Estuaries Vol. 26, No. 5, p. 1255–1268 October 2003

Interactive Effects of Light and Salinity Stress on the Growth, Reproduction, and Photosynthetic Capabilities of *Vallisneria americana* (Wild Celery)

GAIL T. FRENCH and KENNETH A. MOORE*

Virginia Institute of Marine Science, Gloucester Point, Virginia 23062

ABSTRACT: The effects of light and salinity on *Vallisneria americana* (wild celery) were studied in outdoor mesocosms for an entire growing season. Morphology, production, photosynthesis, and reproductive output were monitored from sprouting of winter buds to plant senescence and subsequent winter bud formation under four salinity (0, 5, 10, and 15 psu) and three light (2%, 8%, and 28% of surface irradiance) regimes. Chlorophyll *a* fluorescence was used to examine photochemical efficiency and relative electron transport rate. High salinity and low light each stunted plant growth and reproduction. Production (biomass, rosette production, and leaf area index) was affected more by salinity than by light, apparently because of morphological plasticity (increased leaf length and width), increased photosynthetic efficiency, and increased chlorophyll concentrations under low light. Relative maximum electron transport rate (ETR_{max}) was highest in the 28% light treatment, indicating increased photosynthetic capacity. ETR_{max} was not related to salinity, suggesting that the detrimental effects of salinity on production were through decreased photochemical efficiency and not decreased photosynthetic capacity. Light and salinity effects were interactive for measures of production, with negative salinity effects most apparent under high light conditions, and light effects found primarily at low salinity levels. For most production and morphology parameters, high light ameliorated salinity stress to a limited degree, but only between the 0 and 5 psu regimes. Growth was generally minimal in all of the 10 and 15 psu treatments, regardless of light level. Growth was also greatly reduced at 2% and 8% light. Flowering and winter bud production were impaired at 10 and 15 psu and at 2% and 8% light. Light requirements at 5 psu may be approximately 50% higher than at 0 psu. Because of the interaction between salinity and light requirements for growth, effective management of SAV requires that growth requirements incorporate the effects of combined stressors.

Introduction

Distribution and abundance of submersed aquatic vegetation (SAV) in the Chesapeake Bay has fluctuated widely over the last 40 years but continues to remain well below historical levels (Bayley et al. 1978; Orth and Moore 1983, 1984; Twilley and Barko 1990). Declines have been related in large part to water quality conditions that directly or indirectly limit light availability for growth (Kemp et al. 1983; Moore et al. 1996, 1997; Carter et al. 2000). SAV has substantial ecological and societal value because it stabilizes sediment, sequesters nutrients, and provides habitat for fish and waterfowl. Extensive research has been conducted to define optimum habitat characteristics for growth and reproduction. This information has been used to set management goals and to evaluate sites for SAV restoration (Batiuk et al. 1992, 2000).

One little-understood area of SAV ecology is the interaction between light availability and salinity stress on plant response. In estuarine systems such as the Chesapeake Bay turbidity levels are generally found to be inversely related to salinity with higher turbidities occurring in lower salinity regimes (Champ et al. 1980; Stevenson et al. 1993; Moore et al. 1997). Experimental studies indicate that SAV production is greater under elevated light (Barko et al. 1991; Carter et al. 1996; Blanch et al. 1998), but abundance of SAV species found in freshwater and oligohaline regions of the Chesapeake Bay is typically lower at higher salinities (Moore et al. 2000). A greater understanding of the interactive effects of salinity and light availability on SAV growth should provide important insights into the habitat requirements necessary for successful restoration. Will decreased turbidity increase salinity tolerance? Will lower salinity ameliorate light stress? Several short-term studies have indicated that light reduction may compound salinity stress (Kraemer et al. 1999; Ralph 1999a), but the long-term (entire growing season) effects of these two stresses, either individually or together, are poorly resolved. Also unclear is whether the effects of these stresses are interactive or additive.

The effects of different light and salinity regimes on the SAV species *Vallisneria americana* were evaluated throughout the growing season. Plant re-

* Corresponding author: tele: 804/684-7384; fax: 804/684-7752; e-mail: moore@vims.edu

sponse to environmental conditions was examined using various measures of health, including plant morphology and biomass, photosystem characteristics, and reproductive output. Direct measure of chlorophyll *a* fluorescence, a rapid, highly sensitive, and non-intrusive technique that has only recently been applied to SAV, was used to obtain information about photosystem II (PSII) photochemical processes, such as photosynthetic capacity and efficiency.

Materials and Methods

Species Description

V. americana (Michx.), also known as wild celery, tape grass, or eel-grass, is a dioecious, perennial, aquatic monocotyledon (Lowden 1982). It has linear submerged or floating leaves and a vertical stem with a short axis that produces stolons and fibrous roots (Lowden 1982). It grows primarily in eastern North America, west from Nova Scotia to South Dakota, and south to the Gulf of Mexico (Korschgen and Green 1989). In the Chesapeake Bay, *V. americana* has historically been one of the dominant freshwater and low salinity species, inhabiting the upper Potomac River, the upper James River, and the upper Chesapeake Bay, including the Susquehanna Flats and the Elk, Sassafras, Northeast, and Susquehanna rivers (Bayley et al. 1978; Haramis and Carter 1983; Twilley and Barko 1990; Moore et al. 2000). For the last 20 years, its distribution has been greatly reduced or absent from these freshwater and low salinity tidal regions (Moore et al. 2000).

Mesocosms

Outdoor mesocosms were employed to study the effects of light and salinity on *V. americana* at the Virginia Institute of Marine Science, Gloucester Point, Virginia (37°14.8′N, 76°30.3′W). *V. americana* was grown in 36, 110-l glass aquaria (60 cm × 30 cm × 60 cm). Six aquaria were positioned in each of 6 tanks. Aquaria and tanks were oriented in approximately an east-west direction. Water flowed directly from the adjacent York River through the tanks to maintain ambient river temperatures. Aquaria were maintained at one of four salinities: 0, 5, 10, and 15 psu, achieved by a combination of York River water and dechlorinated tap water. Salinity was adjusted as necessary with dechlorinated tap water and Forty Fathoms Crystal Sea salt. Water was continuously aerated by a common source and filtered using submerged aquarium filters with polyester fiber and activated charcoal.

Shade levels were achieved via neutral density shade cloth. Tanks were randomly designated high, moderate, and low light treatments (3 aquariums for each light-salinity combination). Light reaching the sediment surface was measured by a LI-COR quantum sensor (LI-192SA, LI-COR, Inc.). The high, moderate, and low light treatments provided at the sediment surface approximately 28%, 8%, and 2% of surface irradiance, or a maximum daily irradiance of 500, 144, and 36 (mol m^{-2} s^{-1} for June–August. Water depth to sediment surface was approximately 46 cm. Surface irradiance was measured continuously throughout the growing season (LI-190SA, LI-COR, Inc.) and reached highest levels in June to early July (40–50 mol m^{-2} d^{-1}), and then gradually declined to 15–20 mol m^{-2} d^{-1} by November. Aquarium water temperature was measured continuously. Summer temperatures reached 30°C and fell to 5°C by December.

Each aquarium contained four 1-l pots. Sediment was a mix of York and James River sediments (1% gravel, 87% sand, 3% silt, and 8% clay). Organic carbon content was 4%, as determined by combustion at 500(C for 5 h. Sediment NH_4^+ concentrations, determined by KCl extraction (Parsons et al. 1984), ranged from 12–111 mmol m^{-2}, with no consistent trends between treatments or over time. NO_3^{-2} is typically minimal in organic-rich freshwater sediments and was not measured (Morlock et al. 1997; Hopkinson et al. 1999). Water column dissolved inorganic nitrogen (DIN) consisting of NO_3^{-2}, NO_2^-, and NH_4^+ were determined spectrophotometrically following the methods of Parsons et al. (1984), and PO_4^{-3} concentrations following the methods of the U.S. Environmental Protection Agency (1979). DIN concentrations were less than 15 μM in the 28 and 8% light treatments and 12–130 μM in the 2% light treatment. DIN was composed primarily of NO_3^{-2}. PO_4^{-3} concentrations were as high as 9 μM in June and were lower at higher salinity. Concentrations were less than 2 μM in subsequent months.

Winter buds were collected in late March, several weeks before sprouting, from a freshwater region in Maryland. After collection, they were stored in the dark in aerated deionized (DI) water at approximately 4°C. Three buds were planted per pot, equivalent to 178 m^{-2}, on May 26 at 5–10 cm under the sediment surface. Buds that were soft or appeared in any other way unhealthy were discarded. Approximately 10–20% of buds were deemed unacceptable for use.

Sampling

Plant morphological measurements were taken once every 2 wk. These measurements include number of rosettes per pot, number of leaves per rosette, and length and width of longest leaf in each rosette. Presence of flowering structures was noted.

A destructive sub-sampling was conducted on August 4. Leaf length and number of rosettes were slightly past their maximum at this time. One pot per aquarium was randomly selected. The plants were washed free of sediment, and leaf surface area was determined (Model 3100 Area Meter, LI-COR, Inc.). Leaf area index (LAI) was determined as m^2 leaf area per m^2 sediment surface. Aboveground and belowground tissues were separated, dried at 50°C for one week, and weighed.

A pulse-amplitude modulated fluorometer (Diving PAM-2000, Heinz Walz GmbH, Germany) was used to measure chlorophyll *a* fluorescence parameters (see Schreiber et al. 1994; White and Chritchley 1999; Maxwell and Johnson 2000 for a more thorough description of the fluorometer and its applications). Two types of measurements were taken: effective and maximal quantum yield, and rapid light curves. Effective quantum yield is a measure of photosynthetic capacity under ambient light, while maximum quantum yield is an indication of maximum photochemical efficiency. Rapid light curves reveal information about light adaptation and overall photosynthetic capacity. All fluorescence measurements were taken under water on one representative (i.e., of typical appearance and intermediary age) leaf per aquarium, approximately 5 cm from the leaf base. Leaf clips were used to block ambient insolation during measurements.

Quantum yield measurements were taken once every 2 wk throughout the experiment. Maximal quantum yield (ratio of variable to maximum fluorescence, or F_v/F_m) was measured after 10 min of dark adaptation. Effective quantum yield ($\Delta F/F_m'$) was measured on the same leaves in ambient light, just prior to dark adaptation.

Rapid light curves were conducted on August 20. Short periods of light (10 s) of increasing intensity were applied to leaves by the PAM fluorometer. Relative electron transport rate (ETR) was calculated at 9 discrete irradiance steps from 0–2,240 (mol m^{-2} s^{-1}. Relative maximum ETR (ETR_{max}) was calculated as the average of the three highest consecutive ETRs per light curve.

At every other maximal quantum yield sampling (i.e., once per month), the leaves used for PAM fluorometry measurements were collected and frozen for chlorophyll analysis. Chlorophyll was extracted by grinding leaves in a solution of 80% acetone, 0.1% diethyl amine (DEA), and DI water while on ice. Solutions were then centrifuged and read on a Shimadzu UV Probe spectrophotometer using wavelengths of 645, 663, and 725 nm. Chlorophyll *a* and *b* concentrations were calculated according to Dennison (1990).

On December 13 after the leaves senesced, the winter buds produced in each treatment were counted, dried at 50°C for 1 wk, and weighed.

Statistical Analysis

The single and interactive effects of light and salinity treatments were determined using Analysis of Variance (ANOVA; StatView for Windows, SAS Institute Inc.). Prior to conducting ANOVA, normality was confirmed visually, and homogeneity of variance was verified with Cochran's test. For all measurements yielding more than one value per aquarium (i.e., length of longest leaf per rosette and other morphological measurements), data were averaged over each aquarium ($n = 3$ per treatment) for use in ANOVA. Factor level means were compared using the Student-Newman-Keuls test (SNK). Repeated measures ANOVA were conducted using a general linear model (GLM; The SAS System for Windows, SAS Institute Inc.). Repeated measures ANOVA was conducted on time series data to determine the effect of time, in addition to light and salinity, on each parameter.

Percent variance attributable to a given factor was the percent sum of squares of that factor contributing to the total sum of squares of all factors in an ANOVA. For repeated measures ANOVA percent variance was calculated separately for the date and non-date components.

Results

Morphology and Production

High salinity and low light each stunted vegetative production (rosette density, biomass, and leaf area) and reproduction (flowering and winter bud production). Rosette density, or the colonizing capability of the plants, ranged from 26–695 rosettes m^{-2}, varied seasonally, and was highly dependent on light and salinity treatments (Fig. 1). Averaged over all dates, rosette density increased with increasing light ($p = 0.0012$; Table 1). Plants in the 2% light treatment typically produced the fewest rosettes (Fig. 1). Plants in the 28% light treatment produced substantially more than either of the other light treatments at salinities ≤ 10 psu. Elevated salinity had a highly significant negative effect on vegetative reproduction ($p = 0.0001$; Table 1).

The effects of salinity and light interacted (Table 1; $p = 0.0012$) such that the effects of salinity were most apparent at 28% light and the light effects were most pronounced at the 0 and 5 psu salinity levels (Fig. 1). Partitioning of variance revealed that salinity accounted for 30% of variance, light accounted for 17%, and the light-salinity interaction accounted for 30%.

Rosette density varied significantly over time ($p < 0.0001$; Table 1), with density generally highest in the middle of July and beginning of August (Fig.

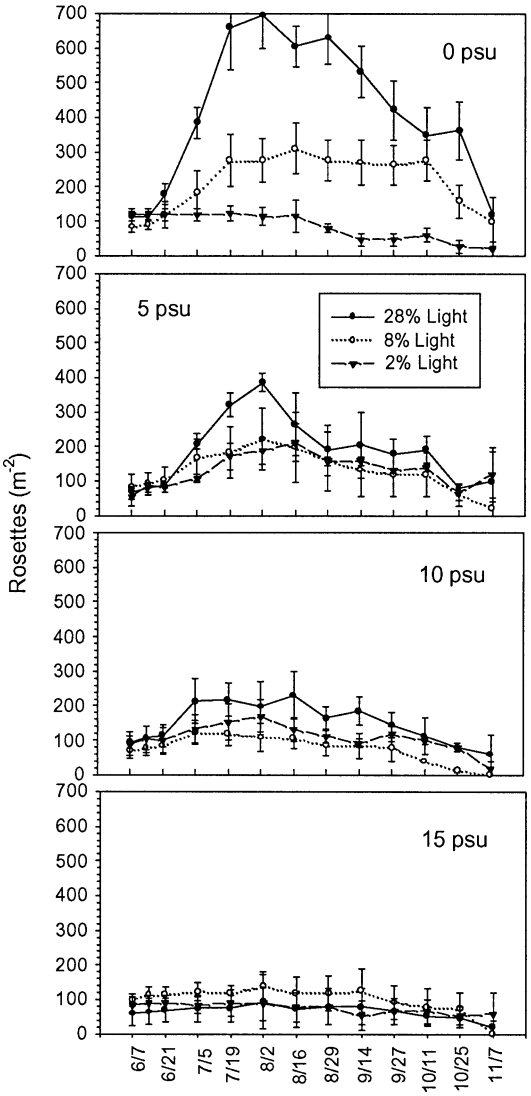

Fig. 1. Mean rosette density (m^{-2}) over time for each light and salinity treatment. Error bars represent standard error.

1). Maximum seasonal densities of 2% and 8% light treatments were not significantly different, nor were those of 10 and 15 psu treatments. Loss of rosettes in the latter part of the season was due to leaf senescence and rosettes coming loose from the sediment. Rosette density increased approximately linearly from the start of vegetative reproduction (June 14) to the approximate date of maximum density (July 19). Rate of rosette production was greatest in the 28% light, 0 psu treatment, averaging a 17% increase per day over initial abundance. Rosette production in the 8% and 2% light, 0 psu treatments was 9% and 3% d^{-1}, respectively. Production rates of 5, 10, and 15 psu treatments ranged from 6–12% d^{-1}, 4–6% d^{-1}, and 0–3% d^{-1}, respectively. Peak density corresponded with the time of maximum seasonal temperature and occurred several weeks after the time of maximum seasonal irradiance.

Leaf area and above- and belowground biomass followed the same general light and salinity patterns as rosette density, each increasing with decreasing salinity and with increasing light. At 28% light, LAI, aboveground biomass, and maximum seasonal rosette production at 15 psu were 2%, 4%, and 17%, respectively, of that at 0 psu. In the 0 psu treatment leaf area index, aboveground biomass, and maximum seasonal rosette density at 2% light were 21%, 9%, and 17%, respectively, of that at 28% light. LAI was directly related to light ($p = 0.0009$; Table 2; Fig. 2), which accounted for 20% of variance. The 28% light treatment produced the greatest LAI ($p < 0.05$), with a trend of 8% light producing greater LAI than 2% light. LAI was inversely related to salinity ($p < 0.0001$), explaining 35% of variance. LAI decreased from low salinity to high, although differences between 0 and 5 psu, and 10 and 15 psu were not significant ($p > 0.05$). The effects of light and salinity interacted ($p = 0.0125$). This interaction accounted for 21% of variance. Aboveground biomass ranged from 1.9–47.0 gdw m^{-2}. Belowground biomass ranged from 1.6–50.7 gdw m^{-2}.

Leaf length increased with decreasing light, although it increased with decreasing salinity ($p < 0.0001$; Table 1; Fig 3). Light treatment did not have a significant effect on leaf length when averaged over time ($p = 0.3728$). Inspection of the data reveals that by mid-summer, plants in the 2% and 8% light levels had produced longer leaves than the 28% light treatment, especially for the 0 psu treatment (Fig. 3).

Analysis of maximum seasonal length confirms this observation and reveals that leaves in the 28% light treatment were significantly shorter than those in the 2% and 8% light treatments ($p < 0.05$). Maximum seasonal length was also inversely related to salinity ($p < 0.0001$), except for no difference between 10 and 15 psu treatments. Light and salinity effects did not interact ($p = 0.2947$). Maximum seasonal length ranged from 5.6–52.3 cm. Adult plants in the 0 psu, 2% and 8% light treatments generally reached the water surface. The majority of variance in maximum seasonal length was attributable to salinity treatment (73.4%; Table 2), while only a small fraction was due to light treatment (5.2%) or the interactive effects of light and salinity (5.3%).

Initial leaf elongation rate was determined as the rate of elongation from planting (length = 0 cm) to maximum seasonal leaf length. Initial elongation rates ranged from 0.18 cm d^{-1}–1.24 cm d^{-1} and followed similar patterns to those of maximum

TABLE 1. General linearized model repeated measures ANOVA tables for leaf length (cm), rosette density (m^{-2}), and effective quantum yield over time. Independent variables are light and salinity. n = 3 for each light-salinity treatment. % Variance is the percent variation attributed to each parameter, as determined by partitioning of variance.

		DF	F	P	% Var.
Rosette Density	Light	2	9.04	0.0012	17.0
	Salinity	3	10.64	0.0001	30.1
	Light × salinity	6	5.36	0.0012	30.3
	Error	24			22.6
	Date	12	32.36	<0.0001	32.9
	Date × light	24	5.66	<0.0001	11.5
	Date × salinity	36	4.94	<0.0001	15.1
	Date × light × salinity	72	2.64	<0.0001	16.1
	Error (Date)	288			24.4
Maximum Leaf Length	Light	2	1.03	0.3728	2.6
	Salinity	3	16.76	<0.0001	64.3
	Light × salinity	6	0.31	0.9233	2.4
	Error	24			30.7
	Date	12	62.46	<0.0001	49.9
	Date × light	24	1.53	0.0579	2.4
	Date × salinity	36	9.92	<0.0001	23.8
	Date × light × salinity	72	0.98	0.5327	4.7
	Error (Date)	288			19.2
Effective Quantum Yield	Light	2	52.216	<0.0001	22.9
	Salinity	3	6.835	0.0002	4.5
	Date	6	14.711	<0.0001	19.4
	Light × salinity	6	2.807	0.0128	3.7
	Date × salinity	18	1.208	0.2616	4.8
	Date × light	12	2.778	0.0019	7.3
	Date × light × salinity	36	0.575	0.9731	4.5
	Error	150			32.9

seasonal leaf length (light, $p = 0.0307$; salinity, $p < 0.0001$).

Leaf width also increased with decreasing salinity ($p = 0.0012$) and displayed a non-significant trend of increasing width with increasing light ($p = 0.0510$). This was most apparent at mid-season ($p = 0.0191$) and in the 0 and 5 psu treatments. Width peaked mid-summer and then declined, from 2.7–4.8 mm in July to 0.9–2.6 mm in November.

The number of leaves per rosette was not affected by light or salinity ($p > 0.05$). However, it fol-

TABLE 2. Two-way ANOVA for light and salinity effects on morphology, production, and photosynthesis characteristics. n = 3 for each light/salinity combination. % Variance is the percent variation attributed to each parameter, as determined by partitioning of variance.

		DF	F	P	% Var.
Leaf Area Index	Light	2	9.614	0.0009	19.5
	Salinity	3	11.48	<0.0001	34.9
	Light × salinity	6	3.502	0.0125	21.3
	Error	24			24.3
Maximum Seasonal Leaf Length	Light	2	3.862	0.0351	5.2
	Salinity	3	36.359	<0.0001	73.4
	Light × salinity	6	1.301	0.2947	5.3
	Error	24			16.2
Winter Bud Density	Light	2	1.652	0.2127	8.8
	Salinity	3	1.757	0.1822	14.0
	Light × salinity	6	0.841	0.5507	13.4
	Error	24			63.8
Relative ETR_{max}, August 20	Light	2	9.33	0.0017	34.1
	Salinity	3	2.504	0.0919	13.7
	Light × salinity	6	1.771	0.1619	19.4
	Error	18			32.8
I_k, August 20	Light	2	8.564	0.0024	73.7
	Salinity	3	1.024	0.4052	8.8
	Light × salinity	6	1.031	0.4372	8.9
	Error	18			8.6

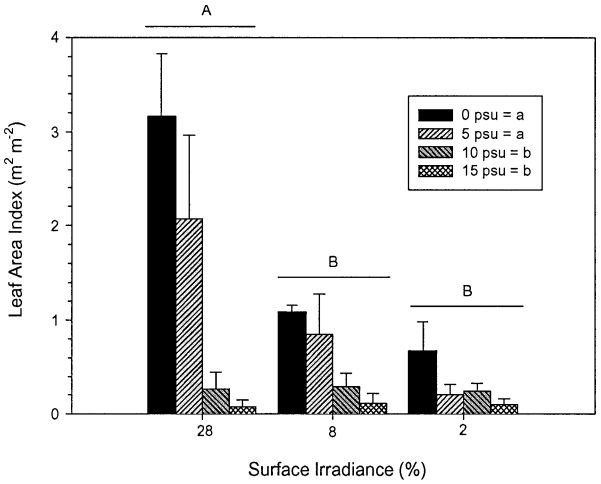

Fig. 2. Mean leaf area index (m^2 m^{-2}) of mid-season sampling (n = 3). Error bars represent standard error. Different letters over bars indicate significant differences between light treatments, while different letters in legend indicate differences between salinity treatments (Student-Newman-Keuls, $p < 0.05$).

lowed the same general seasonal pattern as length and width, averaging 4.7 leaves per rosette in mid-June, increasing to 6.5 in July, and decreasing to 2.9 by November.

Epiphytic growth on leaves was minimal in all treatments.

Reproductive Structures

Flowering occurred in August and September and only in 6 aquaria, all in 28% or 8% light and 0 or 5 psu treatments. Most flowering plants produced seeds later in the season.

End-of-season winter bud density ranged from 66 to 500 buds m^{-2} (Fig. 4). Winter bud density was highly variable within treatments and so was not significantly related to light ($p = 0.2127$; Table 2) or salinity ($p = 0.1822$). There was a clear trend of more buds at higher light and also at lower salinity. Salinity effects were most apparent at 28 and 8% light, and light effects were most prominent at 0 psu.

Total winter bud biomass displayed similar nonsignificant trends. Individual bud weight was 0.019 gdw (SE ± 0.002) and was not related to light or salinity ($p > 0.05$).

Chlorophyll

On August 2 leaf chlorophyll *a* concentrations ranged from 0.49–2.04 mg dm^{-2} and decreased with increasing light ($p = 0.0024$; Table 2; Fig. 5), with the chlorophyll concentrations of plants in the 2% light treatment greater than those in the 8% or 28% light treatment (SNK; $p < 0.05$). Chlorophyll *b* concentrations ranged from 0.39–1.16 mg dm^{-2} and followed the same pattern as chlorophyll *a*, with concentrations at the 2% light treatment greater than those in the 8% or 28% light treatment. Neither chlorophyll *a* nor *b* concentrations were related to salinity ($p > 0.05$). The ratio of chlorophyll *a* to *b* concentrations also was not related to light or salinity.

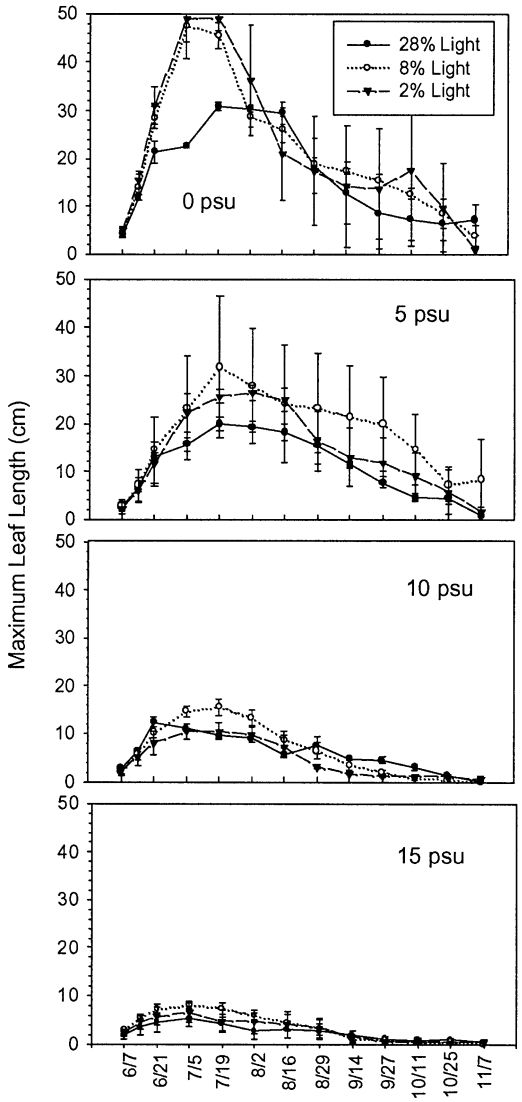

Fig. 3. Mean length of longest leaf per rosette over time for each light and salinity level. Error bars represent standard error.

Leaves were also collected for chlorophyll analysis on July 6, September 1, and September 29. Chlorophyll concentrations did not change appreciably over time and followed similar light and salinity patterns to those of August 2 samples.

PAM Fluorometry

Due to plant dieback throughout the season, there were too many missing quantum yield data

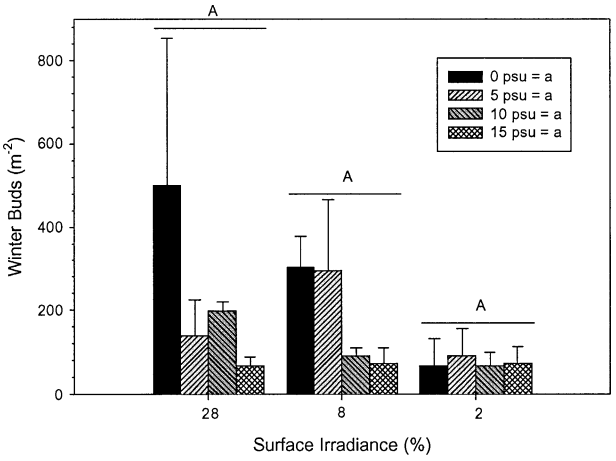

Fig. 4. Mean number of winter buds (m^{-2}) harvested at the end of the growing season. n = 3 for each light-salinity combination. Identical letters over bars and in legend indicate no significant difference between means (Student-Newman-Keuls, $p > 0.05$).

to conduct repeated measures ANOVA using the general linear model for imbalanced design (The SAS System for Windows, SAS Institute Inc.). Three-way ANOVA was conducted to quantify treatment effects instead, with date, light and salinity as factors. Due to small sample size on September 29 (n = 16), this sampling date was eliminated from analyses.

Effective quantum yield ranged from 0.61–0.75 at the start of the sampling season. High light had a strong negative effect throughout the season ($p < 0.0001$; Table 1; Fig. 6), and explained 23% of variance, more than salinity or any interactive effects. Plants in the 28% light treatment had the lowest effective quantum yield ($p < 0.05$), with yields of plants in the 8% and 2% treatments not significantly different. Effective quantum yield was significantly related to salinity ($p = 0.0002$), but only 5% of variance was attributable to this factor. Plants in the 5 psu treatment had the highest effective quantum yield, followed by 10 psu, 0 psu, and 15 psu. Effective quantum yield also varied significantly over time ($p < 0.0001$), and seasonal effects explained 19% of variance. In general, effective quantum yield was lowest in August, especially for plants in the 28% light treatment. This decline in quantum yield coincided with seasonal dieback.

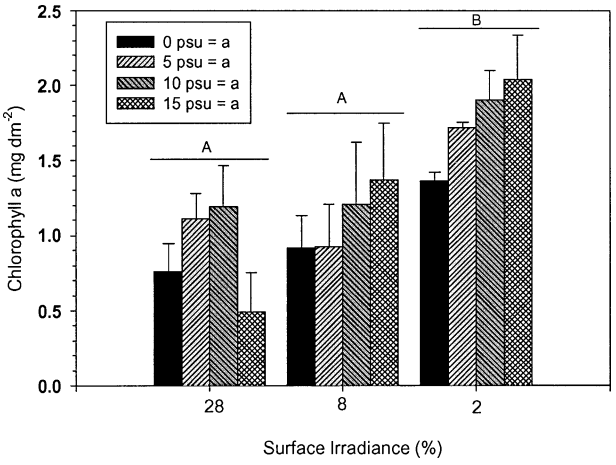

Fig. 5. Mean chlorophyll *a* concentrations (mg dm^{-2}) of leaf tissue harvested on August 2. One leaf was collected per aquarium (n = 3). Error bars represent SE. Different letters over bars indicate significant differences between light treatments, while different letters in legend indicate differences between salinity treatments (Student-Newman-Keuls, $p < 0.05$).

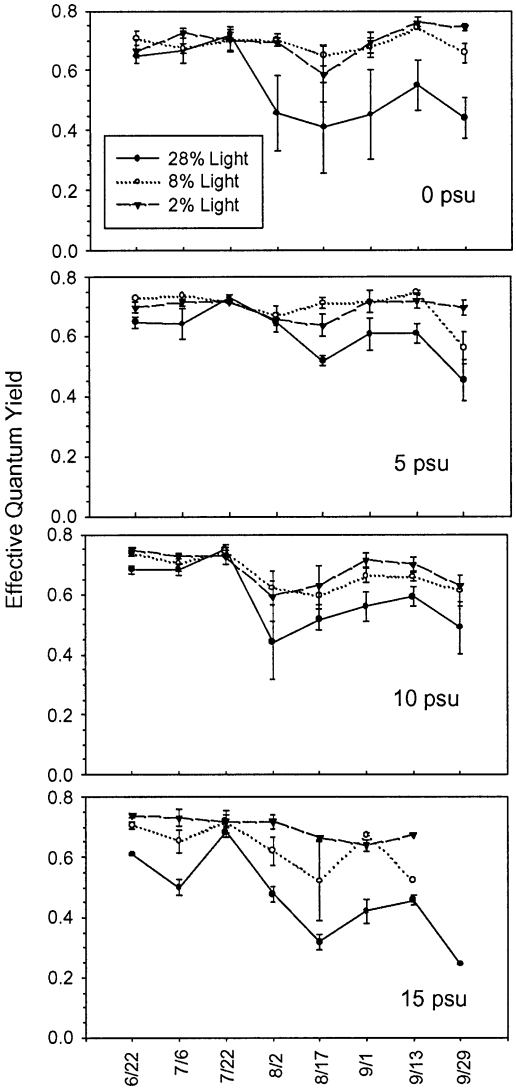

Fig. 6. Mean effective yield for light and salinity treatments over time. Error bars are standard error for a maximum of 3 samples. Sample size is less than 3 on some dates due to insufficient leaf material.

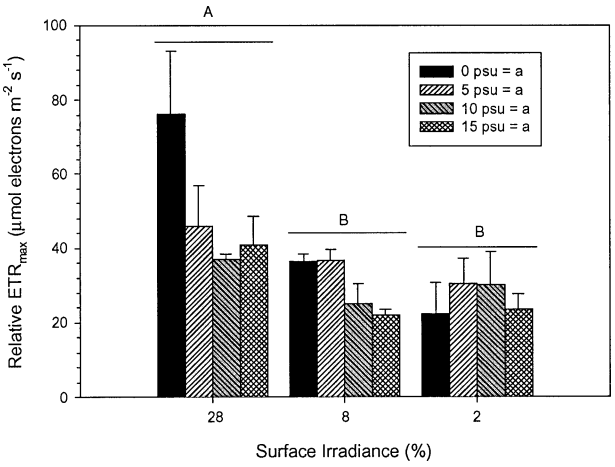

Fig. 7. Mean relative maximum electron transport rate (ETR_{max}) for each treatment on August 20. Sample size is 3 for most light-salinity combinations. It is less than three for several treatments due to insufficient leaf material. Error bars represent SE. Different letters over bars indicate significant differences between light treatments, while different letters in legend indicate differences between salinity treatments (Student-Newman-Keuls, $p < 0.05$).

Effective quantum yields of the 8% and 2% light, 0 and 5 psu plants largely recovered by the end of August (0.56–0.74), while effective quantum yields of plants in the other treatments recovered only partially, if at all (minimum of 0.25). A large portion (33%) of variance was unexplained by single or interactive effects of light, salinity, or date.

Effective quantum yield of plants in the 28% light, 0 psu treatment was low by the August 2 sampling date (0.45; Fig. 6) due to very low effective quantum yields of plants in two of the three aquaria. Leaves of these plants had become very thin, pale, and brown by this time. Maximal quantum yield, a measure of maximum photochemical efficiency, followed light, salinity, and seasonal patterns similar to those of effective quantum yield.

Relative ETR_{max} was directly related to light availability ($p = 0.0017$; Table 2; Fig. 7) and was significantly higher in the 28% light treatment than in the 8% or 2% light treatment ($p < 0.05$; Fig. 7). Salinity did not significantly affect ETR_{max} ($p = 0.0919$), although ETR_{max} in the 28% light, 0 psu treatment was greater than that in any other light or salinity treatment. Light explained 34% of variance, and 33% was not attributable to light or salinity. The minimum saturating irradiance (I_k) was significantly greater in the 28% light treatment than in the 8% or 2% light treatments ($p = 0.0024$; Table 2), but was not related to salinity. The initial slope of the rapid light curve (α) was not significantly affected by light or salinity ($p > 0.05$).

Discussion

Morphology and Production

Although both light and salinity markedly influenced *V. americana* production, salinity exerted a stronger control for the ranges considered. This suggests that salinity is the primary factor controlling the distribution of *V. americana,* as it generally only occurs in salinities up to 5 psu in the Chesapeake Bay region. The upper salinity tolerance for production reported here was between 5 and 10 psu, while the lower light requirement was more difficult to define. The smaller effect of light reduction, compared to salinity stress, on production reflects both morphological and photosynthetic adaptations to low light, such as increased elongation and photochemical efficiency. In contrast, the morphological and photosynthetic responses to salinity, such as reduced elongation and photochemical efficiency, appeared to compound other salinity effects.

Light and salinity levels strongly influenced morphology, which in turn altered the plants' ability to capture available light. Leaves of plants grown at higher salinity were shorter and narrower than those in lower salinity and thus had less leaf area over which to capture light. The reduced ability to capture light due to less leaf area was further compounded by the shorter canopy height of the higher salinity treatments. In contrast to salinity stress, reduced light resulted in taller leaves, enabling more effective light capture. Plants in the 2% and 8% light treatments were up to 66% taller than those in the 28% treatment. Leaf elongation is a common response to low light for *V. americana* (Barko et al. 1982, 1991), as well as other SAV species (Stross 1979; Barko and Smart 1981; Barko et al. 1982). Although leaf lengths in the 2% and 8% light treatments were not statistically different, lengths under 8% light were longer at mid-season than those in 2% light for all but the 0 psu treatment. These results suggest that elongation capacity is diminished at some level of irradiance below 8% surface irradiance, particularly when plants are under salinity stress.

Leaf width increased with increasing light, particularly by mid-season. The opposite responses of length and width to light resulted in no significant difference in surface area per leaf across light levels, but a greater proportion of leaf surface area near the water's surface at reduced light. Plants in lower light appeared to shift resources away from rosette production, biomass, and total leaf area to elongation. Light stress elicited a response that maximized light capture per unit of production. The phenotypic plasticity in response to light, in contrast to salinity, may be considered adaptive, as

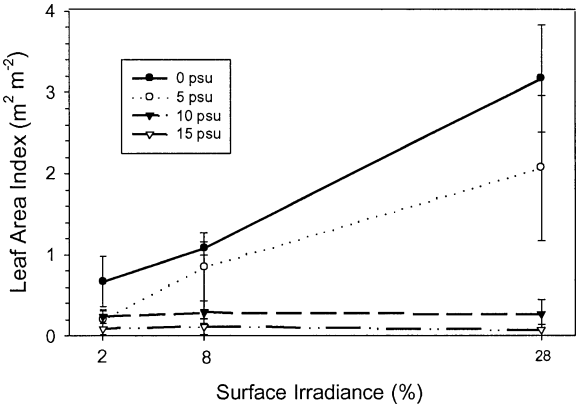

Fig. 8. Interaction plot for leaf area index at mid-season harvest. Error bars represent SE.

it appears to facilitate *V. americana* survival in a variety of environments. It may contribute to the species' wider tolerance to light, relative to salinity.

Interactive Effects

The effects of light and salinity interacted for all production measures (rosette density, above- and belowground biomass, and LAI; Fig. 8). The nature of the interaction was that the effect of one factor was greatest when the other was not limiting. Similar responses of *V. americana* have been found for light and temperature (Barko et al. 1982) and light, CO_2, and nutrients (Barko et al. 1991). High light ameliorated salinity stress at 5 psu. Salinity tolerance is contingent on light availability in the 0 to 5 psu range. Production at 10 and 15 psu did not respond to additional light. Low salinity reduced light demand at 8% and 28% light, while production at 2% light didn't respond to decreased salinity.

The effects of light and salinity did not interact for the two morphological measurements leaf length and width. Light and salinity effects for these characteristics were instead additive. This difference between responses of production and shoot morphology may be due to the effects of light and salinity stress on one morphological characteristic compounding effects on another morphological characteristic or on photosynthesis, thus disproportionately decreasing production. It has been determined for other SAV species that environmental stressors such as light and salinity can increase light requirements through increasing maximum photosynthetic rate (P_{max}), compensating irradiance (I_c), saturating irradiance (I_k), or quantum yield (Goodman et al. 1995; Masini et al. 1995; Ralph 1999a). Salinity stress induces photosystem changes that may result in decreased biomass under low light. Salinity and light stress also elicit morphological changes, primarily reduced LAI (caused by light and salinity stress) and reduced canopy height (caused by salinity stress only), which result in reduced light capture, compounding photosystem stress and, consequently, further reducing production.

Salinity tolerance may have been greater under high light due to the higher light providing additional energy for osmotic regulation. This mechanism was proposed by Kraemer et al. (1999), who observed similar light-salinity interactions.

Seasonal Response

The seasonal pattern of growth suggests that the beginning of the season may be the most important time for population establishment and resource procurement. Elongation and colonization were most rapid May through July, at which point plants reached maximum length and density, and treatment effects were greatest. This period of maximum canopy height during near-maximum irradiance is likely a critical period for plants to build up resources for vegetative reproduction and turion formation. The rate of leaf elongation was faster in the higher light and lower salinity treatments, enabling plants to capture more resources earlier in the season, thus bestowing an additional benefit to plants in these treatments. The rate of vegetative reproduction varied similarly by treatment, which may confer the added advantage to low salinity and high light plants of early modification of the local environment toward conditions more favorable for survival. Long-term survival thus appears to be particularly sensitive to stress during the first few months of growth. Restoration may be more successful when planting occurs in these early months, as vegetative spreading is greatest then.

Photosynthesis

Measures of photosynthetic performance provide insight into mechanisms by which growth and production are affected by environmental conditions. In this study, chlorophyll *a* and *b* concentrations increased with decreasing light. This is a common adaptive plant response (Dring 1986; Hale and Orcutt 1987; Kirk 1994) and can result in greater light capture. Chlorophyll *a* and *b* concentrations were not significantly different between the 8% and 28% light treatments, but concentrations in the 2% light treatment were significantly higher. These results are in contrast to growth measurements, for which there were no differences between 2% and 8% light treatments, but for which the 28% light treatment was significantly greater. These results suggest that the increase in chlorophyll concentration at the 2% light level

may have increased total light capture to approximately that of the 8% light treatment, thus obscuring differences in production between the two lower light levels.

The general lack of relationship between salinity and chlorophyll concentrations suggests that elevated salinity does not harm chlorophyll production or maintenance, nor does it stimulate greater chlorophyll production to increase specific rates of photosynthesis in order to ward off salinity stress. Twilley and Barko (1990) also found no relationship for *V. americana* between chlorophyll and salinity. Dunton (1996) also found no change in chlorophyll concentrations in *Halodule wrightii* growing along a salinity gradient. Chlorophyll concentrations have been found to increase with increasing salinity for many other species, including *Hydrilla verticillata*, *Najas indica*, and *Najas gramenia* (Rout and Shaw 2001), *Halophila ovalis* (Ralph 1998), and for many terrestrial plants (Asch et al. 2000; Romero-Aranda et al. 2001; Wang et al. 2001).

While the chlorophyll *a*:*b* ratio was not related to light in this study, many other investigators have reported a decreasing ratio with decreasing light (Dring 1986; Hale and Orcutt 1987; Ralph 1999b), and others have found contrary results (Dunton 1996; Ralph 1999a). It may be that in the current study light intensity in the 28% light treatment was not great enough to affect chlorophyll *b* levels.

The increase in photochemical efficiency and capacity, like the response of chlorophyll, also appeared to facilitate survival across a range of light regimes. Photosynthetic response to salinity stress did not appear to confer advantage to high salinity plants. The salinity-induced stress to the photosynthetic apparatus may have been the primary cause of stunted growth at high salinities. Like chlorophyll concentrations, and unlike growth measurements, light had a stronger effect on quantum yield than did salinity.

The difference in quantum yield between light levels may be due to either photoinhibition at higher light or an increase in efficiency at lower light to sustain adequate photosynthesis (Ralph and Burchett 1995; Ralph 1998b). It was more likely due to an increase in photochemical efficiency at the 2% and 8% light levels than to photoinhibition at the 28% light level. Photoinhibition was unlikely because relative ETR_{max}, which decreases with photoinhibition (White and Critchley 1999), was highest in the 28% light treatment (Fig. 7). The observed increase in efficiency under low light, along with greater leaf elongation and chlorophyll concentrations, may facilitate plant growth under low light conditions.

Although light was the primary factor controlling photochemical efficiency, salinity also had a strong negative effect. These results indicate salinity-induced stress to the photosystem and support the widespread findings that photochemical efficiency typically declines with environmental stress (Havaux 1992; Schreiber et al. 1994; Ralph 1998, 1999a; Ralph and Burchett 1998; Maxwell and Johnson 2000). This decrease in efficiency may be caused by sodium influx, potassium deficiency, intracellular ionic competition, and membrane rupture and permeability (Ralph 1998). PAM fluorometry studies on freshwater SAV are scant, but hypo- and hypersaline stress in the seagrasses *H. ovalis* (Ralph 1998) and *Zostera marina* (Kamermans et al. 1999) have also been found to decrease quantum yield. The decrease in photochemical efficiency at high salinity could be one, if not the primary, mechanism leading to reduced growth.

The one important exception to the trend of decreasing photochemical efficiency with increasing salinity is found in the 28% light, 0 psu treatment, which was lower than the 5 psu and 10 psu treatments of the same light level by the beginning of August. Although this treatment supported the most vigorous growth, leaves were very thin and pale by this time. Chlorophyll *a* and *b* concentrations on August 2 were also lower at 0 psu than at 5 psu or 10 psu in the 28% light treatment, indicating chlorosis. Quantum yield measurements mirrored physical signs of stress and decline for this treatment, too.

Quantum yield, especially effective quantum yield, was generally lowest in August, after the production peak, particularly for the 28% light treatment. This depression suggests stress at this time, possibly from an increase in respiration caused by seasonally high temperatures. Elevated temperature can have a more negative effect on photochemical efficiency than high light (Ralph 1999a). High temperatures appear primarily to affect temperature-sensitive enzymes, which in turn produce a secondary photoinhibitory response (Koroleva et al. 1994). The stress and resulting reduction in photochemical efficiency at this time of year may increase the vulnerability of *V. americana* to other stressors. The August depression was not due to a change in chlorophyll concentrations, as concentrations were not appreciably lower in August than in July.

Although quantum yield measurements were correlated with physical signs of salinity stress, they did not predict physical decline. Yield did not decrease prior to plant dieback. Rosette density, leaf elongation, and leaf width all peaked in mid-July to early August, whereas yield did not decline until early August. Long-term PAM fluorometry studies, particularly ones that compare yield measurements

with survival and other physical measurements, are rare but support the lack of strong correlations of the present study (Moore unpublished data). Other physiological measurements may provide a better indication of imminent decline. Kraemer et al. (1999) found that all physiological indices (glutamine synthetase activity and protein content in shoots, and carbohydrates, total nitrogen and carbon in shoot and subterranean tissues) except photosynthetic rate (as measured as μmol O_2 g^{-1} FW h^{-1}) declined under salinity stress. Photosynthetic rate remained high until death. The authors suggested that shoots of *V. americana* are adapted to maintain photosynthetic output as long as possible under hypersaline stress.

Plants displayed photoadaptation, as evidenced by a higher maximum rate of relative electron transfer (ETR_{max}; Fig. 7) and higher minimum I_k in the 28% light treatment. A higher relative ETR_{max} indicates a greater photosynthetic capacity. ETR is generally directly related to O_2 evolution, particularly at low light intensities (Beer and Bjork 2000; Beer et al. 2000). An increase in relative ETR_{max} under high light conditions is a common response found in a variety of plants (Beer et al. 1998; Kromkamp et al. 1998; Steindler et al. 2001), which allows them to take advantage of greater light availability. Although photochemical efficiency was lower at the 28% light level, photosynthetic capacity was greater, which is consistent with the augmented production at this light level. The increase in I_k under high light found in the current study is another common adaptive response (Beer and Ilan 1998; Steindler et al. 2001).

Salinity stress did not appear to reduce overall photosynthetic capacity. The lack of consistent relationship between salinity and relative ETR_{max} is further supported by Kraemer et al. (1999), who found no relationship between salinity level and O_2 evolution rates. While relative ETR_{max} reveals information about light adaptation, it does not appear to be a good indicator of salinity stress. It is possible that more extreme salinity stress would result in a decrease in relative ETR_{max}. Reduced plant production at higher salinity levels in the present study was likely due to the decrease in photochemical efficiency, and not a reduction in overall photosynthetic capacity.

Reproduction

Long-term survival under light and salinity stress may be diminished due to reduced winter bud production. The absence of flowering, observed here in the 2% light and 10 psu and 15 psu treatments, may also hamper long-term population survival and dispersal and maintenance of genetic diversity. Although little has been documented of the effects of salinity on *V. americana* reproduction, the negative effects of reduced light on winter bud production have been demonstrated (Korschgen et al. 1997). Although more winter buds may be produced in less stressful conditions, this study indicates that buds will not be larger in less stressed conditions, and thus may not confer any additional benefit per bud.

Light and salinity effects on winter bud production may have been partially obscured by other factors. For instance, stressed plants appeared to devote a higher proportion of resources to winter bud production, and less to shoot production, compared to less stressed plants. This plasticity in allocation of resources may be an adaptive strategy to promote long-term survival. The upper range of end-of-season winter bud density reported here was at least twice that found in several field studies (Korschgen and Green 1989). Winter bud production in the most favorable environmental conditions may have been constrained by the size of the pots, nutrient limitation, or some other factor.

Defining Light and Salinity Requirements

Production was greatly reduced at lower light levels, but did not differ between 2% and 8% surface irradiance, suggesting some threshold level between 8% and 28% light. Under 2% and 8% light environments plants were moderately productive, and even produced winter buds. Although growth was more vigorous at 28% light, growth at 2% and 8% light may have been adequate to ensure population survival to at least the next growing season. These results attest to the remarkable shade tolerance of the species, which appears to be due, at least in part, to its ability to increase chlorophyll production and photochemical efficiency and to its moderate morphological plasticity. Light requirements may be greater in the field, where more organic carbon may be lost to herbivory, leaf sloughing and fragmentation, or other factors. Episodic events as well as physical factors such as tidal or riverine currents may further stress plants (Batiuk et al. 2000; Doering et al. 2001; Koch 2001). The accuracy of both light and salinity requirements determined from this mesocosm study are improved by our study throughout an entire growing season. Most controlled studies on *V. americana* have been of much shorter duration (Haller et al. 1974; Barko et al. 1982, 1991; Staver 1986; Twilley and Barko 1990; Blanch et al. 1998; but see Kimber et al. 1995; Korschgen et al. 1997; Spencer et al. 2000). These studies typically fail to capture the effects of stress on early life stages or on other potentially sensitive periods.

Salinity tolerance is somewhere between 5 and 10 psu at 28% surface irradiance. Although it is

unclear whether survival in subsequent years would be substantially diminished at 10 and 15 psu, all vegetative production characteristics were greatly reduced at these higher levels, and thus populations likely would not adequately provide important ecosystem functions. This salinity tolerance is in concordance with that determined by most other studies (Bourn 1932, 1934; Haller et al. 1974; Davis and Brinson 1976; Staver 1986). However, it is lower than the tolerance of 12 psu or more suggested by Twilley and Barko (1990) and 15 psu or more proposed by Kraemer et al. (1999). The higher tolerance in these two studies compared to the present study were likely due in large part to their shorter timescale (Doering et al. 2001) and use of adult plants, which likely would have had stored reserves to help compensate for salinity stress. In addition, the experimental methodology of gradual acclimation likely increased tolerance in the short term in the Twilley and Barko (1990) study. Kraemer et al. (1999) observed no net growth at 15 psu. These results are not in contrast to the findings of the present study, as *V. americana* also generally survived at 15 psu. Results of this study indicate that 15 psu should not be considered an upper tolerance level, as plants sprouting from winter buds in salinities 10 and 15 psu remained very small. Kraemer et al. (1999, p. 146–147) stated that "[i]t is clear that the salinity tolerances of *V. americana* need to be revised."; "[w]hether *V. americana* could survive and reproduce after longer exposures to elevated salinity is not known." The results of the present study suggest that *V. americana* indeed could not survive at salinities of 10 psu or higher in the long term, at least not robustly.

The tolerance of *V. americana* to moderate salinity stress was dependent on the concurrent light regime. Assuming production is directly related to available light, aboveground biomass in the 5 psu treatment at 28% light may be equivalent to biomass at approximately 20% light in the 0 psu treatment (Fig. 8). This implies that *V. americana* may colonize more saline regions if turbidity were decreased. Production remained low at the highest salinity levels, regardless of light level, and it is unlikely that increased light availability would have any effect on growth or potential growth in areas where salinities are 10–15 psu.

V. americana light requirements were dependent on salinity. Although the effects of environmental conditions on photosynthesis have been widely acknowledged, primarily through photosynthesis versus irradiance measurements (Kerr and Strother 1985; Goodman et al. 1995; Masini and Manning 1997; Carter et al. 2000), this information has not been used to refine light requirements (Batiuk et al. 1992, 2000). *V. americana* light requirements generally have been defined as approximately 9% light (Batiuk et al. 1992, 2000), regardless of salinity or other environmental conditions. The results of the current study support a light requirement somewhere between 8%–28% light in 0 psu. Light requirements for *V. americana* growing at 5 psu may be approximately 50% higher than for plants growing at 0 psu.

The relationships between light and salinity must be taken into account in the development of *V. americana* growth requirements. The combined effects of environmental stressors on SAV are rarely studied (but see Goodman et al. 1995; Ralph 1999a) and are largely ignored in the development of habitat requirements for restoration. Different combinations of stressors should be studied for a variety of SAV species and for at least one growing season in order to develop more accurate growth requirements and to better manage this valuable resource.

Acknowledgments

We would like to thank everyone who helped with mesocosm construction and maintenance, data collection, and laboratory analyses, including Whitney Bishop, David Combs, Sara Everett, Curtis Copeland, Amy MacDonald, Alan Moore, Betty Berry Neikirk, Kevin Segerblom, Eric Slominski, Rachel Smith, Amy Tillman, Schuyler Van Montfrans, and Denise Wilson. Special thanks are extended to Dr. Robert Orth for support and advice, James Fishman for input on various aspects of this project, Dr. Linda Schaffner for her comments on an earlier draft of this manuscript, David Wilcox for computer help, Dr. Peter Ralph for his instruction and insight on chlorophyll *a* fluorescence, and Dr. Jennifer Rhode, Dr. Rom Lipcius, and Scott Marion for statistics help. Financial support for portions of this study was received from Norfolk Southern Foundation and the City of Hopewell, Virginia. This is contribution No. 2499 from the Virginia Institute of Marine Science, School of Marine Science, College of William and Mary.

Literature Cited

Asch, F., M. Dingkuhn, and K. Dorffling. 2000. Salinity increases CO_2 assimilation but reduces growth in field-grown, irrigated rice. *Plant and Soil* 218:1–10.

Barko, J. W., D. G. Hardin, and M. S. Matthews. 1982. Growth and morphology of submersed freshwater macrophytes in relation to light and temperature. *Canadian Journal of Botany* 60: 877–887.

Barko, J. W. and R. M. Smart. 1981. Comparative influences of light and temperature on the growth and metabolism of selected submersed freshwater macrophytes. *Ecological Monographs* 51:219–235.

Barko, J. W., R. M. Smart, and D. G. McFarland. 1991. Interactive effects of environmental conditions on the growth of submersed aquatic macrophytes. *Journal of Freshwater Ecology* 6: 199–207.

Batiuk, R. A., P. Bergstrom, M. Kemp, E. Koch, L. Murray, J. C. Stevenson, R. Bartleson, V. Carter, N. B. Rybicki, C. Gallegos, L. Karrh, M. Naylor, D. Wilcox, K. Moore, S. Ailstock, and M. Teichberg. 2000. Chesapeake Bay submerged aquatic vegetation water quality and habitat-based requirements and restoration targets: A second technical syn-

thesis. U.S. Environmental Protection Agency, Chesapeake Bay Program, Annapolis, Maryland.

Batiuk, R. A., R. J. Orth, K. A. Moore, W. C. Dennison, J. C. Stevenson, L. Staver, V. Carter, N. Rybicki, R. E. Hickman, S. Kollar, S. Bieber, P. Heasly, and P. Bergstrom. 1992. Chesapeake Bay submerged aquatic vegetation habitat requirements and restoration targets: A technical synthesis. CBP/TRS 83/92. U.S. Environmental Protection Agency, Chesapeake Bay Program, Annapolis, Maryland.

Bayley, S., V. D. Stotts, P. F. Springer, and J. Steenis. 1978. Changes in submerged aquatic macrophyte populations at the head of the Chesapeake Bay, 1958–1974. *Estuaries* 1:171–182.

Beer, S. and M. Bjork. 2000. Measuring rates of photosynthesis of two tropical seagrasses by pulse amplitude modulated (PAM) fluorometry. *Aquatic Botany* 66:69–76.

Beer, S. and M. Ilan. 1998. In situ measurements of photosynthetic irradiance responses of two Red Sea sponges growing under dim light conditions. *Marine Biology* 131:613–617.

Beer, S., M. Ilan, A. Eshel, A. Weil, and I. Brickner. 1998. Use of pulse amplitude modulated (PAM) fluorometry for in situ measurements of photosynthesis in two Red Sea faviid corals. *Marine Biology* 131:607–612.

Beer, S., C. Larsson, O. Poryan, and L. Axelsson. 2000. Photosynthetic rates of *Ulva* (Chlorophyta) measured by pulse amplitude modulated (PAM) fluorometry. *European Journal of Phycology* 35:69–74.

Blanch, S. J., G. G. Ganf, and K. F. Walker. 1998. Growth and recruitment in *Vallisneria americana* as related to average irradiance in the water column. *Aquatic Botany* 61:181–205.

Bourn, W. S. 1932. Ecological and physiological studies on certain aquatic angiosperms. *Contributions from Boyce Thompson Institute* 4:425–496.

Bourn, W. S. 1934. Sea-water tolerance of *Vallisneria spiralis* L. and *Potamogeton foliosus* Raf. *Contributions from Boyce Thompson Institute* 6:303–308.

Carter, V., N. B. Rybicki, J. M. Landwehr, and M. Naylor. 2000. Light requirements for SAV survival and growth, p. 11–33. *In* Chesapeake Bay SAV Water Quality and Habitat-based Requirements and Restoration Targets: A Second Technical Synthesis. U.S. Environmental Protection Agency, Chesapeake Bay Program, Annapolis, Maryland.

Carter, V., N. B. Rybicki, and M. Turtora. 1996. Effect of increasing photon irradiance on the growth of *Vallisneria americana* in the tidal Potomac River. *Aquatic Botany* 54:337–345.

Champ, M. A., G. A. Gould, III, W. E. Bozzo, S. G. Ackleson, and K. C. Vierra. 1980. Characterization of light extinction and attenuation in Chesapeake Bay, August 1977, p. 263–277. *In* V. S. Kennedy (ed.), Estuarine Perspectives. Academic Press, New York.

Davis, G. J. and M. M. Brinson. 1976. The submersed macrophytes of the Pamlico River estuary, North Carolina. University of North Carolina, Water Resources Research Institute Report No. 112. Raleigh, North Carolina.

Dennison, W. C. 1990. Chlorophyll content, p. 83–86. *In* R. C. Phillips and C. P. McRoy (eds.), Seagrass Research Methods. UNESCO, Paris, France.

Doering, P. H., R. H. Chamberlain, and J. M. McMunigal. 2001. Effects of simulated saltwater intrusions on the growth and survival of wild celery, *Vallisneria americana,* from the Caloosahatchee estuary (South Florida). *Estuaries* 24:894–903.

Dring, M. J. 1986. The Biology of Marine Plants. Edward Arnold, London, U.K.

Dunton, K. H. 1996. Photosynthetic production and biomass of the subtropical seagrass *Halodule wrightii* along an estuarine gradient. *Estuaries* 19:436–447.

Goodman, J. L., K. A. Moore, and W. C. Dennison. 1995. Photosynthetic responses of eelgrass (*Zostera marina* L.) to light and sediment sulfide in a shallow barrier island lagoon. *Aquatic Botany* 50:37–47.

Hale, M. G. and D. M. Orcutt. 1987. The Physiology of Plants Under Stress. John Wiley and Sons, Inc., New York.

Haller, W. T., D. L. Sutton, and W. C. Barlowe. 1974. Effects of salinity on growth of several aquatic macrophytes. *Ecology* 55:891–894.

Haramis, G. M. and V. Carter. 1983. Distribution of submersed aquatic macrophytes in the tidal Potomac River. *Aquatic Botany* 15:65–79.

Havaux, M. 1992. Stress tolerance of photosystem II in vivo. *Plant Physiology* 100:424–432.

Hopkinson, Jr., C. S., A. E. Giblin, J. Tucker, and R. H. Garritt. 1999. Benthic metabolism and nutrient cycling along an estuarine salinity gradient. *Estuaries* 22:863–881.

Kamermans, P., M. A. Hemminga, and D. J. Jong. 1999. Significance of salinity and silicon levels for growth of a formerly estuarine eelgrass (*Zostera marina*) population (Lake Grevelingen, The Netherlands). *Marine Biology* 133:527–539.

Kemp, W. M., R. R. Twilley, J. C. Stevenson, W. R. Boynton, and J. C. Means. 1983. The decline of submerged vascular plants in upper Chesapeake Bay: Summary of results concerning possible causes. *Marine Technology Society Journal* 17: 78–89.

Kerr, E. A. and S. Strother. 1985. Effects of irradiance, temperature and salinity on photosynthesis of *Zostera muelleri*. *Aquatic Botany* 23:177–184.

Kimber, A., C. E. Korschgen, and A. G. Van der Valk. 1995. The distribution of *Vallisneria americana* seeds and seedling light requirements in the Upper Mississippi River. *Canadian Journal of Botany* 73:1966–1973.

Kirk, J. T. O. 1994. Light and Photosynthesis in Aquatic Ecosystems. Cambridge University Press, Cambridge, U.K.

Koch, E. W. 2001. Beyond light: Physical, geological and geochemical parameters as possible submersed aquatic vegetation habitat requirements. *Estuaries* 24:1–17.

Koroleva, O. Y., W. Bruggemann, and G. H. Krause. 1994. Photoinhibition, xanthophyll cycle and in vivo chlorophyll fluorescence quenching of chilling-tolerant *Oxyria digyna* and chilling sensitive *Zea mays*. *Physiologia Plantarum* 92:577–584.

Korschgen, C. E. and W. L. Green. 1989. American wild celery (*Vallisneria americana*): Ecological considerations for restoration work. Fish and Wildlife technical report. U.S. Fish and Wildlife Service, Washington, D.C.

Korschgen, C. E., W. L. Green, and K. P. Kenow. 1997. Effects of irradiance on growth and winter bud production by *Vallisneria americana* and consequences to its abundance and distribution. *Aquatic Botany* 58:1–9.

Kraemer, G. P., R. H. Chamberlain, P. H. Doering, A. D. Steinman, and M. D. Hanisak. 1999. Physiological responses of transplants of the freshwater angiosperm *Vallisneria americana* along a salinity gradient in the Caloosahatchee estuary (southwestern Florida). *Estuaries* 22:138–148.

Kromkamp, J., C. Barranguet, and J. Peene. 1998. Determination of microphytobenthos PSII quantum efficiency and photosynthetic activity by means of variable chlorophyll fluorescence. *Marine Ecology Progress Series* 162:45–55.

Lazar, A. C. and C. J. Dawes. 1991. A seasonal study of the seagrass *Ruppia maritima* L. in Tampa Bay, Florida. Organic constituents and tolerances to salinity and temperature. *Botanica Marina* 34:265–269.

Lowden, R. M. 1982. An approach to the taxonomy of *Vallisneria* L. (Hydrocharitaceae). *Aquatic Botany* 13:269–298.

Masini, R. J., J. L. Cary, C. J. Simpson, and A. J. McComb. 1995. Effects of light and temperature on the photosynthesis of temperate meadow-forming seagrasses in Western Australia. *Aquatic Botany* 49:239–254.

Masini, R. J. and C. R. Manning. 1997. The photosynthetic re-

sponses to irradiance and temperature of four meadow-forming seagrasses. *Aquatic Botany* 58:21–36.

MAXWELL, K. AND G. N. JOHNSON. 2000. Chlorophyll fluorescence—A practical guide. *Journal of Experimental Botany* 51: 659–668.

MEYER, B. S., F. H. BELL, L. C. THOMPSON, AND E. I. CLAY. 1943. Effect of depth of immersion on apparent photosynthesis in submersed vascular aquatics. *Ecology* 24:393–399.

MOORE, K. A., H. A. NECKLES, AND R. J. ORTH. 1996. *Zostera marina* (eelgrass) growth and survival along a gradient of nutrients and turbidity in the lower Chesapeake Bay. *Marine Ecology Progress Series* 142:247–259.

MOORE, K. A., R. L. WETZEL, AND R. J. ORTH. 1997. Seasonal pulses of turbidity and their relations to eelgrass (*Zostera marina* L.) survival in an estuary. *Journal of Experimental Marine Biology and Ecology* 215:115–134.

MOORE, K. A., D. J. WILCOX, AND R. J. ORTH. 2000. Analysis of abundance of submersed aquatic vegetation communities in the Chesapeake Bay. *Estuaries* 23:115–127.

MORLOCK, S., D. TAYLOR, A. GIBLIN, C. HOPKINSON, AND J. TUCKER. 1997. Effect of salinity on the fate of inorganic nitrogen in sediments of the Parker River estuary, Massachusetts. *Biological Bulletin* 193:290–292.

ORTH, R. J. AND K. A. MOORE. 1983. Chesapeake Bay: An unprecedented decline in submerged aquatic vegetation. *Science* 222:51–53.

ORTH, R. J. AND K. A. MOORE. 1984. Distribution and abundance of submerged aquatic vegetation in Chesapeake Bay: A historical perspective. *Estuaries* 7:531–540.

PARSONS, T. R., Y. MAITA, AND C. M. LALLI. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, New York.

PEREZ, M. AND J. ROMERO. 1992. Photosynthetic response to light and temperature of the seagrass *Cymodocea nodosa* and the prediction of its seasonality. *Aquatic Botany* 43:51–62.

RALPH, P. J. 1998. Photosynthetic response of *Halophila ovalis* (R. Br.) Hook. f. to osmotic stress. *Journal of Experimental and Marine Biology and Ecology* 227:203–220.

RALPH, P. J. 1999a. Photosynthetic response of *Halophila ovalis* (R. Br.) Hook. f. to combined environmental stress. *Aquatic Botany* 65:83–86.

RALPH, P. J. 1999b. Light-induced photoinhibitory stress responses of laboratory-cultured *Halophila ovalis*. *Botanica Marina* 42:11–22.

RALPH, P. J. AND M. D. BURCHETT. 1995. Photosynthetic responses of the seagrass *Halophila ovalis* (R. Br.) Hook. f. to high irradiance stress, using chlorophyll *a* fluorescence. *Aquatic Botany* 51:55–66.

RALPH, P. J. AND M. D. BURCHETT. 1998. Impact of petrochemicals on the photosynthesis of *Halophila ovalis* using chlorophyll fluorescence. *Marine Pollution Bulletin* 36:429–436.

ROMERO-ARANDA, R., T. SORIA, AND J. CUARTERO. 2001. Tomato plant-water uptake and plant-water relationships under saline growth conditions. *Plant Science* 160:265–272.

ROUT, N. P. AND B. P. SHAW. 2001. Salt tolerance in aquatic macrophytes: Possible involvement of the antioxidative enzymes. *Plant Science* 160:415–423.

SCHREIBER, U., W. BILGER, AND C. NEUBAUER. 1994. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. *Ecological Studies: Analysis and Synthesis* 100:49–70.

SPENCER, D. F., G. G. KSANDER, J. D. MADSEN, AND C. S. OWENS. 2000. Emergence of vegetative propagules of *Potamogeton nodosus, Potamogeton pectinatus, Vallisneria americana,* and *Hydrilla verticillata* based on accumulated degree-days. *Aquatic Botany* 67:237–249.

STAVER, L. W. 1986. Competitive interactions of submersed aquatic vegetation under varying nutrient and salinity conditions. Master's Thesis, University of Maryland, College Park, Maryland.

STEINDLER, L., S. BEER, A. PERETZMAN-SHEMER, C. NYBERG, AND M. ILAN. 2001. Photoadaptation of zooxanthellae in the sponge *Cliona vastifica* from the Red Sea, as measured in situ. *Marine Biology* 138:511–515.

STEVENSON, J. C., L. W. STAVER, AND K. W. STAVER. 1993. Water quality associated with survival of submersed aquatic vegetation along an estuarine gradient. *Estuaries* 16:346–361.

STROSS, R. G. 1979. Density and boundary regulations of the *Nitella* meadow in Lake George, New York. *Aquatic Botany* 6: 285–300.

TITUS, J. E. AND M. S. ADAMS. 1979. Coexistence and the comparative light relations of the submersed macrophytes *Myriophyllum spicatum* L. and *Vallisneria americana* Michx. *Oecologia* 40:273–286.

TWILLEY, R. R. AND J. W. BARKO. 1990. The growth of submersed macrophytes under experimental salinity and light conditions. *Estuaries* 13:311–321.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Manual of methods for chemical analysis of water and wastes. EPA-600-4-79-020. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

VAN DIJK, G. M. 1991. Survival of a *Potamogeton pectinatus* L. population under various light conditions in a shallow euthrophic lake (Lake Veluwe) in the Netherlands. *Aquatic Botany* 39:121–129.

WANG, D., M. C. SHANNON, AND C. M. GRIEVE. 2001. Salinity reduces radiation absorption and use efficiency in soybean. *Field Crops Research* 69:267–277.

WHITE, A. J. AND C. CRITCHLEY. 1999. Rapid light curves: A new fluorescence method to assess the state of the photosynthetic apparatus. *Photosynthesis Research* 59:63–72.

Received for consideration, June 27, 2002
Revised, September 30, 2002
Accepted for publication, November 5, 2002