 6 \times moderate shade: $t = 3.88$, $df = 36$, $p = 0.0004$; week 9 \times moderate shade: $t = 2.66$, $df = 36$, $p = 0.011$). When shades were removed and plants allowed to recover, chlorophyll concentration in moderate shade plots did not differ from unshaded plots at either 16 or 26 weeks (week 16 \times moderate shade: $t = 0.973$, $df = 36$, $p = 0.337$; week 26 \times moderate shade: $t = 1.26$, $df = 36$, $p = 0.216$; Fig. 5a).

In contrast to the spring experiment, chlorophyll concentration in seagrass leaves in both shaded and unshaded plots did not change significantly over the experiment (overall ANOVA week \times shade: $F_{6,27} = 1.01$, $p = 0.439$; Fig. 5b). However, chlorophyll concentration either decreased (moderate shade plots) or was maintained at the same level (low shade plots) as in unshaded plots.

Phenolic Acids

In the spring experiment, phenolic concentration in seagrass leaves from unshaded plots increased across the experimental shading period (Fig. 6a), as would be expected given the increase in natural light availability. However, phenolic concentration in shaded plots did not show a similar increase, and remained lower than unshaded plots across the entire shading period (overall ANOVA week \times shade: $F_{10,45} = 2.98$, $p =$

0.006; comparisons: week 9 \times low shade: $t = -2.68$, $df = 45$, $p = 0.010$; week 9 \times moderate shade: $t = -3.96$, $df = 45$, $p = 0.0002$). After 7 weeks of recovery, phenolics in unshaded plots decreased to levels similar to shaded plants (week 16 \times low shade: $t = 0.955$, $df = 45$, $p = 0.344$; week 16 \times moderate shade: $t = 0.123$, $df = 45$, $p = 0.902$; Fig. 6a). Phenolic concentration in all shaded and unshaded plants decreased further after 17 weeks of recovery. Interestingly, after overwintering, phenolic concentration showed a trend in decreasing phenolic concentration with increased shade and was significantly lower in moderate shade plants compared to unshaded plants (week 49 \times moderate shade: $t = -2.18$, $df = 45$, $p = 0.034$; Fig. 6a).

Phenolic concentrations in the fall experiment increased over the shading period in unshaded plots, but only increased slightly in low shade plants or not at all in moderate shade plants (overall ANOVA week \times shade: $F_{8,36} = 2.62$, $p = 0.023$; comparisons: week 9 \times low shade: $t = -2.35$, $df = 36$, $p = 0.024$; week 9 \times moderate shade: $t = -3.03$, $df = 36$, $p = 0.004$; Fig. 6b). After shades were removed and recovery allowed, values were similar among all plots within 6 weeks (week 15 \times low shade: $t = -0.96$, $df = 36$, $p = 0.342$; week 15 \times moderate shade: $t = -0.25$, $df = 36$, $p = 0.802$). After overwintering, phenolic concentration was elevated in all plots relative to the end of the fall recovery period (Fig. 6b).

Rhizome WSC

In the spring experiment, water-soluble carbohydrate (WSC) concentrations in rhizomes in unshaded and low shade plots increased for the first 6 weeks then decreased at the end of the shading period (Fig. 6c), likely related to the high temperature event that occurred during this time (see below). However, WSC concentration in low shade plants remained lower than in unshaded plants (week 6 \times low shade: $t = -1.98$, $df = 45$, $p = 0.050$; week 9 \times low shade: $t = -1.86$, $df = 45$, $p = 0.060$). In moderate shade plants, WSC concentrations did not build during the shading period, and remained lower than unshaded and low shade plants (week 6 \times moderate shade: $t = -2.46$, $df = 45$, $p = 0.017$; week 9 \times moderate shade: $t = -2.01$, $df = 45$, $p = 0.050$). During recovery, WSC concentrations were quickly built in low shade plots to background levels within 6 weeks (week 16 \times low shade: $t = -0.94$, $df = 45$, $p = 0.353$; Fig. 6c). However, although moderate shade plants also built WSCs during the recovery period, concentrations remained lower than in unshaded plants until week 26 of the experiment (week 16 \times moderate shade: $t = -2.13$, $df = 45$, $p = 0.039$; week 26 \times moderate shade: $t = -0.659$, $df = 45$, $p = 0.513$; Fig. 6c), when background concentrations drastically reduced. After overwintering, WSCs were similar among all shaded and unshaded plots (Fig. 6c).

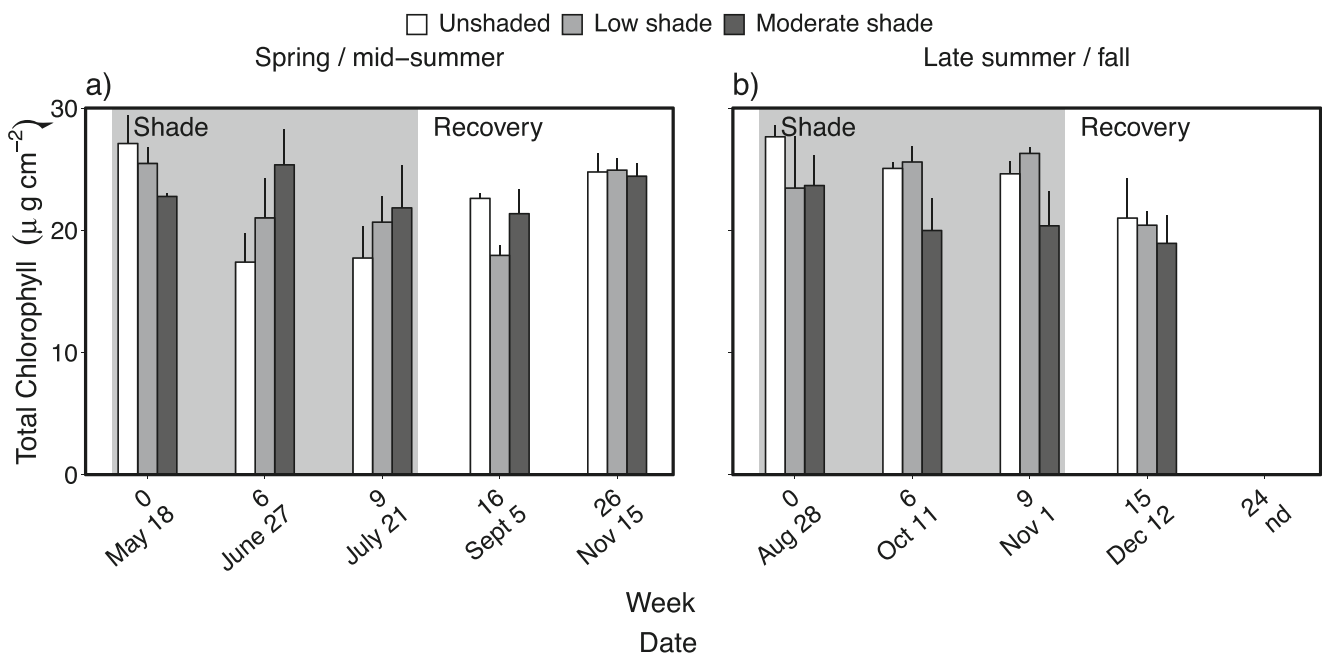


Fig. 5 Mean chlorophyll concentration (± 1 SE) during periods of shading and recovery when shading was implemented in the spring/mid-summer (a) and late summer/fall (b). nd = no data

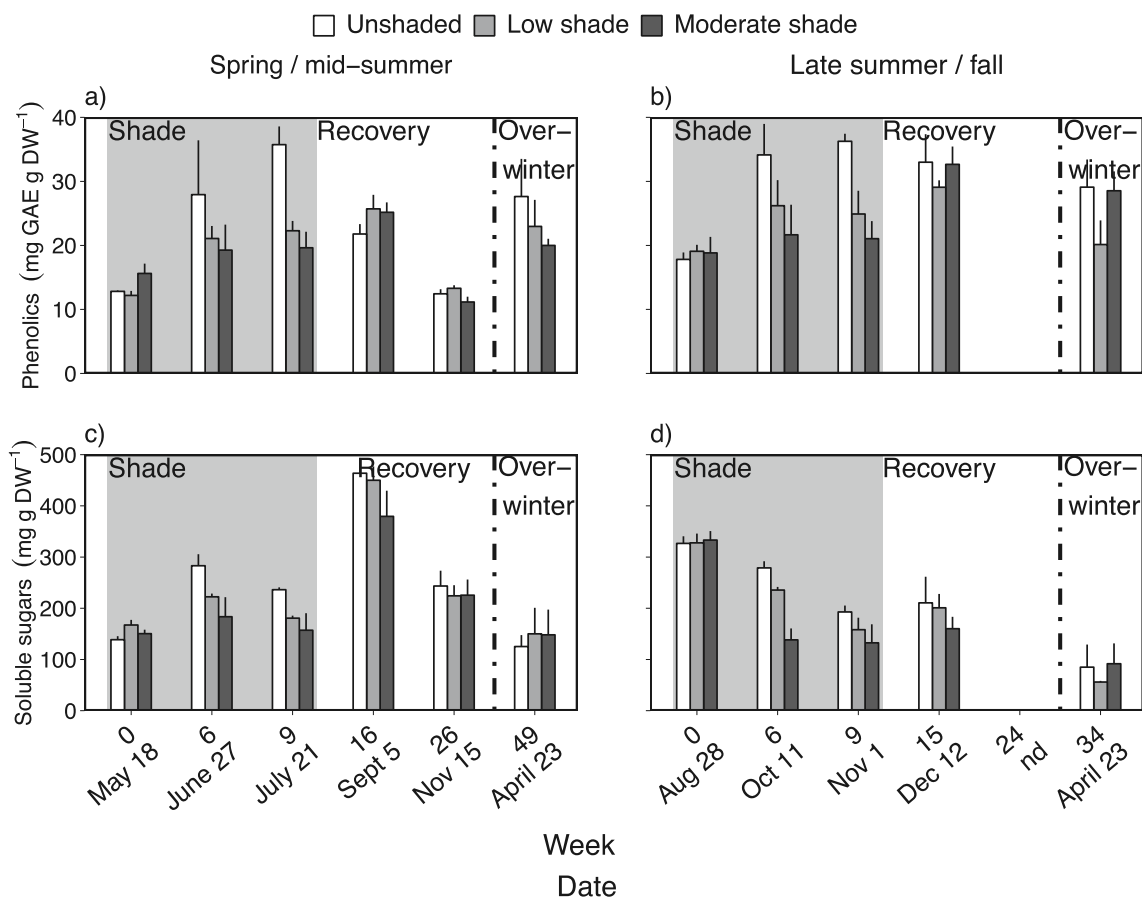


Fig. 6 Mean phenolic acid concentration and rhizome water-soluble carbohydrates (± 1 SE) during periods of shading, recovery, and after overwintering, when shading was implemented in the spring/mid-summer (a, c) and late summer/fall (b, d). nd = no data

In the fall experiment, initial WSC concentrations in all plots were high, indicating that plants were storing excess carbohydrates over the summer months when light levels were not restricting (Fig. 6d). Plants in the unshaded plots did not continue to store carbohydrates during the shading period and started using reserves in October. Despite this, moderate shade plants had lower WSC than plants in unshaded plots after 6 weeks of shading (week 6 \times moderate shade: $t = -5.023$, $df = 36$, $p < 0.0001$). WSC remained significantly lower in moderate shade plots compared to unshaded plots at the end of the shading period, even though carbohydrates in unshaded plots had continued to decrease (week 9 \times moderate shade: $t = -2.28$, $df = 36$, $p = 0.028$). WSC concentration in low shade plots also decreased across the shading period, but did not differ from unshaded plots (week 9 \times low shade: $t = -1.22$, $df = 36$, $p = 0.231$). After 6 weeks of recovery (week 15 of the experiment), plants in moderate shade plots did replace some carbohydrates used during the shading period, although concentrations remained lower than in unshaded plots (week 15 \times moderate shade: $t = -1.95$, $df = 36$, $p = 0.05$; Fig. 6d). After overwintering, WSC concentrations were similar among all plots but were lower than observed after overwintering in the spring experiment (Fig. 6d).

Discussion

Our study showed that the response and resilience of the temperate seagrass *Zostera marina* to light reduction strongly depends on the intensity and seasonal timing of the shading event. Specifically, we found that low and moderate shading in the fall had more severe impacts on *Z. marina* density, morphology, and physiology than shading in the spring. Fall shaded plants decreased faster to lower densities relative to unshaded plants, than observed during spring shading. During fall shading, both low and moderate shading reduced shoot density almost immediately, whereas during spring shading, effects of low shade were only evident after shades were removed. Moderately shaded plants in the fall also reduced growth and chlorophyll content and had high leaf loss, whereas in the spring they increased growth and chlorophyll and had lower leaf loss. Furthermore, moderately shaded plants in the fall utilized more WSC reserves than unshaded plants and did not restore these reserves to background levels during recovery. In contrast, spring shaded plants did not use carbohydrate reserves, either slightly increasing or maintaining initial levels, and built stores to near background levels during recovery. These seasonal effects of shading carried into the next growing season, with fall shaded plants having lower density and carbohydrate stores overall compared to spring shaded plants, at least for 1 year of overwintering. Although we did not replicate the experiment over multiple years, similar temperature and light conditions during the experiment to

baseline values suggests that the seasonal effect of shading would be consistently observed.

The different seasonal responses of seagrass to shading detected in our study have also been observed in other studies. However, in contrast to our results, most found that shading during the main growing season (summer) had more severe consequences than shading in other seasons (Bulthuis 1983; Fitzpatrick and Kirkman 1995; Serrano et al. 2011; Kim et al. 2015; Chartrand et al. 2016). This discrepancy likely results from the more extreme light reductions and higher summer water temperatures in these studies compared to ours. Water temperature influences seagrass response to light limitation, because respiration increases proportionately more relative to photosynthesis as temperature rises (Marsh et al. 1986; Staehr and Borum 2011). Consequently, seagrasses exposed to high water temperature require more light to maintain carbon balance and thus are more susceptible to light reduction (Staehr and Borum 2011; Moore et al. 2012; Kim et al. 2015). Although we observed temperatures in the spring experiment that were above the photosynthetic optimum (23 °C) and also 30 °C where acute mortality occurs (Lee et al. 2007a; Nejrup and Pedersen 2008), these events only had short term impacts for the unshaded and shaded seagrass. In fact, mean water temperature was similar during both the spring and fall experiments. Thus, water temperature was not likely the underlying driver of the observed seasonal shading effects. Instead, seasonal differences in daily photoperiod and hours of photosynthetic light saturation likely played a key role. Although the same degree of experimental shading was imposed in both the spring and fall, fall shaded plants experienced light reduction superimposed onto shorter photoperiods and lower incident PAR. Thus, it was necessary for fall shaded plants, particularly moderately shaded plants, to severely change their density, morphology and physiology in order to maintain carbon balance.

In both experiments, shaded plants employed several common strategies to adapt to light stress, one of which was to lower plant biomass by reducing shoot density and number of leaves. These reductions minimize self shading and thin the canopy, effectively increasing light availability for the remaining shoots (Dalla Via et al. 1998; Collier et al. 2009; Enriquez and Pantoja-Reyes 2005). Removal of plant biomass also reduced respiratory demands and the carbon required to maintain carbon balance (Lee et al. 2007a; Ralph et al. 2007). Given that the total number of hours of daily light saturation (H_{Sat}) during spring shading were higher than in the fall, fall shaded plants had to more drastically reduce shoots and leaves to maintain carbon balance. In fact, higher H_{Sat} in the spring meant that the effects of shading on shoot density were mostly evident for moderate shade plants, whereas both low and moderate shade plants reduced density with shading in the fall. It is not surprising that full mortality of shaded plants was not observed in either experiment, as the percent incident PAR

at the seagrass canopy was within or above the range of the minimal light requirements for mature northern *Z. marina* for all shade levels (12–18 % incident light; Lee et al. 2007a). Higher mortality may have resulted if sexual reproduction was more prominent at the site, as seedlings have higher light requirements than mature vegetative shoots (> 50% surface incidence light, Ochieng et al. 2010).

In addition to reductions in plant biomass, shaded plants also changed leaf morphology and growth rates to compensate for low light conditions. In the spring, increasing photoperiod and H_{Sat} supported faster growth in moderately shaded plants compared to unshaded and low shade plants, resulting in longer leaves that enhanced light capture. Spring shaded plants also enhanced photosynthetic efficiency by increasing chlorophyll content. These are common responses of seagrasses to light limitation, with increased growth often evident during the early stages of shading (Bulthuis 1983; Abal et al. 1994; Longstaff and Dennison 1999; Lee et al. 2007a). Shorter photoperiod and H_{Sat} did not support these responses in the fall experiment, where shaded plants reduced leaf growth and chlorophyll content. This decreased photosynthetic capacity but reduced respiratory demand. Similar results have been observed previously, usually when light was reduced for long periods of time (Collier et al. 2009; Lavery et al. 2009).

Shaded plants in our study further responded to low light conditions by decreasing production of phenolic acids, carbon-based secondary metabolites thought to act as deterrents against fouling, grazing, and disease infection (Vergeer et al. 1995). Phenolic acids typically show seasonal patterns with highest concentrations when plants are actively growing (Harrison and Durance 1989), although this is difficult to discern in our data. Reductions in phenolic acids were evident in shaded plants in both the spring and fall, as observed by Vergeer et al. (1995). This suggests that resistance to disease infection, grazers, and other environmental stressors may have been impaired at these times. However, an alternative view suggests that phenolic acids mainly act as antioxidants, protecting plant tissues from photo-damage (Close and McArthur 2002). If this is the case, reduced levels with shading may simply reflect the reduced potential for photo-damage.

Consistent with previous findings, our study showed that the mobilization of water-soluble rhizome carbohydrate reserves was important for the survival of light stressed *Z. marina* (Burke et al. 1996; Alcoverro et al. 1999). While our unshaded plants showed seasonal patterns in rhizome WSCs typical of northern *Z. marina* (Vichkovitten et al. 2007), spring shaded plants reduced or stopped storing carbohydrates, while fall shaded plants drew down stored reserves. Spring shaded plants did not mobilize stored WSCs because, when combined with reductions in plant biomass, the long photoperiod and high H_{Sat} allowed enough photosynthesis to maintain carbon balance. This contrasts other studies where

higher and longer light reductions and warmer water caused seagrasses to use carbohydrate reserves even when photoperiod and H_{Sat} was naturally high (Collier et al. 2009; Lavery et al. 2009). During fall shading, low and moderate shade plants utilized 14 and 46% more rhizome WSCs, respectively, than unshaded plants, because carbon balance could not be achieved from photosynthesis or changes in plant biomass alone. Clearly, the building of adequate carbohydrate reserves over the spring and summer was important for *Z. marina* resilience to light disturbance in the fall. Extended duration of shading in both experiments would have likely caused higher use of stored carbohydrates, with potentially serious consequences for overwintering.

Our field study is one of only a few to monitor the recovery of seagrass plants after experimental light reduction was ended (Collier et al. 2009; McMahan et al. 2011; Chartrand et al. 2016). In contrast to the impacts of seasonal shading on *Z. marina*, we found that plant recovery was generally similar between both experiments, despite spring shaded plants having a longer recovery under longer photoperiods and higher H_{Sat} than fall shaded plants. Within 6–15 weeks after shades were removed, most physiological metrics were similar to those in unshaded plants, a result of shaded plants changing values to reach those in unshaded plants, or from natural declines in background values. However, this rapid physiological response did not translate immediately into the recovery of shoot density and morphology, a disconnect that has been observed for other seagrass species (Collier et al. 2009; McMahan et al. 2011). Growth of previously shaded plants was slower than unshaded plants during recovery, even for spring plants that originally grew faster in the shade. Mechanisms underlying slow growth of previously light stressed plants remain unclear (Collier et al. 2009; McMahan et al. 2011).

The fact that shoot density did not recover after shades were removed or after the first year of overwintering in both experiments reflects the reliance of *Z. marina* on vegetative growth at our field site. *Z. marina* beds that have high reproductive shoot density and strong seed banks can recover large areas very quickly (Jarvis and Moore 2010; Lee et al. 2007b). However, when vegetative growth is the dominant mechanism, recolonization occurs by rhizome elongation and lateral shoot growth from remaining or neighboring rhizomes, which is a relatively slow process (Boese et al. 2009; Duarte 1991). Boese et al. (2009) found that when all *Z. marina* biomass (above and belowground) was removed from 4 m² plots, recovery was exclusively from rhizome growth of adjacent plants and took 24–48 months, depending on the continuity of the meadow. We might expect shorter recolonization times in our experiment, given the smaller disturbance, high meadow continuity, and the presence of live belowground biomass in the shaded plots (Boese et al. 2009; Smith et al. 2016). Certainly, some recovery was evident after the second

overwintering period, where shoot density in low shade plots was similar to unshaded plots in both experiments. Although shoot density in moderate shade plots tended to be lower than unshaded plots after 2 years, it had increased from the previous year and would be expected to continue recovering.

The results of our study have important implications for the management of human activities that influence light conditions in the marine nearshore, such as dredging and shoreline construction. In corroboration with other studies (e.g., Collier et al. 2009; Lavery et al. 2009; Kim et al. 2015; Chartrand et al. 2016), our study shows that the response of seagrass to light reduction depends on the duration, degree, and timing of the shading event. Shading in the spring/early summer may not be catastrophic if light reduction is relatively low and short in duration, as the naturally long photoperiod and high H_{Sat} can alleviate some shading effects. Recovery during the main growing season would allow rebuilding of carbohydrate stores before overwintering. However, shading that spans the entire growing season when carbohydrate stores are normally built would have serious consequences for overwintering, particularly if light reduction is extreme. In the fall, shading may be tolerated if strong carbohydrate stores were built during the growing season, but short photoperiod and low H_{Sat} during this time further exacerbate shading impacts. Our results suggest that there are periods during the annual phenology of *Z. marina* where light stressors will have less impact. Clearly, management practices that aim to mitigate impacts of light reduction for seagrasses should not only consider shade duration and intensity but also its seasonal timing. Use of such knowledge can delineate appropriate periods where seagrass resilience to the disturbance is maximized, contributing towards the sustainable management of valuable near-shore habitats.

Acknowledgements We thank A. Campbell, J. Hogenbom, D. Krug, B. Roethlisberger, M. Scarrow, and C. Siong for assistance in the field and laboratory. Anonymous reviewers provided helpful comments that improved the manuscript.

Funding Information Funding was provided by Fisheries and Oceans Canada.

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