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Effects of Light and Salinity Stress on Vallisneria americana (Wild Celery)

Gail T. French
College of William and Mary - Virginia Institute of Marine Science

EFFECTS OF LIGHT AND SALINITY STRESS ON VALLISNERIA AMERICANA (WILD CELERY)

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Master of Science

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of

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Gail T. French

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Kenneth A. Moore, Ph.D.
Committee Chairman/Advisor

Robert J. Orth, Ph.D.

James E. Perry, III, Ph.D.

Linda C. Schaffner, Ph.D.

Peter A. van Veld, Ph.D.

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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 tuber sprouting to plant senescence 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 electron transport rate. High salinity and low light each negatively influenced 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. Conversely, high salinity resulted in decreased photosynthetic efficiency, morphological changes that compounded salinity stress (reduced leaf elongation), and no change in chlorophyll concentrations. Light and salinity stresses were additive for morphological and photosynthetic characteristics. Although fluorescence parameters were correlated with physical symptoms of light and salinity stress, they did not predict reduced growth or death. Maximum electron transport rate (ETR_{max}) was highest in the 28% light treatment, indicating increased photosynthetic capacity. ETR_{max} was not, however, 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. The difference between responses of production and morphological measures may be due to the effects of light and salinity stress on a morphological characteristic compounding effects on another morphological characteristic or on photosynthesis, thus disproportionately decreasing production. 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 reduced at 2 and 8% light. The 28% light treatment was approximately at saturating levels, but did not cause photoinhibition. In addition, flowering and tuber production were impaired at 10 and 15 psu and at 2 and 8% light. Thus, upper salinity tolerance was between 5 and 10 psu, and light requirements may have been between 8 and 28% light. However, light requirements at 5 psu may be approximately 50% higher than at 0 psu. Results suggest that improving water clarity in the Chesapeake Bay may increase distribution, but only into regions less than 10 psu. The period May through July appears to be more important for resource procurement and colonization; thus, transplanting may be more successful at this time. 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.

EFFECTS OF LIGHT AND SALINITY STRESS ON *VALLISNERIA AMERICANA* (WILD CELERY)

INTRODUCTION

Over the past thirty years, distribution and abundance of rooted angiosperms, or submersed aquatic vegetation (SAV), in the Chesapeake Bay has fluctuated at levels well below historical levels (Bayley et al. 1978, Orth and Moore 1983, Orth and Moore 1984, Twilley and Barko 1990). Declines have been related in large part to water quality conditions that directly or indirectly limit light availability to the submersed plants for growth (Kemp et al. 1983, Moore et al. 1996, Moore et al. 1997, Carter et al. 2000). Due to the ecological and societal value of these submerged plant communities, extensive research has been conducted to determine the causes of the decline and 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).

One poorly 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). However, distribution of freshwater species generally decreases at higher salinities (Moore et al. 2000a). Therefore, greater understanding of the interactive effects of salinity and light availability on SAV growth can provide important insights into the habitat requirements of SAV which are necessary for improving conditions for restoration. With increasing development along shorelines

throughout the world, including the Chesapeake Bay, turbidity is rising (Dennison et al. 1993). Largely unknown is the effect of this increased turbidity alone. Also unknown is whether there is an interactive effect between light and salinity. For instance, will increased turbidity decrease salinity tolerance? Will lower salinity ameliorate light stress? Answers to these questions will help us better manage the factors affecting SAV and determine suitable areas for transplanting. Several short-term studies have indicated that light stress may compound salinity stress (Kraemer et al. 1999, Ralph 1999c), but little is known about the long-term (entire growing season) effects of these two stresses, either individually or together. Also unclear is whether these stresses are interactive or additive.

In this study I evaluated the effects of different light and salinity regimes on the SAV species *Vallisneria americana* throughout the growing season. I examined plant response to environmental conditions using various measures of health, including plant growth, morphology and biomass, photosystem characteristics, and reproductive output.

Ecology of Vallisneria americana

Taxonomy and morphology

Vallisneria americana (Michx.), also known as wild celery, tape grass, or eel-grass, is a dioecious, freshwater, perennial aquatic plant (Lowden 1982) of the Hydrocharitaceae family. It has linear submerged or floating leaves that can reach lengths of 2 m or more. Its stem is vertical with a short axis and produces stolons and fibrous roots (Lowden 1982). *V. americana* produces two basic forms: narrow- and broad-leaved. The narrow-leaved variety bears leaves less than 10 mm wide with 3 to 5 distinct longitudinal veins and smooth to finely toothed margins. It is found in lakes, lagoons, and

freshwater inland waterways. Leaves of the broad-leaved variety are 10 to 25 mm wide with 5 to 9 veins and prominently toothed margins. It is found in coastal freshwater inlets or spring-fed waterways subject to nearly constant temperature (Lowden 1982). The narrow-leaved variety is the subject of this study.

Distribution

V. americana grows primarily in eastern North America, west from Nova Scotia to South Dakota and south to the Gulf of Mexico (Korschgen and Green 1988). In the Chesapeake Bay, *V. americana* has historically been one of the dominant freshwater and low salinity species, inhabiting the upper Potomac, the upper James, 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. 2000a). It co-occurs most commonly with *Myriophyllum spicatum* and *Hydrilla verticillata* (Moore et al. 2000a).

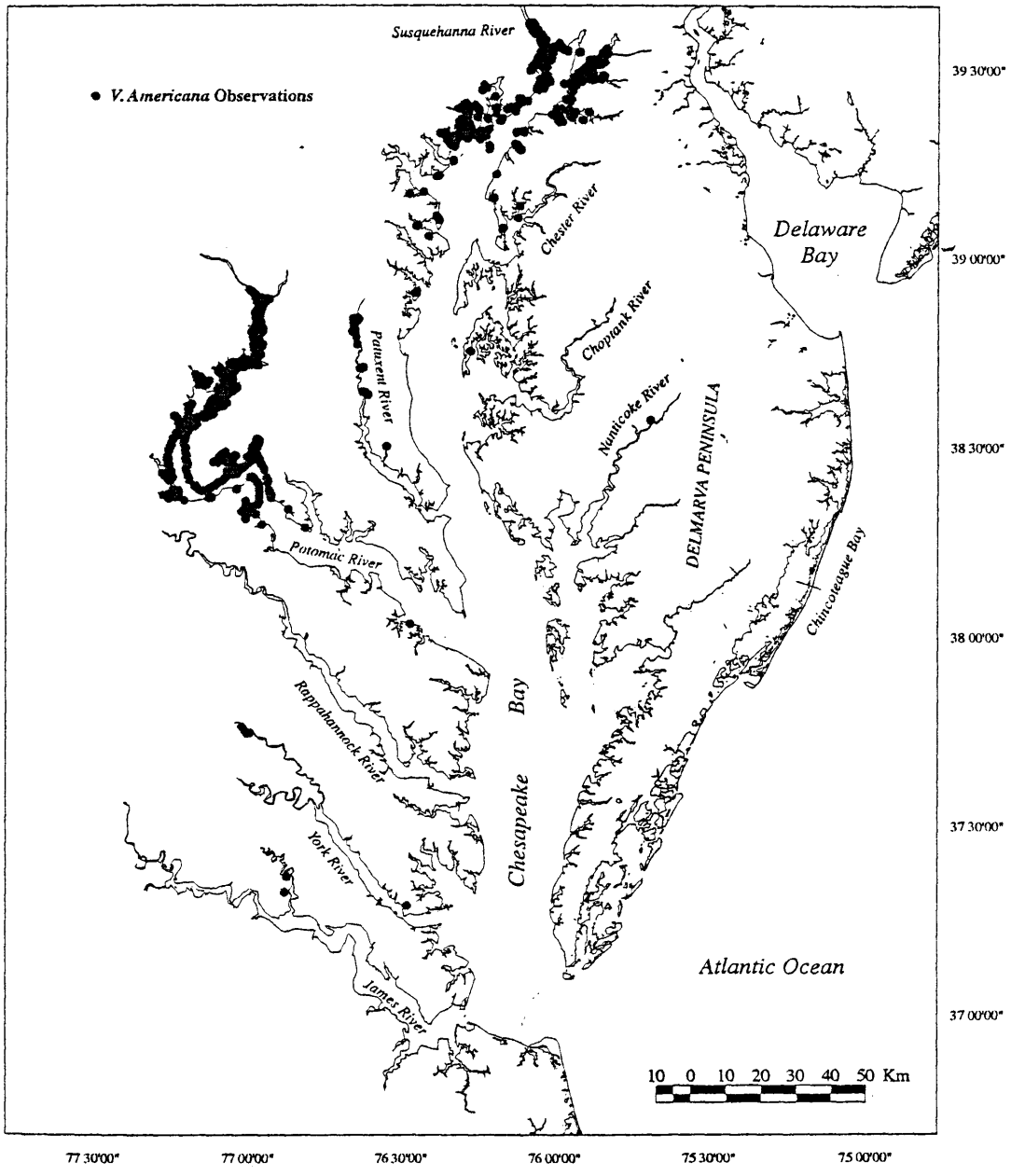
Unfortunately, historical distribution studies do not generally distinguish between *V. americana* and other species in the typical freshwater SAV community (Moore et al. 2000a), so tracing historical changes in *V. americana* abundance requires the assumption that *V. americana* has followed the patterns of other freshwater species.

Throughout the last half century, freshwater SAV abundance in the Chesapeake Bay has fluctuated greatly. For example, in the tidal Potomac River the areal distribution of submersed macrophyte species in 1981 was less than 25% of that in 1960 (Haramis and Carter 1983). This decline has been related to increased nutrient and sediment inputs. This same area experienced SAV resurgence in 1983 and 1984 (Twilley and Barko

1990). This comeback has been attributed to increased water clarity, a result of improvements in sewage treatment and unusual weather conditions (Dennison et al. 1993), as well as the introduction and rapid expansion of the exotic species *Hydrilla verticillata* (Carter and Rybicki 1986, Orth et al. 1994). The tidal freshwater portions of the James River are thought to have supported SAV growth until the mid-1940's. Currently, SAV is found only in some tributary creeks near the Chickahominy River (Orth et al. 1999, Moore et al. 2000b). This decline may be due to any number of factors, including reduced water clarity due to suspended solids and phytoplankton, high epiphyte loads, poor sediment characteristics (i.e., high organic content), or physical limitation due to biological or physical disturbance (Moore et al. 2000b). Although detailed records of SAV distribution in the upper Chesapeake Bay do not exist for the early part of the twentieth century and previously, it is likely that distribution is substantially less today (Orth and Moore 1984). Currently, only 20% of the upper Chesapeake Bay that could potentially support SAV is vegetated, and most vegetated areas are sparsely covered (Dennison et al. 1993). *V. americana* is primarily found in the Susquehanna and Potomac Rivers only (Fig. 1, from Moore et al. 2000a).

Freshwater SAV distribution has exhibited recent periods of increase. For example, from 1985 to 1993 Chesapeake Bay freshwater SAV increased from 3,200 to 6,650 metric tons, or 3200 to 4800 ha (Moore et al. 2000a, Orth et al. 1999), suggesting that water quality may be improving in certain areas.

Figure 1. Distribution of *V. americana* throughout tidal regions of the Chesapeake Bay. Dots indicate all observations of the species made between 1985 and 1999 (Moore et al. 2000a, and updated by VIMS SAV mapping program).


Susquehanna River
• V. Americana Observations
Chester River
Delaware Bay
Choptank River
Patuxent River
Nanticoke River
DELMARVA PENINSULA
Potomac River
Chincoteague Bay
Rappahannock River
Chesapeake Bay
York River
Atlantic Ocean
James River
10 0 10 20 30 40 50 Km
39 30'00"
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77 30'00"
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Reproduction

V. Americana primarily reproduces asexually, although it is capable of sexual reproduction, as well. Shoots emerge in late spring, when temperatures reach 10-14°C, from tubers (also called winter buds or turions) (Korschgen and Green 1988). Tubers are generally buried 5-27 cm deep in the Potomac River (Carter and Rybicki 1985). Near the end of the growing season in late summer, the production of rosettes, or leaf clusters, stops and some rosettes develop one or more tubers on stolons that grow down into the sediment (Titus and Stephens 1983). After tuber formation, the remaining stem tissue degenerates and breaks free of the substrate, floating until it decomposes.

Sexual reproduction occurs more rarely than asexual reproduction, although it may be critical for long distance dispersal and maintenance of genetic diversity. During the 1978 growing season in Chenango Lake, New York, for instance, only 24% of sampled rosettes flowered (Titus and Stephens 1983). In the Pamlico River estuary, North Carolina, no germination from seeds was observed (Korschgen and Green 1988). Flowers are generally produced in summer (Carter and Rybicki 1985, Korschgen and Green 1988). In late summer or early fall, some of the fruit capsules rupture and release a gelatinous matrix containing seeds (Kaul 1978). Other fruits do not rupture until the plants break free of the substrate and float away (Korschgen and Green 1988).

Habitat value

V. americana has many important ecological functions. Like other SAV species, its roots, rhizomes, and stolons provide structural support and habitat for benthic algae and invertebrates, and stabilize nearshore sediments, thus reducing erosion (Sculthorpe

1967). Its foliage provides shelter to many types of organisms and, during daylight hours, a locally enriched oxygen supply (Sculthorpe 1967). It removes inorganic nutrients from the water column, thereby suppressing algal growth (Stevenson et al. 1979). Beds also provide habitat for a number of fish species, including bluegills (*Lepomis macrochirus),* pumpkinseeds (*Lepomis gibbosus*), and yellow perch (*Perca flavescens*). As a food source, *V. americana* is a particularly desirable species for many types of organisms, including common carp (*Cyprinus carpio*), muskrats (*Ondatra zibethicus*), and red-bellied turtles (*Pseudemys rubriventris*), as well as many species of invertebrates (Carter and Rybicki 1985, Korschgen and Green 1988). Waterfowl, particularly the canvasback duck (*Aythya valisineria)* prefer this species to many other SAV species, and use *V. americana* beds as feeding areas during migration. Waterfowl consume both shoot material and tubers (Korschgen and Green (1988).

Salinity tolerance

V. americana is a freshwater species that generally exhibits moderate salinity tolerance. Experimental studies by Bourn (1932, 1934, *op. cit.* Twilley and Barko 1990) found that growth of *V. americana* peaked at 2.8 practical salinity units (psu), and no growth occurred above salinities of 8.4 psu. Laboratory experiments by Haller et al. (1974) showed that growth occurred between 0.17 and 3.33 psu, and no growth occurred at 6.66 psu. In the field, the species has been observed in slightly oligohaline regions of estuaries and saline lakes. Along the north shore of the Pamlico River estuary, for instance, *V. americana* was observed in 78.1% of quadrats with a mean salinity of 5.3 psu (range from 0 psu to 12.8 psu). In regions of a slightly higher salinity (mean of 7.6 psu,

range from 2.2 psu to 13.9 psu), however, no *V. americana* was found (Davis and Brinson 1976, *op. cit.* Twilley and Barko 1990).

Several more recent studies, however, have indicated a higher salinity tolerance for the species. In a mesocosm study by Twilley and Barko (1990), *V. americana* under 8% and 50% of surface irradiance not only grew at 12 psu, but also grew at the same rate as plants at lower salinities. The higher salinity tolerance observed in this study was attributed to experimental methodology, whereby plants were exposed to gradual changes in salinity (1 psu per day up to 6 psu, and then 2 psu per day up to the final salinity of 12 psu). This gradual increase possibly allowed some osmoregulatory mechanism to operate (Twilley and Barko 1990). The highest recorded salinity tolerance, however, exceeded15 psu, observed by Kraemer et al. (1999). In this study adult plants were transplanted to sites in the Caloosahatchee Estuary, FL. At one site (diffuse attenuation coefficient <2 m^{-1}) plants survived up to 12 weeks where salinity ranged from 12 to 20 psu and survived 4-6 weeks when salinities exceeded 15 psu. Additionally, in an unpublished mesocosm experiment Doering observed survival, but no net growth, for 6 weeks at 15 psu (Kraemer et al. 1999).

Light Requirements

The light environment that a plant experiences is a function of many factors, including plant morphology, shoot density, depth, epiphyte accumulation, and light attenuation through the water column. Attenuation of photosynthetically active radiation (PAR) may be affected by the water itself, which absorbs suspended sediments and dissolved substances, which most strongly absorb blue wavelengths (Kirk 1994).

Attenuation can also be increased by algae, which can form mats or blooms that absorb the red and blue wavelengths used for photosynthesis (Korschgen et al. 1997).

V. americana is a relatively shade-tolerant species. In a photosynthesis study using plants in test tubes, Meyer et al. (1943) found that *V. americana* could survive at lower light intensities than any of the four other species tested (*Najas flexilis, Potamogeton Richardsonii, Elodea canadensis,* and *Heteranthera dubia).* In fact, the apparent photosynthesis of *V. americana* was still 25% of that at the surface when plants were receiving only a maximum of 0.5% surface light. In the field, however, its maximum depth of distribution ranges from less than 2 to 9% of surface irradiance (Batiuk et al. 1992, Carter et al. 2000), and it is most commonly found within the 10% photic zone (Carter and Rybicki 1985).

This species' shade tolerance is unexplained by its morphological plasticity. Other SAV species, such as *Myriophyllum spicatum* L. (Titus and Adams 1979), form surface canopies to intercept light under turbid conditions. *V. americana*, in contrast, has a limited potential for elongation and canopy formation (Barko et al. 1991). It is thus hindered in its ability to concentrate photoreceptive biomass near the water surface in low light conditions. It compensates for this morphological disadvantage, however, by a high physiological adaptability to low light. Its low half-saturation constant (60-197 μmol m^{-2} s^{-1}, compared to 164-365 μmol m^{-2} s^{-1} of *Myriophyllum spicatum* L.) indicates that it is efficient at fixing CO_2 at low light intensities (Titus and Adams 1979). It also acclimates very rapidly to increasing light (Titus and Adams 1979).

While *V. americana* is not as morphologically plastic as many other SAV species (Barko et al. 1991), it can undergo moderate change. For instance, it is capable of a

certain degree of stem elongation in reduced light conditions (8% compared to 50% of light; Barko et al. 1982, Barko et al. 1991). Under low light, leaf surface area and length:breadth ratio can also increase (Barko et al. 1982), even while shoot density and biomass decrease (Barko et al. 1982, Barko et al. 1991, but see Twilley and Barko 1990).

The production of reproductive structures by *V. americana* has been found to be affected by light intensity. Number, total biomass, and individual mass of tubers are inversely related to light intensity (Korschgen et al. 1997). Shoots emerging from tubers under laboratory conditions, however, have the capability to grow to a mean length of 44.0 cm in total darkness (Korschgen, unpublished data). Kimber et al. (1995) found that while seed germination was insensitive to light level, seedling survival was higher and growth was greater in the study's higher two light levels (9 and 25% of light, versus 2 and 5%). Tuber production was restricted to these two light levels.

Effects of combined stressors

Several studies have demonstrated the interactive effects of light and other environmental condition, such as temperature, CO_2, or nutrients, on *V. americana*. Barko et al. (1991) studied the interactive effects of light, sediment fertility, and inorganic carbon availability. They found that only under high light (50% of light, versus 8%) did the other two factors affect plant biomass. Other studies have shown that experimental manipulation affects growth more when *V. americana* has adequate light. Barko et al. (1982), for instance, manipulated light and temperature and found that plants were more responsive to differences in temperature at optimal light levels (600 and 1500 μmol m^{-2} s-1, versus 100 μmol m^{-2} s-1), and vice versa.

The demonstrated interactive effects of light and other environmental conditions have led only a few experimenters to investigate the existence of interactive effects of light and salinity. Twilley and Barko (1990) addressed this question in a mesocosm study using light levels of 8 and 50% light and salinities of 0, 2, 4, 6, and 12. After five weeks, they measured indices of survival, including stem density and length, chlorophyll from the apical 10 cm, epiphyte mass, N and P concentrations, and reproductive structures. They found no difference in total biomass (above- or belowground) or stem density among the five salinity treatments in either light treatment. Epiphytic mass increased with increasing salinity. Stem length increased with increasing salinity, except at the highest salinity, at which it decreased. Stem length also increased with decreasing light. There was no difference in number of underground buds between treatments, and there were very few flowers in any treatment. N and P concentrations increased with increasing salinity (except at 12 psu, when P concentration was at its lowest), with no difference between the light levels.

The field transplant study of Kraemer et al. (1999) also examined combined light and salinity effects. Transplants at sites with greater water clarity (approximately 27 to 38% light) were more tolerant of salinity. They suggest that light may moderate hypersaline stress by providing additional energy to maintain an appropriate osmotic potential.

Although salinity-irradiance interactions are beginning to be understood, significant gaps in our knowledge remain. For example, the long-term effects (at least one growing season) of shading and of a range of salinities on growth are unknown. The field study of Kraemer *et al.* (1999) was conducted for two 12-week periods only, and

salinity and light levels varied naturally throughout the seasons. It is thus impossible to distinguish short-term versus long-term effects of the stressors. The mesocosm study of Twilley and Barko (1990) was likely too short-term to produce many significant differences between regimes. In addition, the lowest light level (8%) was higher than many *V. americana* plants in natural conditions experience; thus, light was not likely limiting.

Relevant studies on the effects of light and salinity on *V. americana* are summarized in Table 1.

Photosystem Processes

Pulse-amplitude Modulated Fluorometry

Although monitoring morphological characteristics of SAV may be instructive of a population's health, careful, regular measurements are often cumbersome. In addition, examining purely physical traits may reveal SAV stress only once the population has begun its decline. The sooner managers are able to detect stress, the better chance they have at maintaining populations or identifying environmental stressors. A technique that reveals stress immediately would, therefore, be of great value in SAV research and management. One technique that is gaining popularity for its ease of use and potential predictive capabilities is chlorophyll *a* fluorescence. This technique, which can evaluate photosynthesis *in situ*, has only been applied to SAV in recent years, but has quickly added to our understanding of SAV response to stressors. However, there remains much to learn about its applications.

Table 1. Summary of studies on light and salinity effects on *V. americana*.

Location	Methods	Factors	Results	Reference
Lake Erie, OH	Leaf blades in test tubes submerged at different depths	Light	High rate of photosynthesis at very low light, compared to other freshwater SAV spp.	Meyer 1943
Florida	Adults transplanted to microcosms	Salinity	0, 3 psu: growth; 7, 10 psu: survival but no growth; 13, 17 psu: death	Haller et al. 1974
Pamlico River, NC	Survey of natural plants	Salinity	Survival at 5 psu, not 8 psu	Davis and Brinson 1976
University Bay, WI	Harvested adult plants	Light	Low half-saturation constant, compared to *Myriophyllum*	Titus and Adams 1979
Mississippi	Adults transplanted into tanks	Light, Temp.	Growth increased from 100 to 600 µmol m^{-2} s^{-1}) but did not increase at 1500. Ligth and temp. effects interacted.	Barko et al. 1982
Potomac R.	Survey of natural plants	Light	Plants found only to <10% light	Carter and Rybicki 1985
Maryland	Adults transplanted into microcosms	Salinity, Nutrients	Growth substantially reduced at 6 psu, compared to 0 psu	Staver 1986
Maryland	Adults transplanted into microcosms	Light and Salinity	Few differences in growth at different light (8, 50%) or salinity (0, 2, 4, 6, 12 psu) levels	Twilley and Barko 1990
Mississippi	Adults transplanted into microcosms	Light, CO_2, Nutrients	Growth greater at 550 than 125 µmol m^{-2} s^{-1}	Barko et al. 1991
Upper Mississippi R., WI	Seeds transplanted to outdoor pool at different depths	Light	Seed germination insensitive to light level; seedling survival higher and growth greater at 9 and 25% light, versus 2 and 5% light	Kimber et al. 1995
Upper Mississippi R., WI	Tubers planted in outdoor ponds at different light levels	Light	Tuber production inversely related to light	Korschgen et al. 1997
Caloosahatchee Estuary, SW FL	Adults transplanted along estuarine gradient	Light and Salinity	Salinity tolerance ~ 15 psu at higher light levels	Kraemer et al. 1999
Potomac R.	Transplanted sprigs	Light	Artificial light increased growth	Carter et al. 2000

Chlorophyll *a* fluorescence reveals information about photosystem II (PSII) photochemical processes, such as the light adaptation, photosynthetic capacity, and efficiency of PSII (Ralph et al. 1998). Chlorophyll *a* fluorescence has been used to study terrestrial plants for decades but has only been applied to SAV since 1997, with the development of Diving-PAM (pulse-amplitude modulated), a submersible fluorometer (Walz, Germany). This device provides information *in situ* about the effective quantum yield ($\Delta F/F_m'$), maximal quantum yield (F_v/F_m, ϕ_{PSII}), and electron-transport rate (ETR) of photosystem operation (Genty et al. 1989).

Effective quantum yield, also known as the Genty parameter, is a measure of photochemical energy conversion at PS II reaction centers under ambient light (Genty et al. 1989). It is measured with light-adapted leaves and is dependent on ambient irradiance. Effective quantum yield is determined as follows: initial fluorescence (F) is measured; a saturation pulse is applied; and fluorescence (F_m') is immediately measured again. Yield is the ratio of (F_m'- F) to F_m'.

Quantum yield of PSII is maximized after dark adaptation, when all PSII reaction centers are open (all primary acceptors are oxidized) and heat dissipation is minimal (Genty et al. 1989, Maxwell and Johnson 2000). Maximal quantum yield, then, is determined in the same manner as effective quantum yield, only dark-adapted leaves are used. Maximal quantum yield indicates maximum photochemical efficiency and is affected by photoinhibition and processes related to other stresses, such as heat and salinity (the quantum yield will be lower when a plant is stressed) (Ralph 1998a, Ralph 1998b, Ralph 1999c). The notation for maximal quantum yield is $(F_m-F_0)/F_m$, or F_v/F_m,

where F_m is the maximum fluorescence, F_0 is the minimum fluorescence, and F_v is the variable fluorescence (Genty et al. 1989, Maxwell and Johnson 2000).

The PAM fluorometer may also be used to conduct rapid light curves (RLC). For this technique light is applied to a leaf at nine increasing light intensities over the course of several minutes. At each level ETR is calculated. ETR is a measure of electron transport through the photochemical reactions, resulting in carbon fixation. It is derived from a relationship between irradiance, leaf absorbance and the quantum yield (Ralph et al. 1998). ETR then may be plotted against irradiance to create a RLC. A RLC enables determination of the maximum ETR (ETR_{max}), the minimum saturating irradiance (I_k), and the irradiance at which photoinhibition begins (Walz 1998). RLCs allow for an examination of the photoadaptation of a plant (Ralph, pers. comm.). Chlorophyll *a* fluorescence, then, can be used to detect not only stress but also adaptation.

One advantage of chlorophyll *a* fluorescence is that it provides a convenient means to study photosynthesis *in situ* with minimal disturbance. Prior to the development of Diving-PAM, SAV photosynthesis was measured by enclosing plants in chambers and measuring gas exchange, or bringing samples back to the lab. PAM fluorometry not only provides a quick, *in situ* measurement of photosynthesis but also allows for a study of multiple aspects of photosynthesis, such as heat dissipation and electron transport. The utility of PAM fluorometry as a surrogate for gas exchange studies has been supported by research finding a direct relationship between O_2 evolution and ETR (Ralph and Burchett 1995, Beer and Bjork 2000).

Use of PAM fluorometry in the study of stressors on SAV

Since the development of Diving-PAM, numerous studies have examined the effects of environmental stress on SAV using PAM fluorometry. Most of these studies have used the effective quantum yield and maximal quantum yield as an indication of stress response (Ralph and Burchett 1998a, Ralph and Burchett 1998b, Ralph 1998a, Ralph 1998b, Longstaff et al. 1999, Ralph 1999a, Ralph 1999b, Ralph 1999c, Prange and Dennison 2000). Yield declines with stress. The most common type of stress studied in this context is high light. Other stressors include temperature (Ralph 1998b, Ralph 1999c), salinity (Ralph 1998a, Ralph 1999c), heavy metals (Ralph and Burchett 1998b, Prange and Dennison 2000), herbicides (Ralph 1999a), and petrochemicals (Ralph and Burchett 1998a).

PAM fluorometry studies on SAV have most often examined the effect of short-term light stress on quantum yield. High light can cause photoinhibition, resulting in decreased photochemical efficiency (Ralph and Burchett 1995, Ralph 1999c). The effect of long-term differences in light regime has not been explicitly studied, although Ralph (1999b) examined the effects of changes in light regime for up to 10 days. Plants adapted to low light conditions by increasing photochemical efficiency and increasing total chlorophyll concentrations (Ralph 1999b).

Few SAV studies have been conducted that measure rapid light curves (but see Ralph et al. 1998, Beer et al. 1998, White and Critchley 1999), which can be a good indicator of photoadaptation (Ralph, pers. comm.). Using this technique, for example, Beer et al. (1998) found that the maximum ETR for *Zostera marina*, which grew *in situ* in low light conditions, was substantially lower than that of *Halophila stipulacea*, which

naturally grew in high light conditions. Rapid light curves remain a relatively unexplored tool for studying SAV photosynthesis.

Another area requiring attention is the interactive effect of stresses on photosynthetic processes. Ralph (1999c) examined the short-term effects, individually and combined, of light, salinity, and heat stress on *Halophila ovalis* and found these stresses (as measured by quantum yield) to be additive. Further study is needed, particularly of the long-term effects of these stresses.

In addition, most PAM fluorometry SAV studies have been conducted on *H. ovalis* (Ralph and Burchett 1998a, Ralph and Burchett 1998b, Ralph 1998a, Ralph 1998b, Bjork et al. 1999, Ralph 1999a, Ralph 1999b, Ralph 1999c, Beer and Bjork 2000, Prange and Dennison 2000), and no freshwater species has been examined.

Objectives

The primary objective of this experiment was to elucidate throughout the growing season the single and interactive effects of various light and salinity regimes on *V. americana*. Specific objectives were to:

- Examine the effects of these regimes on various measures of plant development and growth, including number of rosettes produced, number of leaves per rosette, and leaf length and width;
- Study the regimes' effects on photosynthesis using pulse-amplitude modulated fluorometry techniques;
- Determine the effects of the regimes on reproduction by comparing across treatments the tubers and flowering structures produced;

- Discern any interactive effect between light and salinity on the variables measured;
- Using the above information, further clarify light and salinity requirements for survival; and
- Attain a better understanding of the applicability of PAM techniques, including the utility of maximum quantum yield and rapid light curve measurements in describing stress and photoadaptation, the relationship of these data to morphometrics, and PAM's ability to predict changes in growth and reproductive success.

Hypotheses

H1: *V. americana* response to salinity stress will decrease with increasing light level.

H2: *V. americana* response to light limitation will decrease with decreasing salinity.

H3: Plants will be less tolerant of light and salinity stress than previous studies (starting with adult plants) have found.

H4: Yield parameters will accurately predict physical decline due to stress.

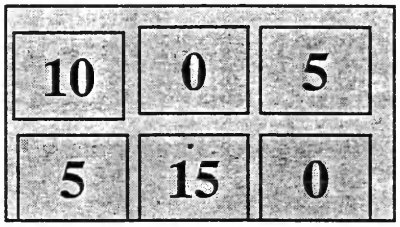
METHODS

Experimental Systems

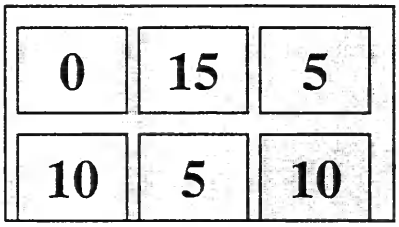
Outdoor mesocosms were employed to study the effects of light and salinity on *V. americana* at the Virginia Institute of Marine Science, Gloucester Point, VA. *V. americana* was grown in 36 110-l glass aquariums (60 x 30 x 60 cm). Six aquariums were positioned in each of 6, 4 x 8 ft. tanks (Fig. 2). Aquariums and tanks were all oriented approximately in an east-west direction. Aquariums were covered with glass tops, slightly raised to allow for air exchange. Water flowed directly from the York River through the tanks to maintain ambient river temperatures. Aquarium water, however, was not changed during the course of the experiment. Aquariums had one of four salinity treatments: 0, 5, 10, and 15 psu, achieved by a combination of York River water and dechlorinated tap water. These levels were selected to represent the range of ideal (0 psu) to stressful, yet still able to support minimal growth (15 psu) (Table 1). Water was continuously aerated and filtered using submerged aquarium filters with polyester fiber and activated charcoal.

Two tanks each were randomly designated high, moderate, and low light treatments. Thus, there were 3 replicates in each of 12 light/salinity treatments. Neutral density shade cloth was used to achieve 28, 8, and 2% of surface irradiance at the sediment surface. Previous studies indicate that 2% light limits growth, but may be sufficient to sustain seasonal survival, 8% is approximately the minimum amount needed for long-term survival, and 28% is saturating for growth (Table 1). Light reaching the sediment surface was measured by a Li-Cor scalar (Li-190SA) meter. Shadecloths alone

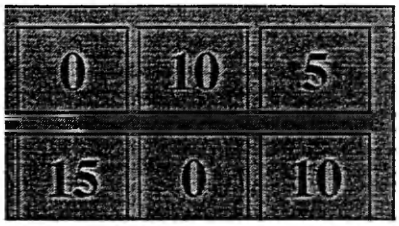
Figure 2. Experimental layout. Large rectangles represent 4' x 8' tanks. Small rectangles represent 30-gallon mesocosm aquariums. Numbers in small rectangles represent salinity level (psu).



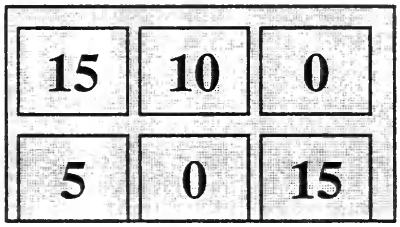
8% Light



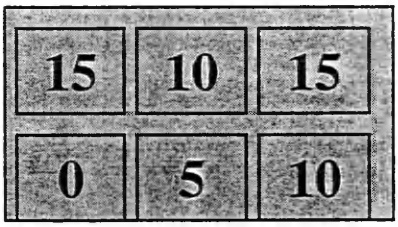
28% Light



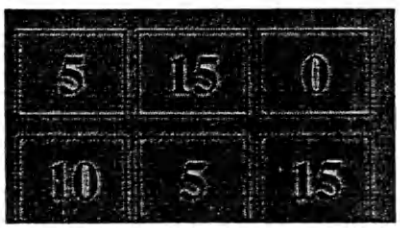
2% Light



28% Light



8% Light



2% Light

blocked 53, 86, and 96% of insolation (Table 2). Glass tops blocked 12%, and the water column attenuated 32% of insolation. Plants experienced some additional shading by the sides of the tanks at low sun angles within 2 hours of sunrise and sunset. Placement of aquariums within the tanks was determined to ensure a regular distribution of salinity regimes with respect to position within the tank.

Each aquarium contained four, 1-l pots. Sediment was a combination of York and James River sediments. The York River sediment consisted of dredged material that had been allowed to dry and weather for over two years. Similar sediments had been used to support two growing seasons of transplanted *V. americana* without nutrient additions. The James River sediment was taken from the freshwater region of the estuary Tar Bay, near Hopewell, VA. Similar sediment has also supported two seasons of *in situ V. americana* transplant growth. The James sediment was collected on May 19 and was kept cold (5°C) and in the dark until use. Pots were filled with 3 parts dried, sieved York sediment mixed with 1 part James sediment. Pots were then left to sit in tap water overnight to release silt. The next day, pots were topped off with a mixture of half York, half James sediment and were then left to soak overnight in water of the appropriate salinity.

Tubers were collected in late March, several weeks before sprouting, from a pond in Maryland. After collection, they were stored in the dark in aerated DI water approximately 4°C. Water was changed once a week. Some (less than 10%) of the 763 tubers began sprouting around April 7 but grew very slowly, reaching maximum shoot lengths of approximately 2 cm. In addition, some tuber tips broke off. Other than sprouting and broken tips, the tubers appeared healthy and did not change in appearance

Table 2. Fraction ambient light passing through: shade cloths of each of the three treatments, glass tops, and water column. Totals for each treatment are calculated light reaching sediment surface, also as a fraction of ambient light. These values are the product of measured fraction ambient light passing through shade cloth, glass top, and water column.

	Mean	Std. Error	N
High Light Shade Cloth	0.47	0.007	12
Medium Light Shade Cloth	0.14	0.004	12
Low Light Shade Cloth	0.04	0.003	12
Glass Top	.88	.032	9
Water Column	.68	.044	9
Total High Light	**.28**		
Total Medium Light	**.08**		
Total Low Light	**.02**		

throughout their storage.

Tubers were planted on May 26. Three tubers were planted per pot, equivalent to 178 m^{-2}. This density is at the upper range of natural densities (Korschgen and Green 1988). Tubers were planted approximately 5-10 cm under the surface, at the shallow end of the range of tuber depth measured *in situ* (Carter et al. 1985). Tubers that were soft or appeared in any other way unhealthy were discarded. Approximately 10 to 20% of tubers were deemed unacceptable for use.

Water depth to sediment surface was approximately 46 cm.

Aquariums were scrubbed as needed, approximately once a week. Submersed filters (Lee's triple flow medium corner filters with polyester fiber and Aquarium Pharmaceuticals, Inc. professional grade activated carbon) were employed on July 22, when algal blooms began to reduce visibility. Filter fiber was cleaned or changed as needed, at least once a week.

Measurements

Morphometrics

Plant morphological measurements were taken once every two weeks. These measurements include number of rosettes per pot, which is a measure of vegetative growth and colonizing capacity; number of leaves per rosette, which may provide a means to augment production and light capture; and length and width of the longest leaf in each rosette. Because leaf width was measured as the width of the longest leaf per rosette, it provides an approximation of maximum leaf width per rosette. Changes in leaf width may alter shoot surface area, and thus light capture. Leaf length provides a measure

of plant elongation and a means of comparing the amout of resources a plant puts into reaching the surface.

Leaf length data were analyzed two ways in this study. Leaf length was defined as the length of the longest leaf per rosette on each sampling date throughout the season. This measurement estimates maximum canopy height throughout the season and can provide a measure of relative depth of the leaf canopy and thus capacity to capture light. Since leaf length peaked on different dates for different aquariums, maximum seasonal leaf length was used to estimate treatment effects on leaf elongation. Maximum seasonal leaf length is defined as the maximum value per aquarium of the longest leaf per rosette (n=3).

Initial leaf elongation rate is the rate of elongation from planting (length=0 cm) to maximum seasonal leaf length, as determined above. Initial leaf elongation rate can indicate resource allocation for early season light capture. The mean length of longest leaf per rosette of each date from planting to date of maximum length was used in elongation rate analysis. A mean elongation rate was determined for each aquarium (n=3).

Presence of flowering structures were also noted. Flower production is an indication of sexual reproduction.

Mid-Season Harvest

Once during the experiment, on August 4, a biomass subsampling was conducted. One pot per aquarium was randomly selected. Leaf length and number of rosettes were slightly past their maximum at this time. The plants were washed free of sediment, and

leaf surface area was determined using a Li-Cor 3100 Area Meter. Above- and belowground vegetation were separated, dried at 50°C for one week and weighed. Aboveground biomass is a general measure of production and an indication of how much resources plants are devoting to shoot material. Belowground biomass is an indication of the resources plants are putting into roots and rhizomes, which contribute to plant stability and resource storage. Leaf surface area is a measure of plant production and potential for light capture. Leaf area at mid-season represents approximately the maximum seasonal area over which plants can conduct photosynthesis.

PAM Fluorometry

A pulse-amplitude modulated fluorometer (Diving PAM-2000, Walz, Germany) was used to measure fluorescence parameters. Two types of measurements were taken: effective and maximal quantum yield and rapid light curves. All fluorescence measurements were taken under water, on representative (i.e., of typical appearance and intermediary age) leaves, approximately 5 cm from the leaf base.

Quantum Yield

Quantum yield measurements were taken once every two weeks throughout the duration of the experiment. One leaf was sampled from each aquarium (n=3). Maximal quantum yield (F_v/F_m) was measured after 10 minutes of darkness achieved by dark-adaptation clips. Effective quantum yield ($\Delta F/F_m'$) was measured on the same leaves, just prior to dark adaptation in ambient light. Maximal quantum yield is a measure of

photochemical efficiency and of stress to PSII. Effective quantum yield is a measure of photosynthetic capacity under ambient light.

Rapid Light Curves

Short periods of light (10 s) of increasing intensity were applied to leaves by the PAM fluorometer. Nine discrete irradiance steps from 0 to 2240 μmol m^{-2} s^{-1} were used, and ETR was calculated by the fluorometer at each step. Leaf portions were shaded from ambient light by leaf clips so that they only experienced the actinic light provided by the PAM fluorometer. Again, one representative leaf was selected from each aquarium. Rapid light curves were obtained on four occasions throughout the course of the experiment. Maximum ETR, determined as the average of the three highest consecutive ETRs per light curve, is an indication of maximum photosynthetic capacity.

Chlorophyll

At every other maximal quantum yield sampling (i.e., once per month), the leaves on which the PAM fluorometry measurements were taken were collected. These leaves were frozen for chlorophyll analysis.

Chlorophyll was extracted by grinding leaves in a solution of 80% acetone, 0.1% diethyl amine (DEA), and deionized (DI) water while on ice. Solutions were then centrifuged and read on a Shimadzu UV Probe spectrophotometer using wavelenghts of 645, 663, and 725 nm. Chlorophyll *a* and *b* concentrations were calculated according to Dennison (1990).

Tuber Harvest

After the leaves senesced at the end of the growing season on December 13, the tubers produced in each treatment were counted, dried at 50°C for one week, and weighed. Tuber production is a measure of vegetative reproduction and survival into the next season.

Environmental Measurements

Mesocosm water samples were collected for nutrient analysis once a month. Samples were filtered and frozen for future analysis. Nitrite, nitrate, and ammonium concentrations were determined spectrophotometrically following the methods of Parsons et al (1984) and inorganic phosphorus following the methods of USEPA (1979). Sediment cores were extracted at the beginning, middle, and end of the experiment. Half of each core was sampled for bulk density, dry weight after 96 h at 60°C, and organic content after ashing at 500°C for 5 h. Nutrients were extracted from the other half of each core using 1 M KCl. Samples were frozen and were analyzed for ammonium (Parsons et al. 1984) and inorganic phosphorus (USEPA 1979). Porewater NO_3^{-2} is typically minimal in freshwater areas and was not measured (Hopkinson et al. 1999, Morlock et al. 1997).

Eight pots were randomly selected for grain size analysis. Analysis was conducted by VIMS Analytical Service Center via the wet sieve, pipette method.

Salinity was measured 16 times throughout the season using a Vista A366ATC Portable Refractometer, a YSI Model 33 Salinity-Conductivity-Temperature probe, and a Hydrolab MiniSonde Water Qualitly Multiprobe. Salinity was adjusted when necessary using dechlorinated tap water and Forty Fathoms Crystal Sea salt. pH was measured four

times using a Fisher Scientific Accumet Portable AP10 and a Hydrolab MiniSonde Water Qualitly Multiprobe.

Temperature was continuously recorded by two HOBO H8 sensors and two TidbiT sensors (Onset Computer Corporation) starting on June 17. Temperature was recorded once at midday on June 10 using a YSI Model 33 Salinity-Conductivity-Temperature probe. Ambient downwelling light and light under the shade cloths were also recorded continuously using Li-Cor scalar meters. The light meter under the shade cloths was rotated between tanks every week.

Statistical Analyses

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 ANOVAs, normality was confirmed visually, and homogeneity of variance was verified with Cochran's test. For all measurements yielding more than one value per aquarium (e.g., length of longest leaf per rosette and other morphological measurements), data were averaged over each aquarium (n=3 per treatment) for use in ANOVAs. Factor level means were compared using the Student-Newman-Keuls test (SNK). Percent variance attributable to a given factor was the percent sum of squares (SS) 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.

Repeated measures ANOVAs were conducted using a general linear model (GLM) (The SAS® System for Windows, SAS Institute Inc.). Repeated measures

ANOVAs were run on time series data to determine the effect of time, in addition to light and salinity, on each parameter.

The maximum ETR (ETR_{max}) for each sample was determined by averaging the three highest consecutive ETRs per RLC. The initial slope of each curve (α) was determined by running a simple linear regression on ETR data up to and including the first ETR used for ETR_{max} calculation. Minimum saturating irradiance (I_k) was calculated using the equation of the line determined for α calculation and the first ETR used for ETR_{max} calculation.

RESULTS

Environmental Conditions

Light

Total daily downwelling insolation (averaged at five-day intervals) increased rapidly to highest levels in June through early July (40 to 50 mol m^{-2} d^{-1}), then gradually declined throughout the remainder of the growing season (Fig. 3). Maximum daily irradiance (six-minute interval) was high from June through August, averaging approximately 1800 µmol m^{-2} s^{-1}, then declined to 500-700 µmol m^{-2} s^{-1} by November. Maximum daily irradiance at the sediment surface in the high, moderate, and low treatments averaged approximately 500, 144, and 36 µmol m^{-2} s^{-1} for June through August.

Salinity

Salinity was measured a total of 16 times throughout the season (App. 1). Salinities averaged 1.3, 5.3, 9.8, and 14.2 psu for treatments 1 through 4 (Table 3).

Sediment

Sediment was comprised of 1% (SE $\pm$ 0.80) gravel, 87% (SE $\pm$ 1.89) sand, 3% (SE $\pm$ 0.55) silt, and 8% (SE $\pm$ 0.30) clay (Table 4). Mean organic carbon content over three sampling dates was 1.4% (SE $\pm$ 0.022) and did not vary appreciably with depth (App. 2). Sediment NH_4^+ concentrations ranged from 12 to 111 mmol m^{-2}, with no

Figure 3. Total daily downwelling irradiance and maximum daily irradiance (six-minute interval) at study site, averaged at 5-day intervals.

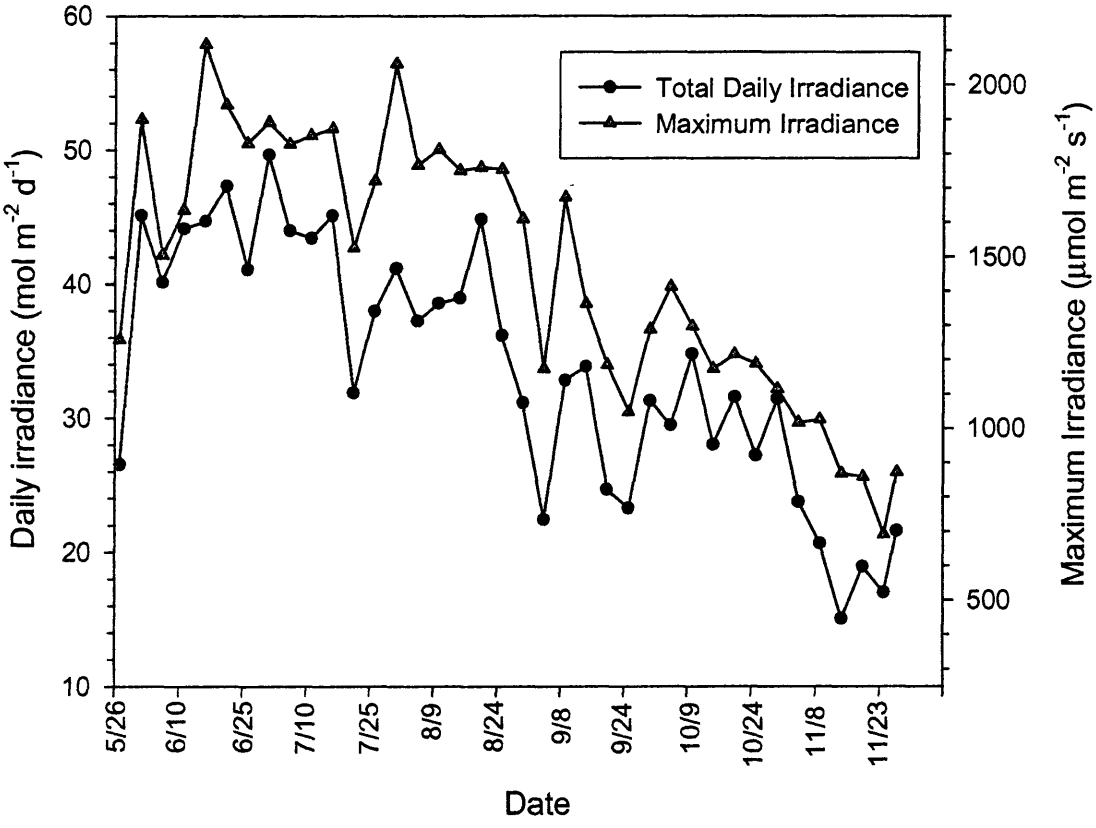

Total Daily Irradiance
Maximum Irradiance
Daily irradiance (mol m-2 d-1)
Maximum Irradiance (µmol m-2 s-1)
Date
60
50
40
30
20
10
2000
1500
1000
500
5/26
6/10
6/25
7/10
7/25
8/9
8/24
9/8
9/24
10/9
10/24
11/8
11/23

Table 3. Average treatment salinity (psu) throughout the growing season

	Mean	Std. Error	N
0 psu	1.3	0.19	92
5 psu	5.3	0.09	130
10 psu	9.8	0.09	133
15 psu	14.3	0.13	132

Table 4. Grain size partitioning (%) for eight randomly selected pots.

	Mean	Std. Error	N
Clay	8.0	0.30	8
Silt	3.1	0.53	8
Sand	87.4	1.19	8
Gravel	1.5	0.80	8

consistent trends between treatments or over time.

Water Column Nutrients

Water column dissolved inorganic nitrogen (DIN) concentrations were less than 15μM in the 28 and 8% light treatments (Fig. 4). They ranged from 12 to 130 in the 2% light treatment and increased from relatively low levels near the start of the experiment in June to higher levels, especially in the 10 and 15 psu treatments, from July through October. Highest concentrations were observed in August. DIN was composed of approximately 61% NO_3^{-2}, 35% NH_4^+, and 4% NO_2^-. There were no consistent trends across treatments. PO_4^{-3} concentrations were as high as 9 μM in June and decreased with increasing salinity (Fig. 5). They were less than 2 μM in subsequent months.

Temperature

Temperature on June 10 was 23.4°C (SE ± 0.067). Aquarium water temperature varied seasonally (Fig. 6), with summer temperatures ranging from 25 to 28°C and winter lows of 5°C. Temperature varied between the 4 sensors employed by a maximum of 1.5°C and a mean maximum daily difference of 0.35°C). Diurnal variation averaged 1.7°C, with a maximum of 4.3°C and a minimum of 0°C.

Figure 4. Monthly water column DIN concentrations (μM). Error bars represent standard error. n=3 for each light/salinity treatment.

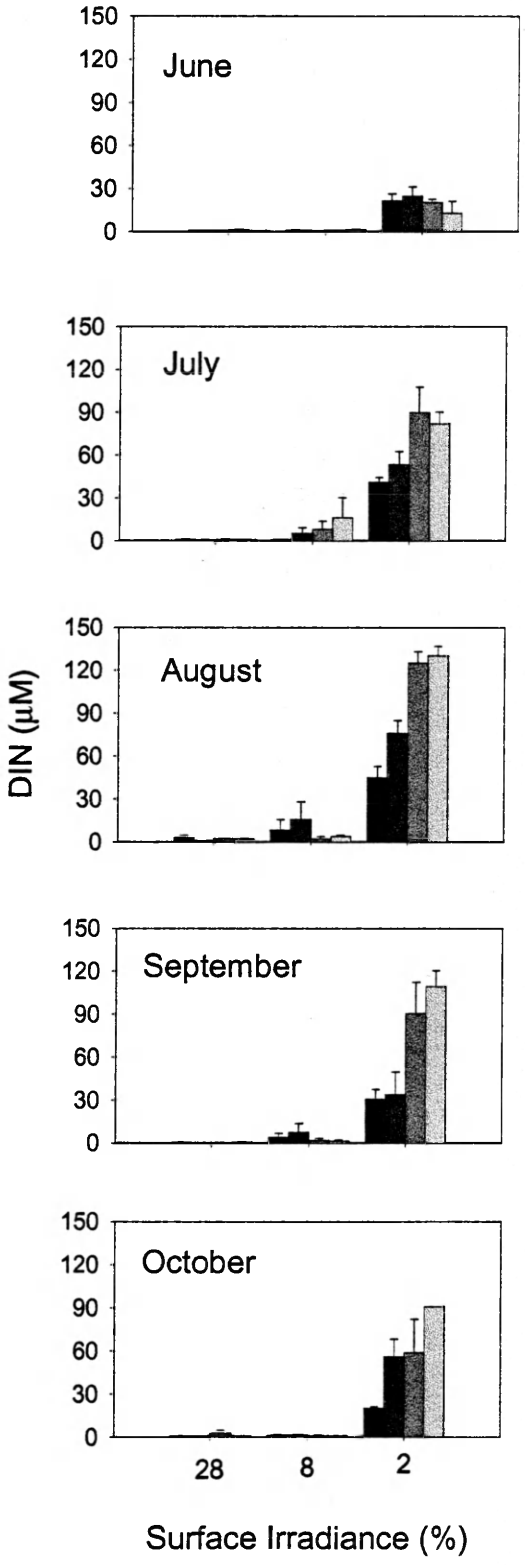

June
July
August
September
October
150
120
90
60
30
0
DIN (µM)
28
8
2
Surface Irradiance (%)

Figure 5. Monthly water column PO_4^{-3} concentrations (μM). Error bars represent standard error. n=3 for each light/salinity treatment.

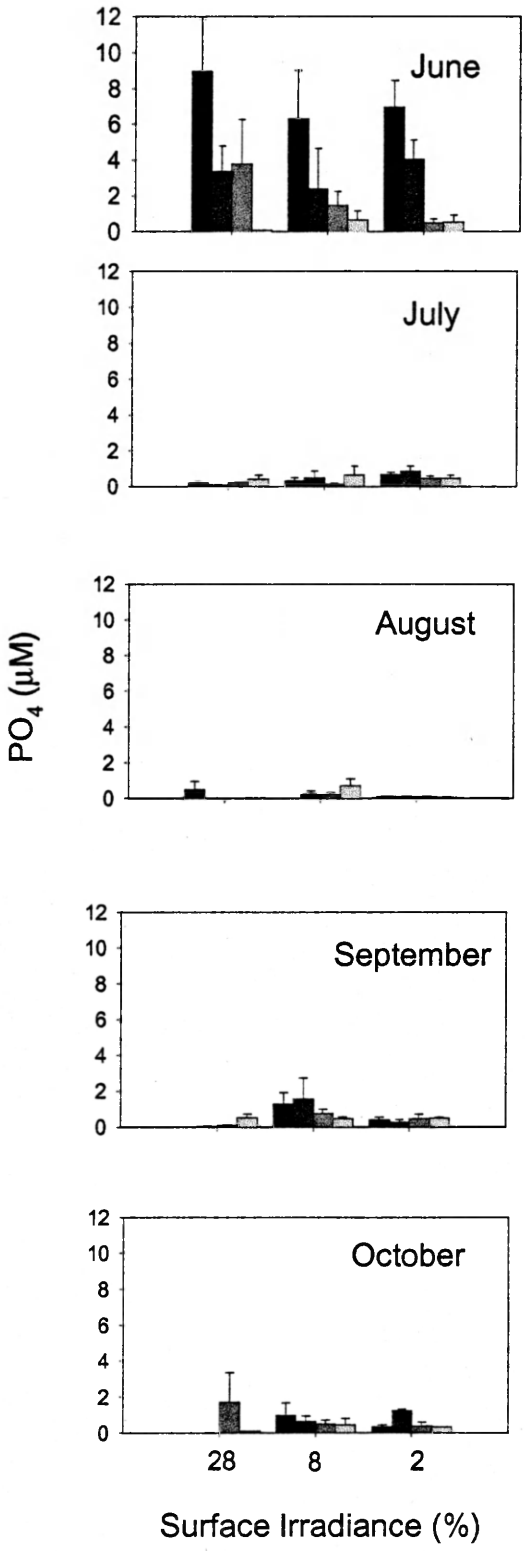
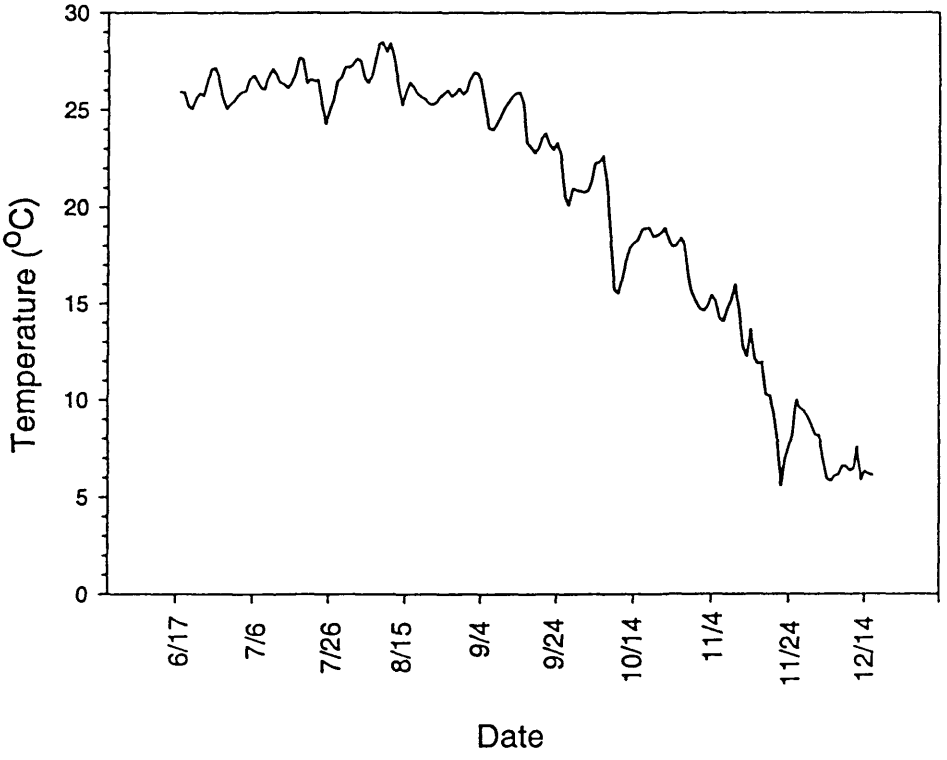

June
July
August
September
October
12
10
8
6
4
2
0
PO_4 (µM)
28
8
2
Surface Irradiance (%)

Figure 6. Temperature (°C) of aquarium water throughout the season, averaged across 4 temperature sensors. Each sensor was in a different tank and recorded temperature every half hour throughout the season.


30
25
20
15
10
5
0
Temperature (°C)
6/17
7/6
7/26
8/15
9/4
9/24
10/14
11/4
11/24
12/14
Date

pH

Aquarium water pH was measured four times throughout the season at approximately midday (App. 3) Mean pH was 7.7 (SE $\pm$ 0.052) and did not change appreciably throughout the season. pH decreased with increasing salinity (Table 5, ANOVA, $p<0.0001$), except for the 15 psu treatment, which was greater than the 10 psu treatment, although not significantly so (SNK>0.05). One aquarium (#28, 8% light, 10 psu salinity) had a pH of 5.04 on July 27. Water was promptly changed.

Morphology and Production

Sprouting

The incidence of tuber sprouting was determined by counting the number of rosettes on June 14 (19 days after planting) relative to number of tubers planted. An early sampling date was selected for analysis in order to avoid mistaking seasonal vegetative reproduction for tuber sprouting. An average of 94.92 (SE $\pm$ 5.45) tubers m^{-2} sprouted, or 53% of those planted. Sprouting incidence did not vary by treatment ($p>0.05$).

Leaf Elongation

Leaf length was highly dependent on salinity (Fig 7). There was a strong inverse relationship between salinity and length ($p<0.0001$, Table 6). Contrast variables indicate changes in salinity effects between each of the first six sampling dates (except for July 19) and its consecutive sampling date. Visual inspection suggests that the effects of salinity increase over time as leaves elongate but then level out after August 16 as leaves senesce.

Table 5. Mean pH per salinity treatment, with associated standard error. Seasonal means of aquariums were used for mean salinity treatment values (n=12).

Salinity (psu)	Mean	SE
0	7.99	0.067
5	7.74	0.081
10	7.40	0.094
15	7.54	0.058

Figure 7. Mean length of longest leaf per rosette over time for each light and salinity level. Longest leaves were averaged over each aquarium to obtain one value per aquarium. These values were then averaged within treatments (n=3). Error bars represent standard error.

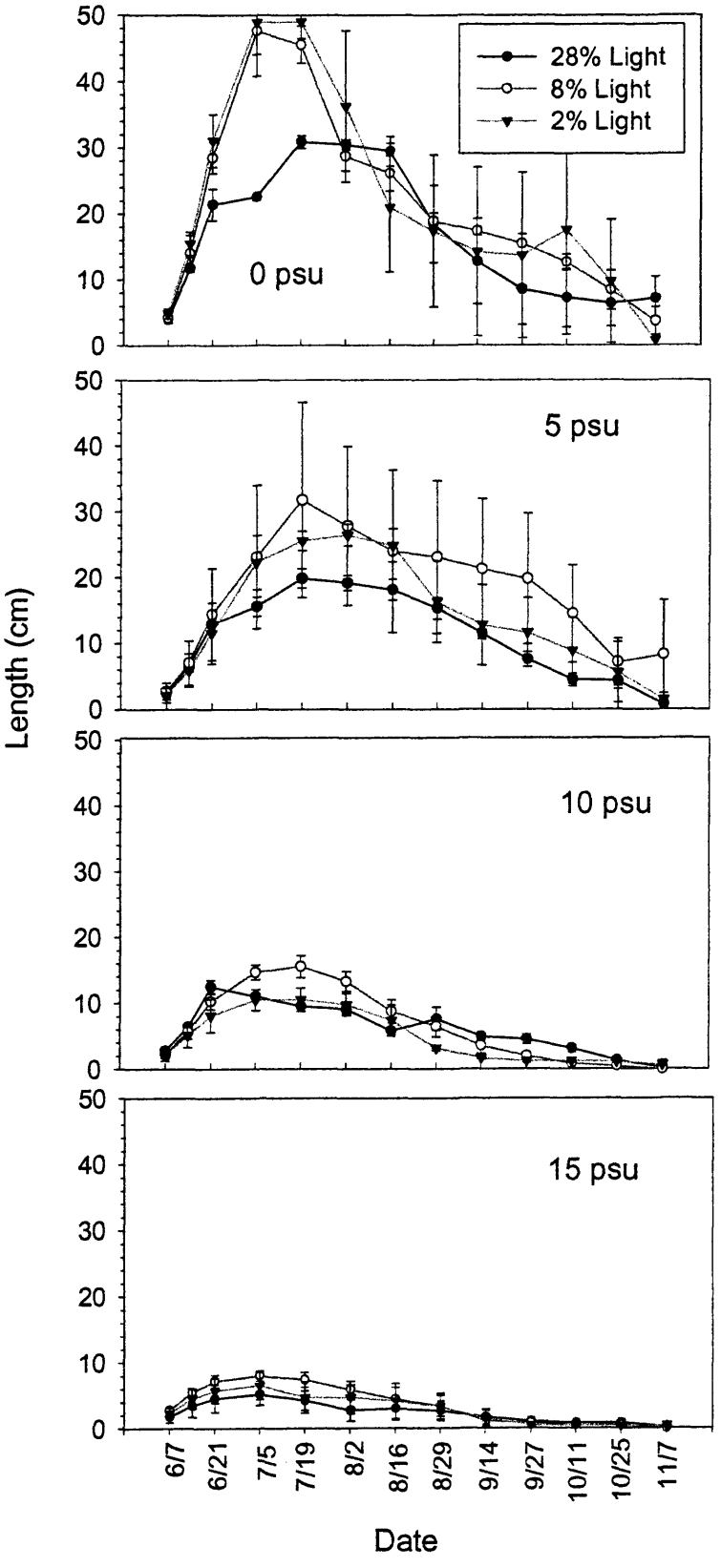

28% Light
8% Light
2% Light
0 psu
5 psu
10 psu
15 psu
Length (cm)
0
10
20
30
40
50
6/7
6/21
7/5
7/19
8/2
8/16
8/29
9/14
9/27
10/11
10/25
11/7
Date

Table 6. General linearized model repeated measures ANOVA tables for various plant characteristics 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.	Wilk's Lambda P
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	**<.0001**	49.9	**<.0001**
	Date * Light	24	1.53	0.0579	2.4	**0.0129**
	Date * Salinity	36	9.92	**<.0001**	23.8	**<.0001**
	Date * Light * Salinity	72	0.98	0.5327	4.7	0.1408
	Error (Date)	288			19.2	
Leaf Width	Light	2	3.85	0.051	14.3	
	Salinity	3	10.35	**0.0012**	57.4	
	Light * Salinity	6	0.55	0.7584	6.2	
	Error	12			22.2	
	Date	11	41.03	**<.0001**	61.1	**0.048**
	Date * Light	22	0.74	0.7898	2.2	0.1423
	Date * Salinity	33	1.4	0.0952	6.3	**0.046**
	Date * Light * Salinity	66	1.41	**0.0487**	12.6	0.145
	Error (Date)	132			17.9	
Leaves per Rosette	Light	2	0.74	0.4936		
	Salinity	3	1.81	0.1913		
	Light * Salinity	6	0.92	0.5073		
	Error	14				
	Date	11	50.55	**<.0001**	60.0	**0.0049**
	Date * Light	22	4.21	**<.0001**	10.0	0.0504
	Date * Salinity	33	2.04	**0.002**	7.3	0.332
	Date * Light * Salinity	66	0.86	0.762	6.1	0.3268
	Error (Date)	154			16.6	
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	
	Time	12	32.36	**<.0001**	32.9	**<.0001**
	Time * Light	24	5.66	**<.0001**	11.5	**0.0073**
	Time * Salinity	36	4.94	**<.0001**	15.1	**0.0004**
	Time * Light * Salinity	72	2.64	**<.0001**	16.1	**0.015**
	Error(Time)	288			24.4	

Light treatment did not have a significant effect on leaf length consistently over time ($p=0.3728$). However, visual inspection of the data reveals that mid-summer, plants in the 2 and 8% light levels produced longer leaves than the 28% light treatment, especially for the 0 psu treatment (Fig. 7). Contrast variables support this observation and indicate that the effects of light changed between July 4 and July 19 ($p=0.0002$) and between August 8 and August 16 ($p<0.0001$). The interactive effects of light and salinity also changed at these two periods ($p<0.0001$ and $p=0.0013$), supporting the observation that mid-summer, light effects were most apparent in the 0 psu treatment.

Leaf length varied significantly over time ($p<0.0001$, Table 6, Fig. 7), with length greatest in July (5.2 to 49.1 cm) and steadily declining until November (0 to 8.3 cm). Primary causes of shortening of longest leaves in the latter part of the season were decaying and breaking off at the distal end, and leaf senescence. Leaf senescence was preceded by loss of chlorophyll, general decay, and/or breakage of leaf at base. The effects of salinity varied among dates ($p<0.0001$). Paritioning of variance indicated that date had the greatest effect on length, explaining 50% of the variance. The interaction between salinity and date accounted for 23.8% of the variance, and the other interactions had relatively minor effects. The effect of date was apparent in all light and salinity treatments (Wilk's test, Table 6).

Maximum seasonal length ranged from 5.6 cm to 52.3 cm (Fig. 8). Both light ($p=0.0351$) and salinity ($p<0.0001$) were significantly related to maximum leaf length (Table 7). Leaves in the 28% light treatment were significantly shorter than those in the 2 and 8% light treatments (Fig. 8, SNK, $p<0.05$). Salinity was inversely related to length, although 10 and 15 psu treatments were not different. Adult plants in the 0 psu, 2 and 8%

Figure 8. Maximum seasonal leaf length for each treatment. Length of longest leaf per rosette was averaged for each aquarium for each date. The maximum value for each aquarium was then averaged within each treatment (n=3). Different letters over bars indicate significant differences between light treatments, while different letters in legend indicate differences between salinity treatments (Student-Newman-Keuls, $p<0.05$).

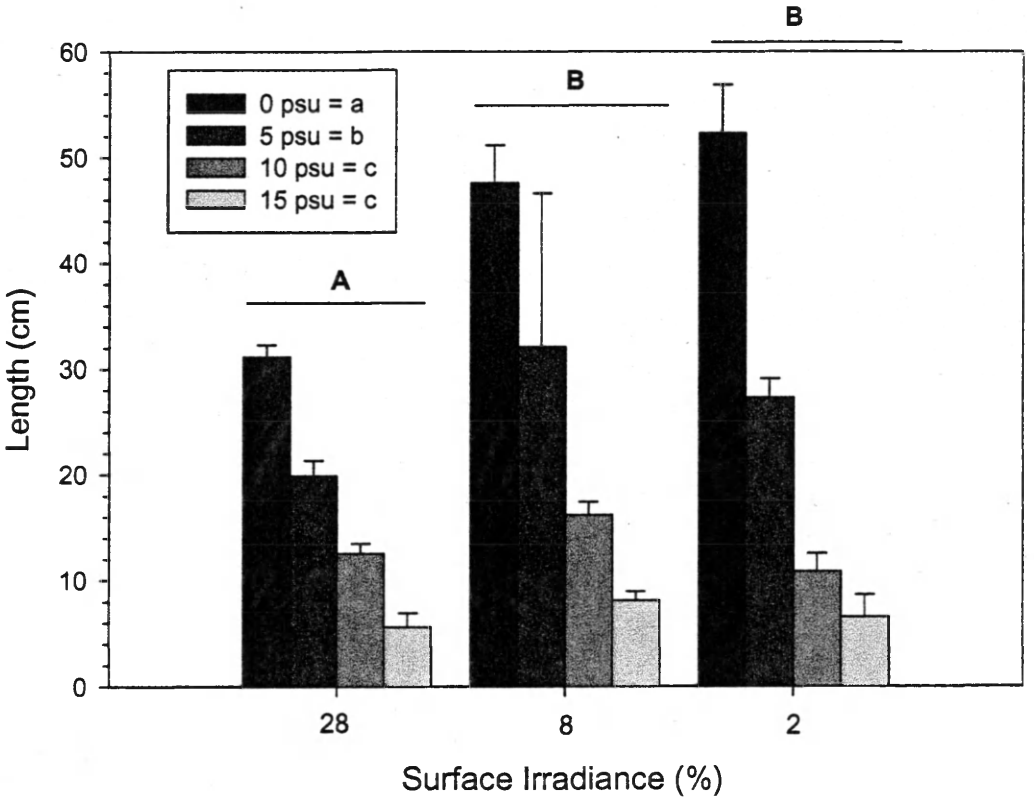

B
B
A
0 psu = a
5 psu = b
10 psu = c
15 psu = c
60
50
40
30
20
10
0
Length (cm)
28
8
2
Surface Irradiance (%)

Table 7. 2-way ANOVA for light and salinity effects on morphology and production 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.
Maximum Seasonal Leaf Length	Light	2	3.862	**0.0351**	5.2
	Salinity	3	36.359	**<.0001**	73.4
	Light * Salinity	6	1.301	0.2947	5.3
	Error	24			16.2
Initial Elongation Rate	Light	2	4.042	**0.0307**	5.4
	Salinity	3	32.472	**<.0001**	65.2
	Light * Salinity	6	3.317	**0.016**	13.3
	Error	24			16.1
Leaf Width at Maximum Length	Light	2	4.688	**0.0191**	16.7
	Salinity	3	3.543	**0.0297**	18.9
	Light * Salinity	6	2.018	0.1026	21.6
	Error	24			42.8
Maximum Seasonal Rosette Density	Light	2	15.937	**<.0001**	24.8
	Salinity	3	11.864	**<.0001**	27.7
	Light * Salinity	6	6.163	**0.0005**	28.8
	Error	24			18.7
Aboveground Biomass	Light	2	7.209	**0.0035**	19.4
	Salinity	3	6.765	**0.0018**	27.3
	Light * Salinity	6	2.602	**0.0437**	21.0
	Error	24			32.3
Aboveground Biomass per Rosette	Light	2	1.156	0.3323	
	Salinity	3	2.649	0.0729	
	Light * Salinity	6	0.770	0.6010	
	Error	24			
Belowground Biomass	Light	2	7.848	**0.0024**	23.5
	Salinity	3	3.319	**0.0368**	14.9
	Light * Salinity	6	2.857	**0.0303**	25.7
	Error	24			35.9
Belowground Biomass per Rosette	Light	2	3.667	**0.0415**	14.0
	Salinity	3	1.629	0.2102	8.8
	Light * Salinity	6	2.997	0.0259	33.3
	Error	23			43.9
Aboveground to Total Biomass Ratio	Light	2	0.765	0.4778	
	Salinity	3	1.612	0.2166	
	Light * Salinity	6	2.057	0.1025	
	Error	21			
Leaf Area Index	Light	2	9.614	**0.0009**	19.5
	Salinity	3	11.48	**<.0001**	34.9
	Light * Salinity	6	3.502	**0.0125**	21.3
	Error	24			24.3

Table 7 (continued).

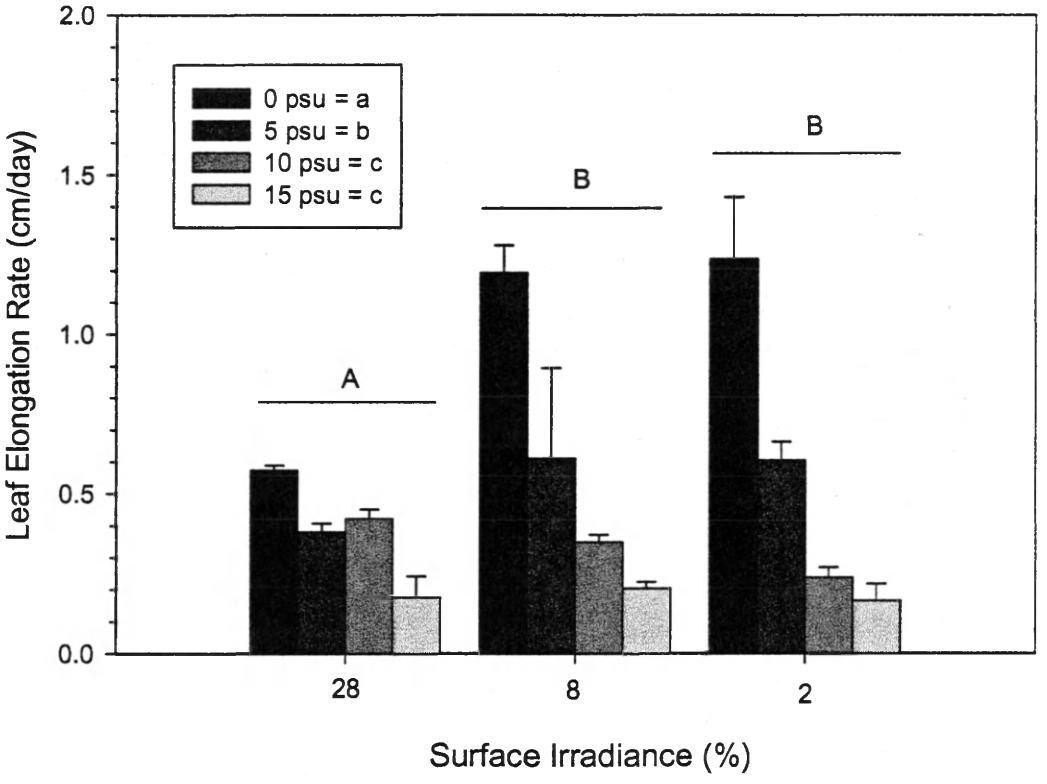
		DF	F	P	% Var.
Aboveground biomass per Leaf Area	Light	2	0.748	0.4857	
	Salinity	3	2.095	0.1314	
	Light * Salinity	6	0.582	0.7405	
	Error	24			
Leaf Area per Rosette	Light	2	0.244	0.7858	1.1
	Salinity	3	4.442	**0.0133**	31.2
	Light * Salinity	6	0.975	0.4644	13.7
	Error	23			53.9
Leaf Length * Width	Light	2	1.579	0.2269	2.6
	Salinity	3	31.069	**<.0001**	75.8
	Light * Salinity	6	0.445	0.8415	2.2
	Error	24			19.5
Tuber 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
Total Tuber Biomass	Light	2	1.444	0.2558	8.2
	Salinity	3	1.607	0.214	13.7
	Light * Salinity	6	0.582	0.7409	9.9
	Error	24			68.2

light treatments generally reached the water surface. Light and salinity effects did not interact ($p=0.2947$). The majority of the variance in length is attributable to salinity treatment (73.4%, Table 7), while only a small fraction is due to light treatment (5.2%) or the interactive effects of light and salinity (5.3%).

Maximum seasonal length was generally achieved between July 5 and July 19. Date of maximum length did not consistently differ with light ($p=0.3435$) or salinity treatment ($p=0.6536$). However, in the 2 and 8% light treatments maximum length occurred 2 to 4 weeks later in the 5 psu treatment than in the 0 psu treatment. In the 0 psu treatment, maximum length occurred 2 weeks later in the 28% light treatment than in the 2 and 8% light treatment.

A linear model best described elongation. Slope was determined by best-fit line (simple linear regression, StatView, Inc.). Initial elongation rates ranged from 0.18 cm/d to 1.24 cm/d (Fig. 9). Elongation rate followed a similar pattern to that of maximum seasonal leaf length. Light ($p=0.0307$) and salinity ($p<0.0001$) each were significantly correlated with elongation rate (Table 7). Plants in the 2 and 8% light treatments elongated significantly faster than 28% light plants did, and salinity differences were most pronounced at the lower two light levels. Elongation rate significantly decreased with each increasing salinity level, although the difference between 10 and 15 psu treatments was not significant (Fig. 9, SNK, $p<0.05$). Light and salinity effects interacted ($p=0.016$). As with maximum seasonal leaf length, most of the variance was due to salinity treatment (65.2%), while very little was attributable to light treatment (5.4%).

Figure 9. Mean initial leaf elongation growth rate (cm/day), measured as slope of growth curve from the start of the experiment (planting) to date of longest leaf length. Slope of line was determined for each aquarium by regression (n=3). Different letters over bars indicate significant differences between light treatments, while different letters in legend indicate differences between salinity treatments (Student-Newman-Keuls, $p<0.05$).


2.0
1.5
1.0
0.5
0.0
Leaf Elongation Rate (cm/day)
0 psu = a
5 psu = b
10 psu = c
15 psu = c
A
B
B
28
8
2
Surface Irradiance (%)

Leaf Width

Throughout the course of the study width of the longest leaf per rosette decreased with increasing salinity ($p=0.0012$, Table 6, Fig. 10). Light had a borderline significant positive effect ($p=0.0510$), which was only apparent in the 0 and 5 psu treatments. Width also varied significantly with time ($p<0.0001$). Width generally decreased throughout the season, although it first increased to a mid-summer peak in the 0 and 5 psu, 28% light treatments and the 5 psu, 8% light treatments. For example, in the 15 psu treatments, average leaf width, which ranged from 2.7 to 3.3 mm in June, decreased to 0.9 to 1.8 mm in November. In contrast, in the 0 psu, 28% light treatment leaf width increased from 4.0 to 4.8 mm from June to July and then decreased to 2.6 mm by November. The pattern for the observed decreasing width was that the older, wider leaves senesced and were replaced with more narrow leaves. The effects of time did not interact with the effects of salinity ($p=0.0952$) or light ($p=0.7898$). There was no interactive effect between light and salinity ($p=0.7584$), but there was between light, salinity, and time ($p=0.0487$).

During the period of maximum seasonal leaf length (as described above) leaf width again decreased with increasing salinity ($p=0.0297$) and with decreasing light availability ($p=0.0191$, Table 7, Fig. 11). Light and salinity accounted for approximately the same amount of variance (18.9% for salinity and 16.7% for light). However, a large portion of the variance (42.8%) could not be explained by the single or interactive effects of light or salinity.

The product of length and width provides an approximation of shoot surface area. Length * width at the time of maximum seasonal leaf length ranged from 1.8 to 19.2 cm^2.

Figure 10. Mean width (mm) of the longest leaf per rosette in each light and salinity treatment. Data were averaged within aquariums to yield one value per aquarium. These values were then averaged within treatments (n=3). Error bars represent standard error.

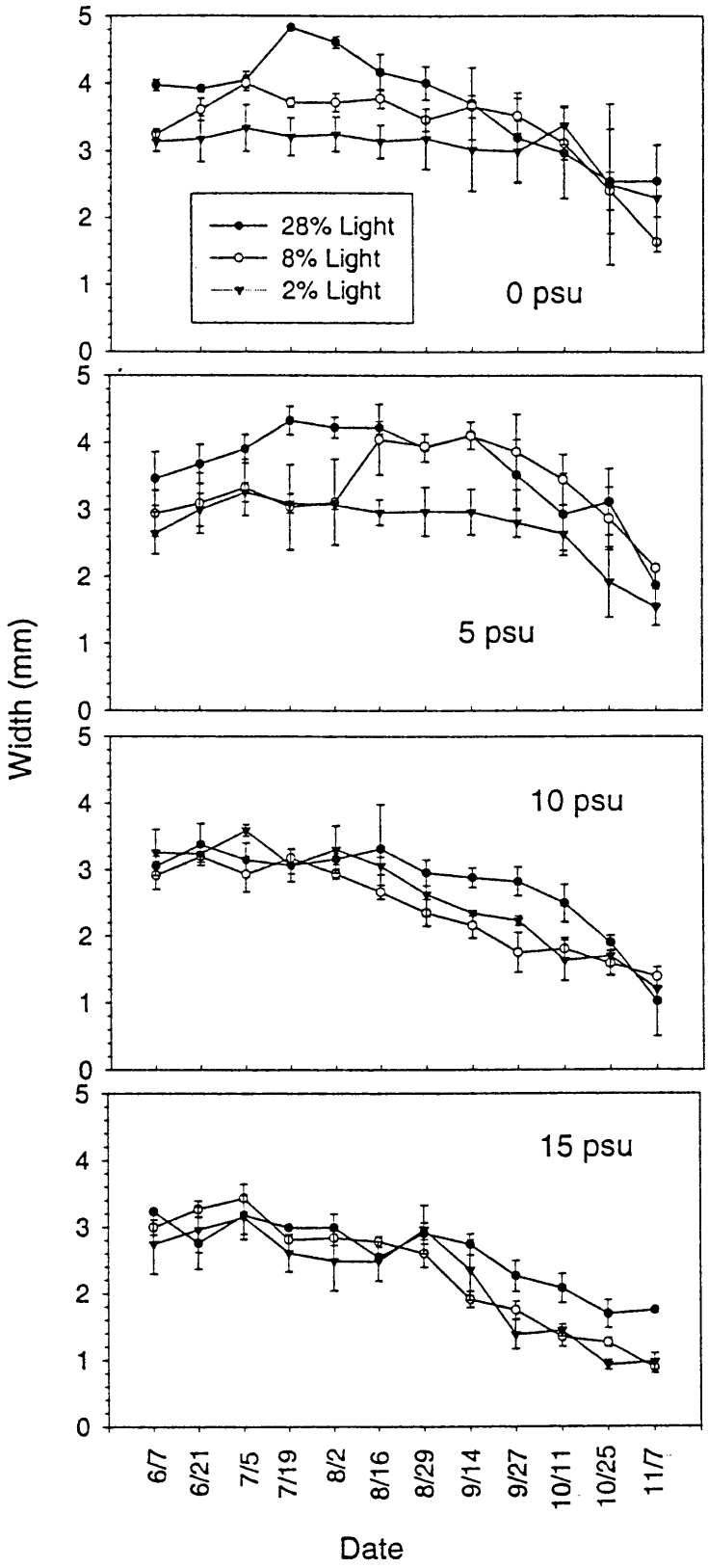
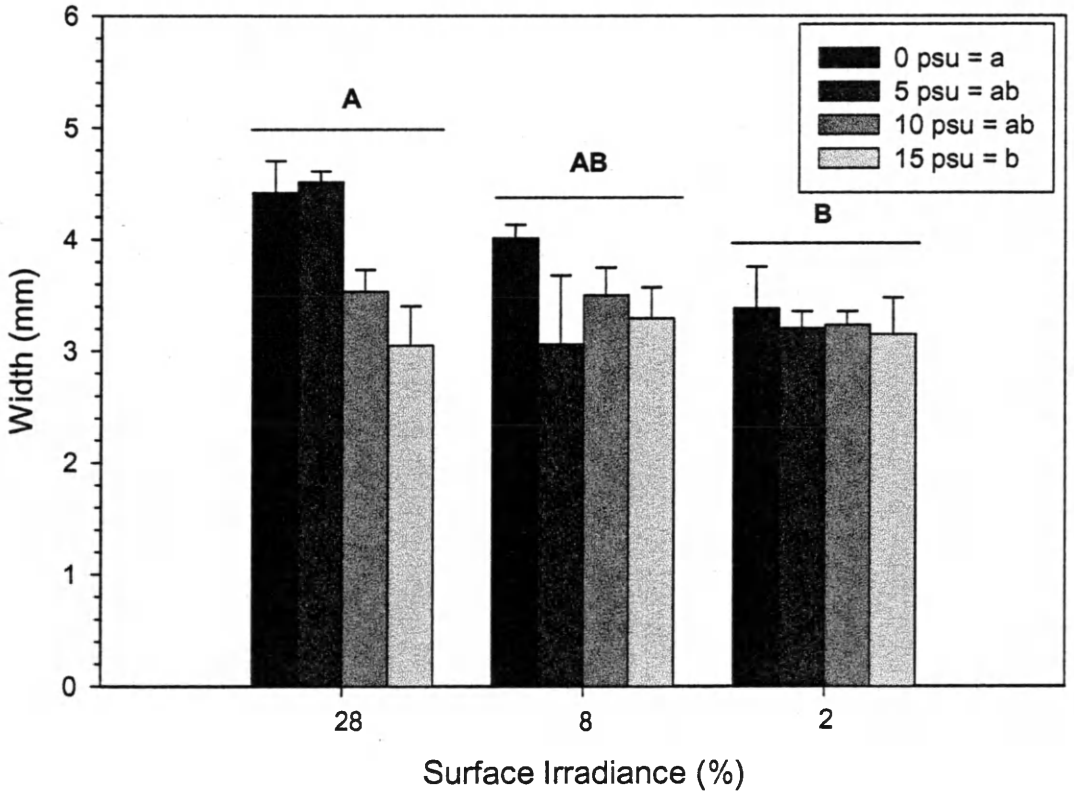

Width (mm)
28% Light
8% Light
2% Light
0 psu
5 psu
10 psu
15 psu
6/7
6/21
7/5
7/19
8/2
8/16
8/29
9/14
9/27
10/11
10/25
11/7
Date

Figure 11. Mean leaf width (mm) at maximum seasonal leaf length for each light/salinity combination (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, $p<0.05$).


0 psu = a
5 psu = ab
10 psu = ab
15 psu = b
A
AB
B
Width (mm)
0
1
2
3
4
5
6
28
8
2
Surface Irradiance (%)

Length * width decreased with increasing salinity ($p<0.0001$) and was not related to light ($p=0.2269$).

Leaf Production

Neither light nor salinity had a consistent effect on leaf production per rosette ($p>0.05$, Table 6). Number of leaves per rosette did, however, vary over time ($p<0.0001$). By mid-June, plants averaged 4.7 leaves per rosette (Fig. 12). By early July, leaf production increased to 6.5 leaves per rosette. Leaf abundance steadily declined after that peak, to a low of 2.9 leaves per rosette in November. The effects of time also interacted with both light ($p<0.0001$) and salinity ($p=0.002$). However, these interactive effects explained much less of the variance (10.0% for time*light and 7.3% for time*salinity) than time alone (60.0%). The interactive effects did not exhibit any clear pattern.

Vegetative Reproduction

Rosette production varied seasonally and was highly dependent on light and salinity treatments (Fig. 13). Averaged over all dates, vegetative reproduction increased with increasing light ($p=0.0012$, Table 6). Plants in the 2% light treatment typically produced the fewest rosettes (Fig. 13). Plants in the 28% light treatment produced substantially more than either of the other light treatments at salinities less than or equal to 10 psu. Salinity had a highly significant negative effect on vegetative reproduction ($p=0.0001$, Table 6). Rosette abundance was highly variable throughout the season and between treatments, ranging from 26 to 695 rosettes m^{-2}.

Figure 12. Seasonal change in number of leaves per rosette, averaged across all treatments (n=36). Data were pooled because repeated measures ANOVA indicated no statistical difference between treatments ($p>0.05$).

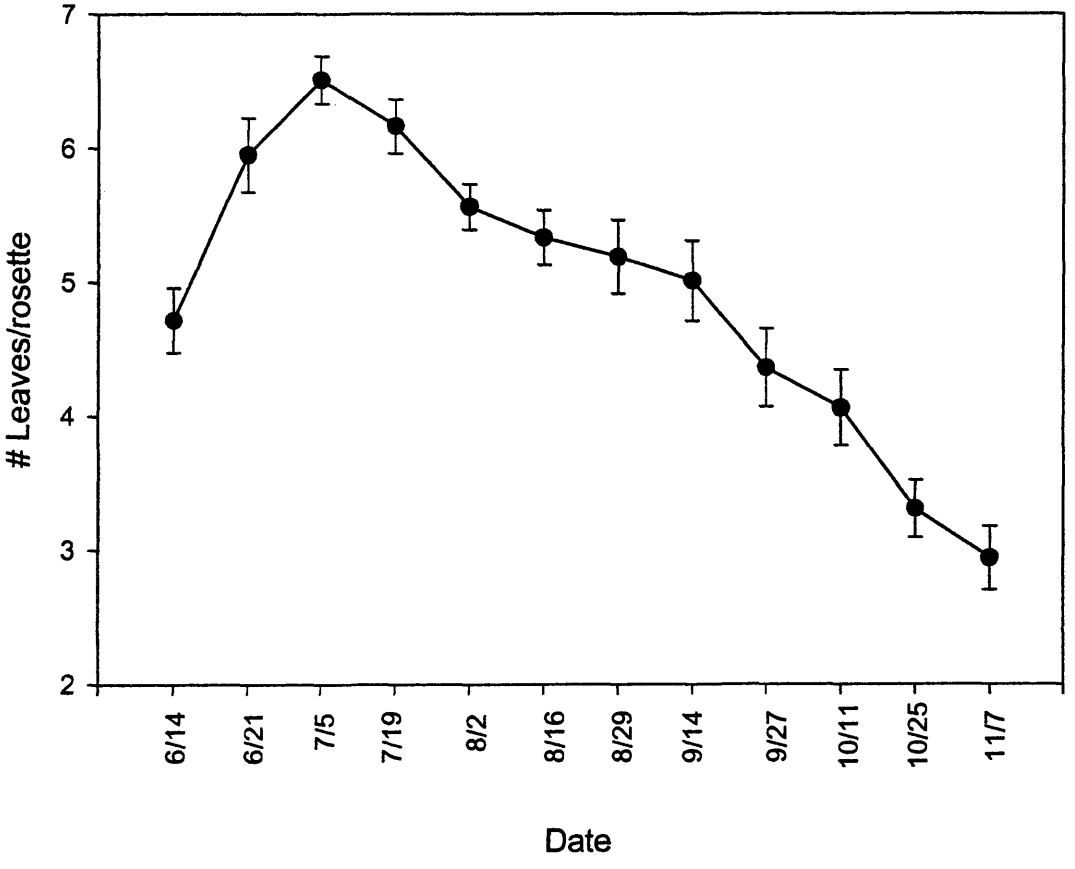
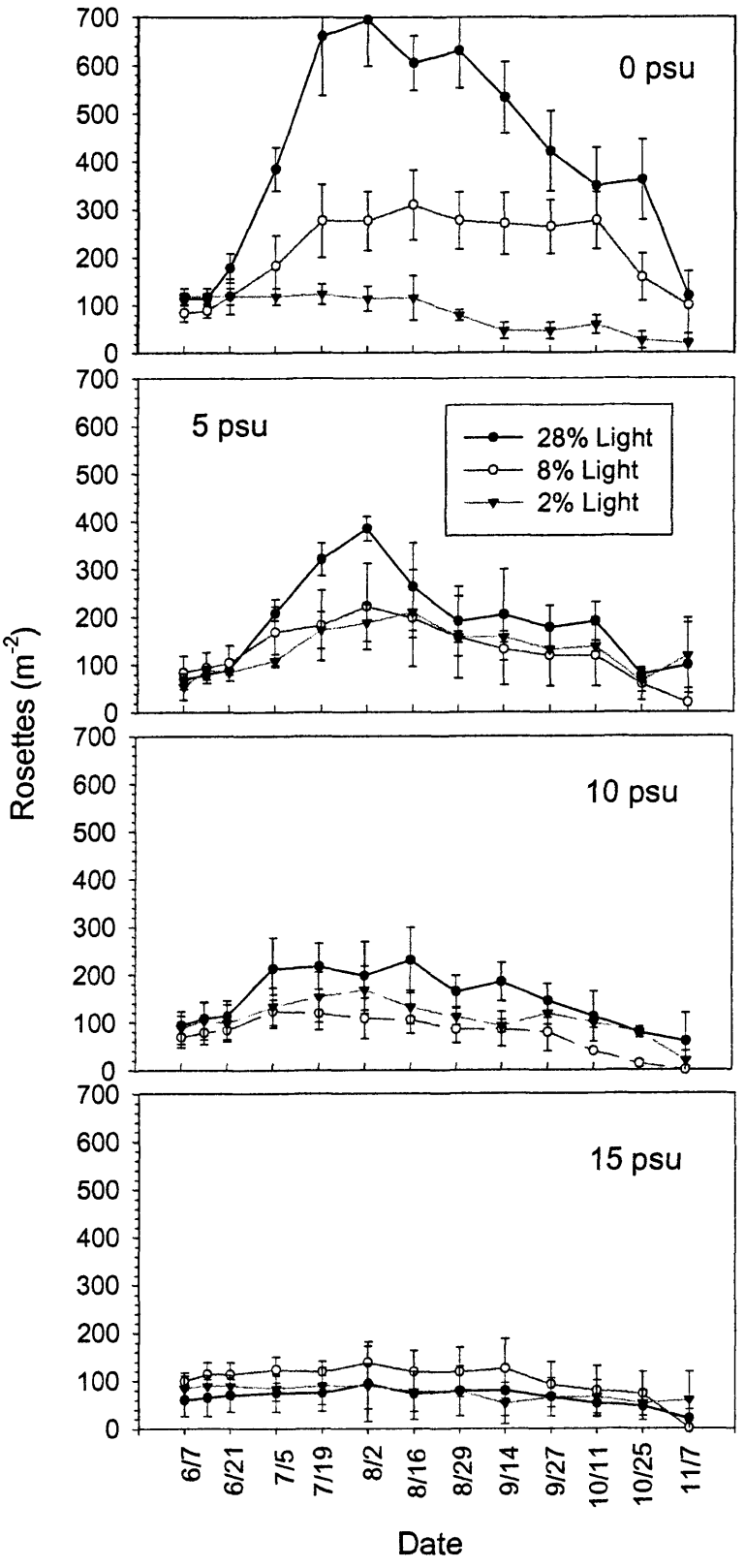

Leaves/rosette
7
6
5
4
3
2
6/14
6/21
7/5
7/19
8/2
8/16
8/29
9/14
9/27
10/11
10/25
11/7
Date

Figure 13. Mean rosette density (m^{-2}) over time for each light and salinity treatment. Numbers of rosettes were averaged to obtain one value per aquarium. These values were then averaged across treatments (n=3). Error bars represent standard error.

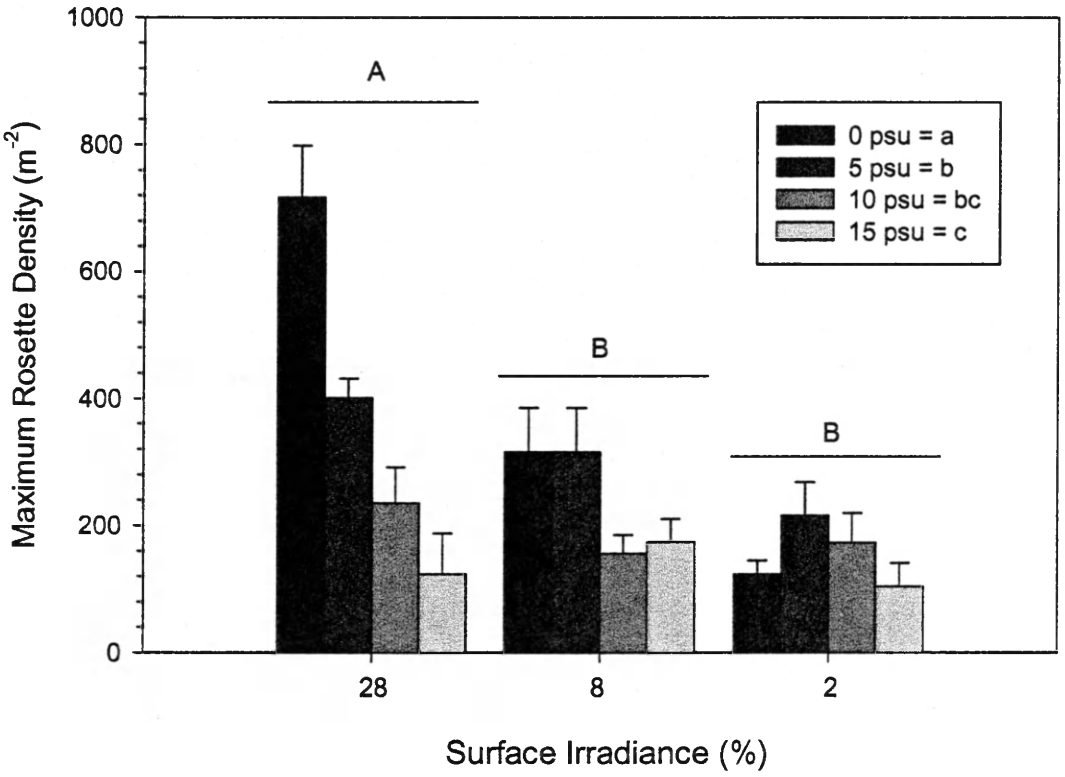

0 psu
5 psu
10 psu
15 psu
28% Light
8% Light
2% Light
Rosettes (m-2)
0
100
200
300
400
500
600
700
6/7
6/21
7/5
7/19
8/2
8/16
8/29
9/14
9/27
10/11
10/25
11/7
Date

There was a strong interaction of the effects of salinity and light (Table 6, $p=0.0012$) such that the effects of salinity were most apparent in 28% light treatment and the light effects were most pronounced at the 0 and 5 psu salinity levels (Fig. 13). Partitioning of variance revealed that light accounted for 17% of variation, while salinity accounted for 30%. The light*salinity interaction accounted for 30%, and the remainder (23%) was not attributable to either factor.

Rosette density varied significantly over time ($p<0.0001$, Table 6), with density generally highest in the middle of July and beginning of August (Fig. 13). Maximum rosette density was also dependent on light ($p<0.0001$) and salinity ($p<0.0001$) (Table 7, Fig. 14). Plants in the 28% light, 0 psu treatment produced a maximum of 717 rosettes m^{-2}. Maximum production of plants in the low light, 15 psu treatment was the lowest, at 104 rosettes m^{-2}. Light and salinity strongly interacted for this parameter ($p=0.0005$). Loss of rosettes in the latter part of the season was due to leaf senescence and rosettes coming loose from the sediment. The effect of date was apparent in all light and salinity treatments (Wilk's test, Table 6).

Rosette density increased approximately linearly from the start of vegetative reproduction (June 14) to the approximate date of maximum density (July 19). Rosette production in the 28% light, 0 psu treatment increased at the greatest rate, 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. Growth rate of 5, 10, and 15 psu treatments ranged from 6 to 12% d^{-1}, 4 to 6% d^{-1}, and 0 to 3% d^{-1}.

Figure 14. Mean maximum seasonal rosette density. Numbers of rosettes per pot were converted to m-2 and then averaged for each aquarium. Maximum values were then averaged for each treatment (n=3). Different letters over bars indicate significant differences between light treatments, while different letters in legend indicate differences between salinity treatments (Student-Newman-Keuls, $p<0.05$).


1000
800
600
400
200
0
Maximum Rosette Density (m-2)
A
B
B
0 psu = a
5 psu = b
10 psu = bc
15 psu = c
28
8
2
Surface Irradiance (%)

The effects of light and salinity on rosette density varied over time, as did the interaction between light and salinity ($p<0.0001$ for each). Partitioning of variance (Table 7) indicated that date alone had the greatest effect on vegetative reproduction, explaining 33% of the variance. The interactions between date and light (12%), date and salinity (15%), and date, light, and salinity (16%) were less pronounced.

Contrast variables reveal the times at which the effects of light and salinity on rosette production changed. A critical period was June 14 to June 21, when the effects of light ($p<0.0001$), salinity ($p<0.0001$), and light*salinity ($p=0.0002$) all changed. Visual inspection of the data (Fig. 13) indicates minimal growth prior to this period and thus little, if any, light or salinity effects. Once rosette production increased, light and salinity effects became pronounced. The light and salinity factors then interacted such that light effects were most apparent at lower salinities and salinity effects were most prevalent for the 28% light treatment.

Another critical period was July 19 to August 2, when again, the effects of light ($p=0.0021$), salinity ($p=0.0070$), and light*salinity ($p=0.0058$) all changed. This two-week period of the life history of *V. americana* generally marks the beginning of the decrease in rosette density. This decrease is most apparent in the lower salinity and higher light treatments.

The effects of salinity were more variable over time than were the effects of light. Salinity effects changed often throughout the season. In addition to the highly significant changes at the dates above, its effects also changed between sampling dates August 16 and 29 ($p=0.0247$), September 27 and October 11 ($p=0.0296$), and October 11 and 25 ($p=0.0119$).

Mid-Season Harvest

Biomass

Aboveground biomass was directed related to light availability ($p=0.0035$, Table 7, Fig. 15) and inversely related to salinity ($p=0.0018$). Treatment means ranged from 1.9 to 47.0 gdw m^{-2}. Aboveground biomass of plants in the 28% light treatment was significantly greater than that of the other two light levels (SNK, $p<0.05$), with a trend of greater biomass under 8% light than under 2% light. Aboveground biomass also generally increased with decreasing salinity, with 0 and 5 psu significantly different than 10 and 15 psu (SNK, $p<0.05$). Light and salinity effects interacted ($p=0.0437$). The effects of salinity were most apparent in the 28% light treatment, and the light effects were only apparent at the 0 and 5 psu levels. Light explained 19% of the variance in aboveground biomass, and salinity explained 27%. The interaction between light and salinity accounted for 21%, and 32% of the variance was not attributable to either of the factors (Table 7).

Examination of aboveground biomass per rosette removes the effect of vegetative reproduction and is, therefore, a measure of shoot growth alone. Aboveground biomass per rosette was not consistently related to light or salinity ($p>0.05$, Table 7, Fig. 16), although it displayed a downward trend with increasing salinity, particularly in the 28% light treatment. It also exhibited a downward trend with decreasing light in the 0 and 5 psu treatments.

Belowground biomass followed the same general pattern as aboveground biomass. Treatment means ranged from 1.6 to 50.7 gdw m^{-2}. Belowground biomass was

Figure 15. Mean aboveground biomass (g dry weight) per m^2, of August 4 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, $p<0.05$).

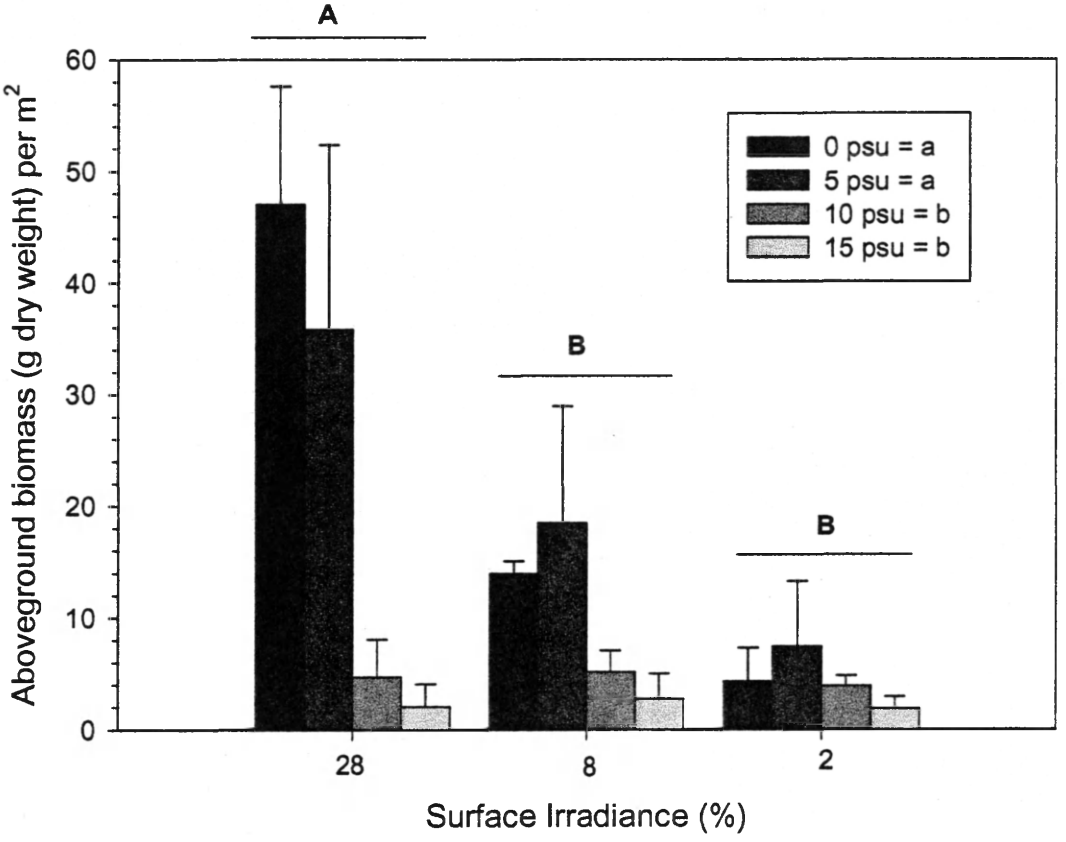
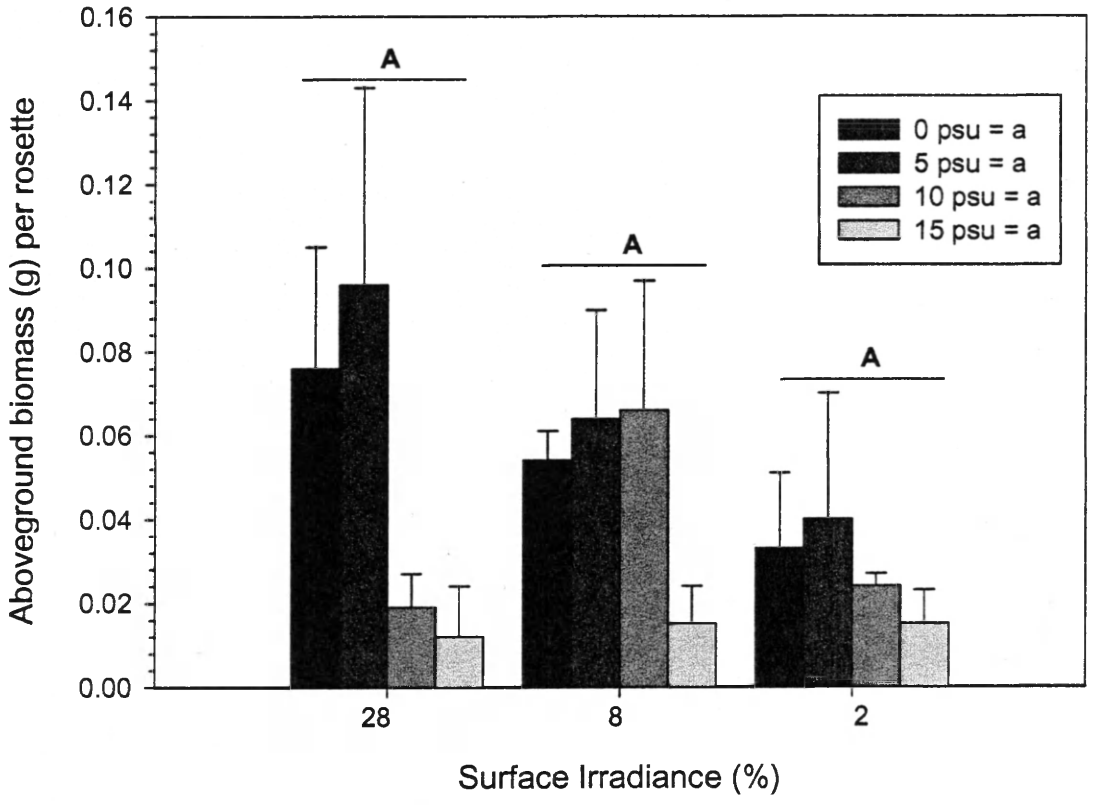

A
B
B
60
50
40
30
20
10
0
Aboveground biomass (g dry weight) per m2
0 psu = a
5 psu = a
10 psu = b
15 psu = b
28
8
2
Surface Irradiance (%)

Figure 16. Mean aboveground biomass (g dry weight) per rosette from mid-season harvest. n = 3 for most light/salinity combinations. Identical letters over bars and in legend indicate no significant difference between means (Student-Newman-Keuls, $p>0.05$).


0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Aboveground biomass (g) per rosette
A
A
A
0 psu = a
5 psu = a
10 psu = a
15 psu = a
28
8
2
Surface Irradiance (%)

directly related to light ($p=0.0024$, Table 7, Fig. 17), with biomass in the 28% light treatment significantly greater than in the 8% or 2% light treatments (SNK, $p<0.05$). Belowground biomass was inversely related to salinity ($p=0.0368$). The one notable exception was the 28% light, 5 psu treatment, where the biomass was greater than in the 28%, 0 psu treatment. Plants in one aquarium contributed to the high biomass of this treatment. The only statistically significant difference for salinity treatments was between the 5 and 15 psu treatments. The effects of light and salinity interacted ($p=0.0303$), with the effects of salinity generally more apparent in the 28% light treatment and the light effects most apparent in the 0 and 5 psu treatments. In contrast to the aboveground biomass response, light had more of an effect on belowground biomass than did salinity, explaining 24% of variance compared to 15% for salinity (Table 7). The interaction between light and salinity explained even more variance (26%), and 36% of the variance could not be explained by either of the factors.

Belowground biomass per rosette was directly related to light ($p=0.0415$, Table 7, Fig. 18), with values in the 28% light treatment significantly greater than those of the 2% light treatment (SNK, $p<0.05$). This difference, however, appears to be primarily due to the very high value of the 28% light, 5 psu treatment. Salinity did not have a consistent effect on belowground biomass per rosette.

Aboveground-to-total biomass ratio ranged from 0.32 to 0.82 and was not statistically different between treatments (light: $p=0.4778$; salinity: $p=0.2166$; light*salinity=0.1025, Table 7), with no apparent trends. The aboveground-to-total biomass ratio per rosette was also not related to light or salinity ($p>0.05$).

Figure 17. Mean belowground biomass (g dry weight) per m^2 of August 4 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, $p<0.05$).

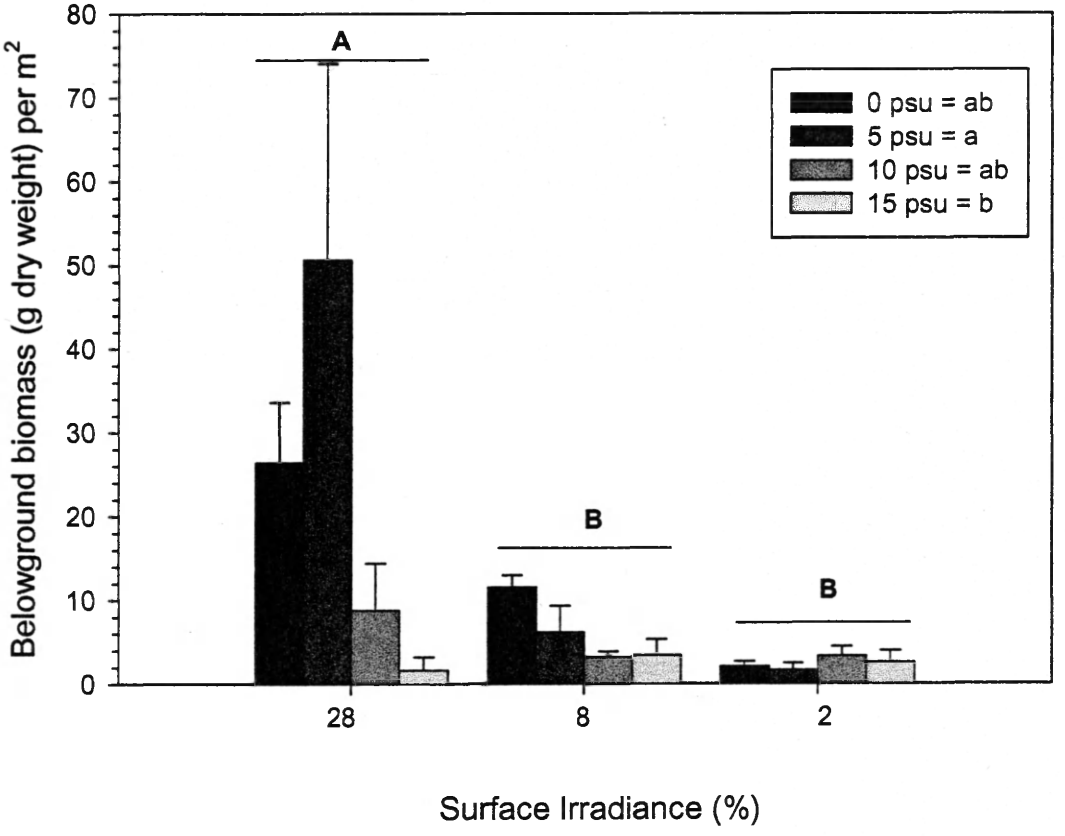
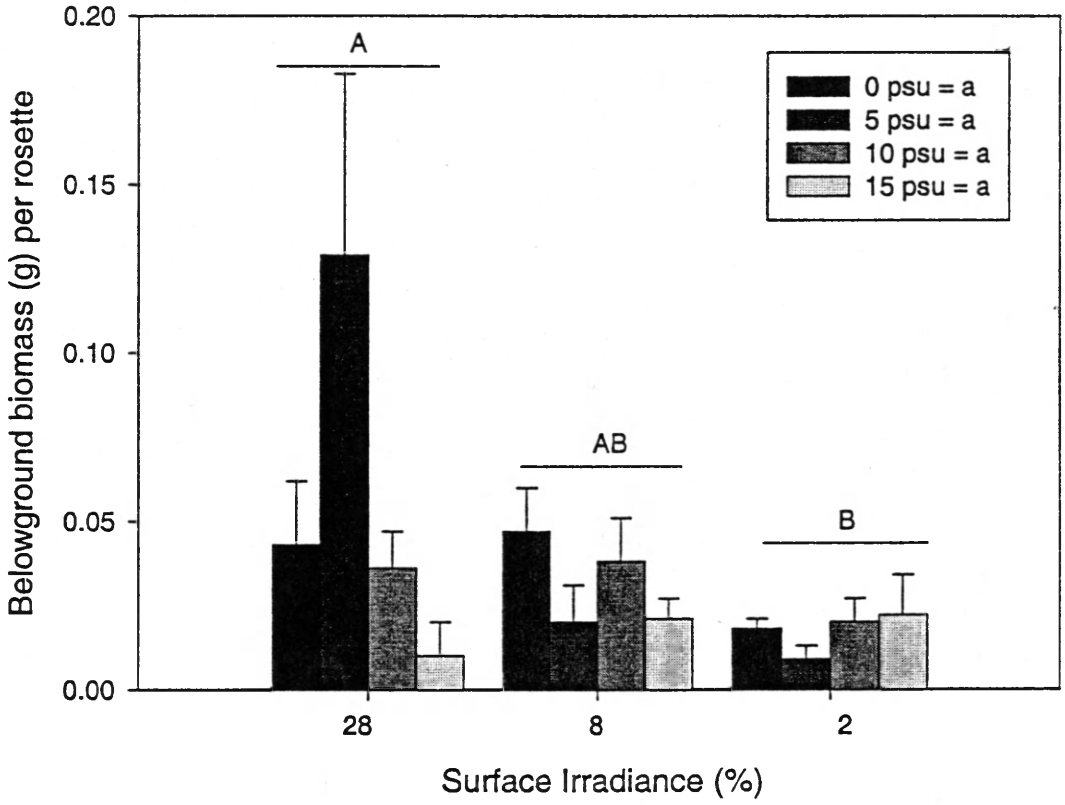

Belowground biomass (g dry weight) per m2
80
70
60
50
40
30
20
10
0
A
B
B
0 psu = ab
5 psu = a
10 psu = ab
15 psu = b
28
8
2
Surface Irradiance (%)

Figure 18. Mean belowground biomass (g dry weight) per rosette from mid-season harvest. n = 3 for most light/salinity combinations. Different letters over bars indicate significant differences between light treatments, while different letters in legend indicate differences between salinity treatments (Student-Newman-Keuls, $p<0.05$).


0.20
0.15
0.10
0.05
0.00
Belowground biomass (g) per rosette
A
AB
B
0 psu = a
5 psu = a
10 psu = a
15 psu = a
28
8
2
Surface Irradiance (%)

Leaf Area

Leaf area followed the same general pattern as above- and belowground biomass, although the effects of light and salinity were even more pronounced. Leaf Area Index varied greatly between treatments (0.08 to 3.2). Light was directly related to leaf area ($p=0.0009$, Table 7, Fig. 19) and accounted for 19% of variation. The 28% light treatment produced the greatest leaf area (SNK, $p<0.05$), with a trend of 8% light producing greater leaf area than 2% light. Salinity was inversely related to leaf area ($p<0.0001$) and explained 35% of variation. Leaf area was greatest from low salinity to high, although differences between 0 and 5 psu, and 10 and 15 psu were not significant (SNK). The effects of light and salinity interacted ($p=0.0125$). This interaction accounted for 21% of variation.

Aboveground biomass per leaf area, an indication of leaf thickness, was not related to light ($p=0.4857$, Table 7, Fig. 20). It was also not significantly related to salinity ($p=0.1314$), although there was a trend of increased thickness at higher salinity.

Leaf area per rosette was not related to light ($p>0.05$, Table 7, Fig. 21), but was related to salinity ($p=0.0133$). Leaf area per rosette generally decreased with increasing salinity, although the only significant difference was between 0 and 15 psu (SNK, $p<0.05$).

The product of leaf length and width, a measure of leaf area per shoot (for the longest leaf per rosette) was not significantly related to light ($p>0.05$, Table 7, Fig. 22) but decreased with increasing salinity ($p<0.0001$).

Figure 19. Mean leaf area (cm^2 per cm^2 sediment surface) 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, $p<0.05$).

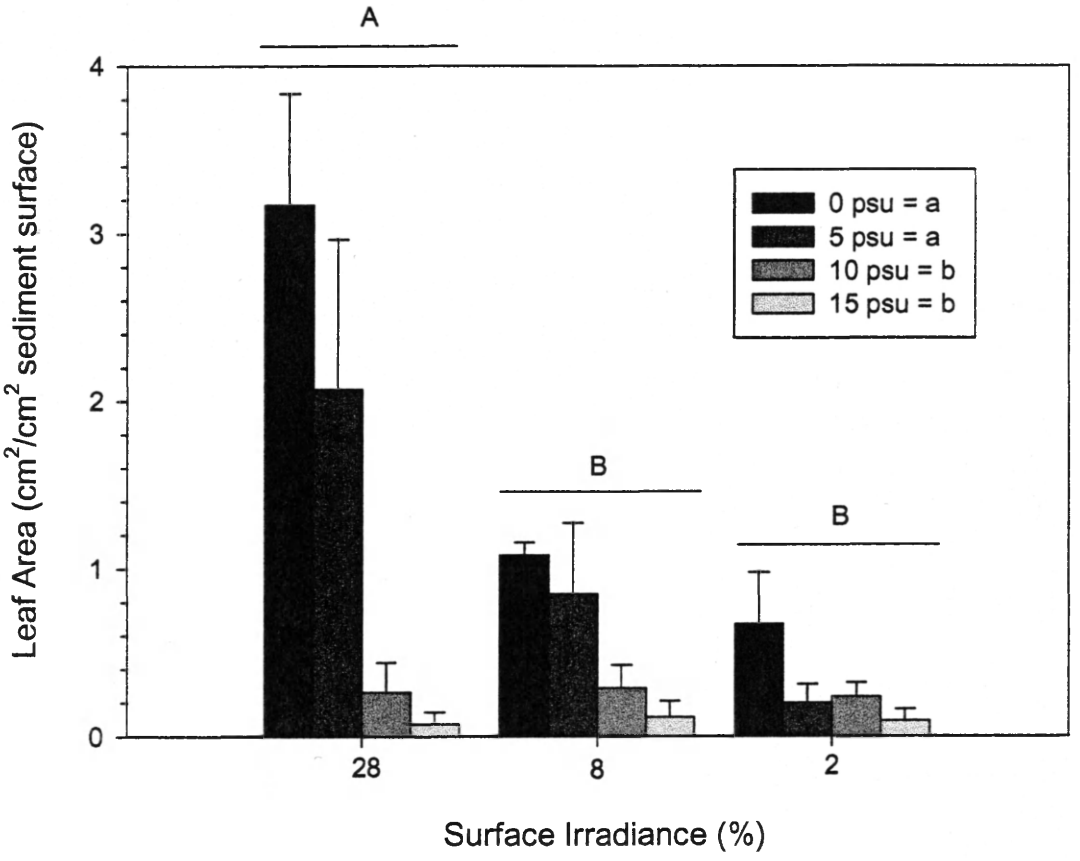

A
B
B
0 psu = a
5 psu = a
10 psu = b
15 psu = b
Leaf Area (cm²/cm² sediment surface)
0
1
2
3
4
28
8
2
Surface Irradiance (%)

Figure 20. Aboveground biomass/leaf area (mg/cm^2), an indication of leaf thickness, from mid-season harvest. n=3 for most light/salinity combinations. Identical letters over bars and in legend indicate no significant difference between means (Student-Newman-Keuls, $p>0.05$)

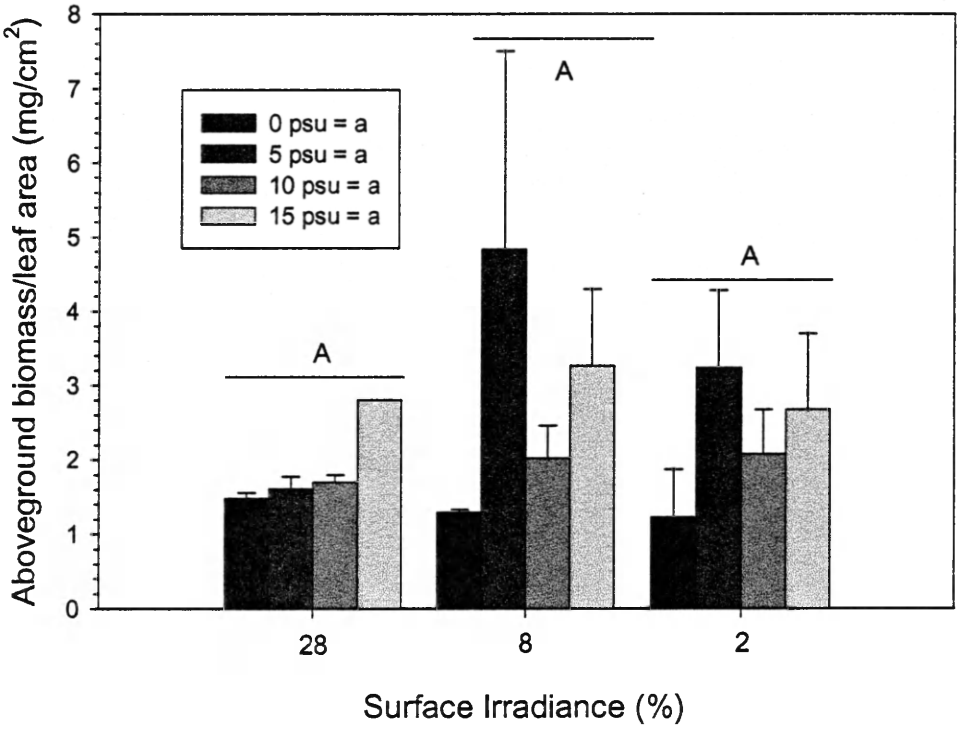

Aboveground biomass/leaf area (mg/cm^2)
0
1
2
3
4
5
6
7
8
0 psu = a
5 psu = a
10 psu = a
15 psu = a
A
A
A
28
8
2
Surface Irradiance (%)

Figure 21. Mean leaf area (cm^2) per rosette from mid-season harvest. n=3 for most light/salinity combinations. Different letters over bars indicate significant differences between light treatments, while different letters in legend indicate differences between salinity treatments (Student-Newman-Keuls, $p<0.05$).

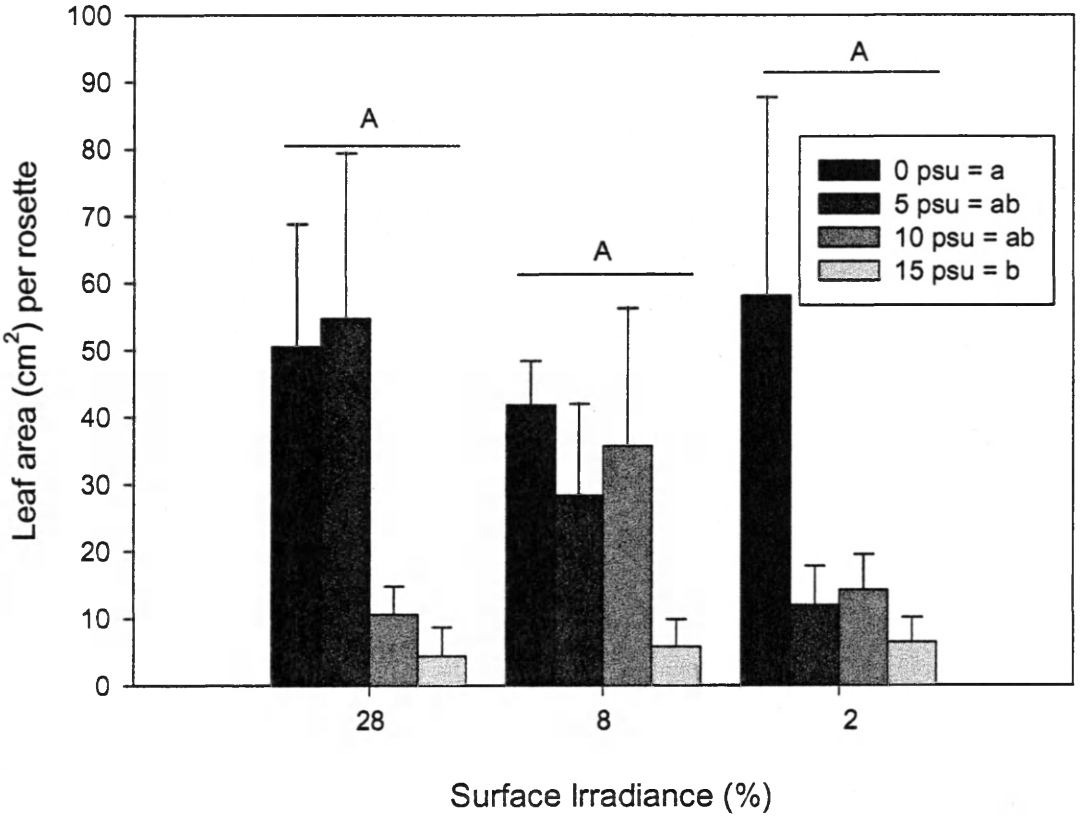
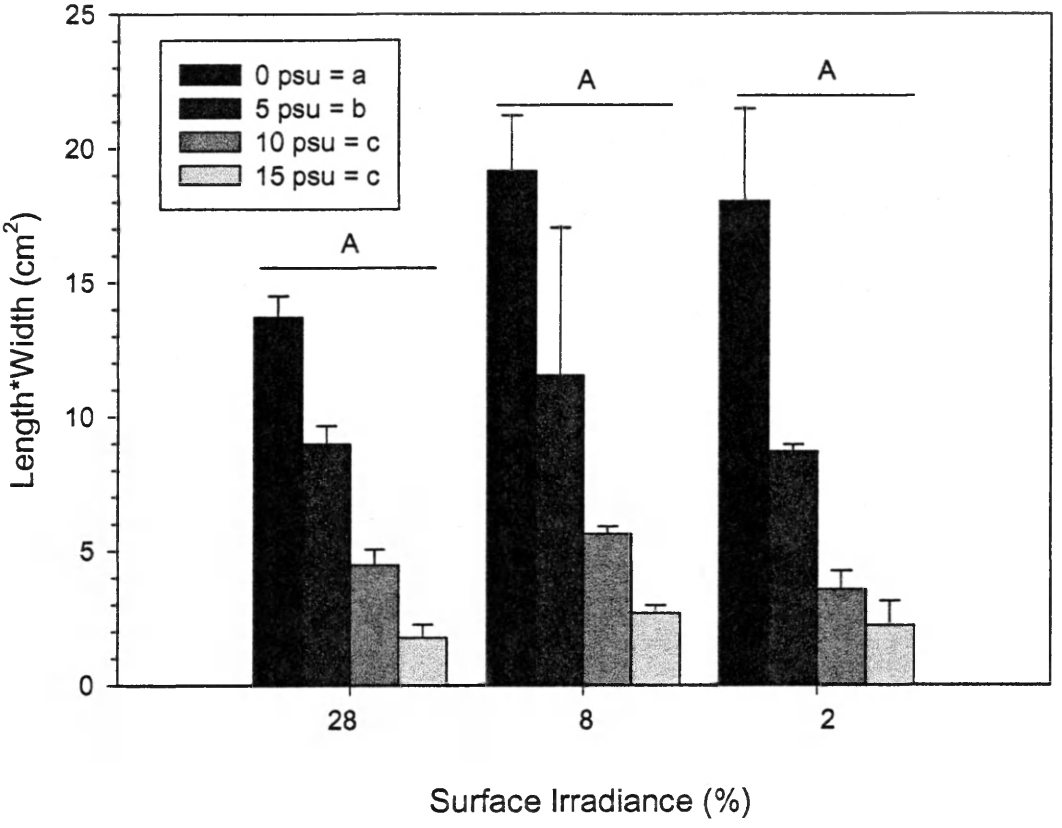

100
90
80
70
60
50
40
30
20
10
0
Leaf area (cm^2) per rosette
A
A
A
0 psu = a
5 psu = ab
10 psu = ab
15 psu = b
28
8
2
Surface Irradiance (%)

Figure 22. Mean length * width (cm^2) at maximum seasonal leaf length for each light/salinity combination (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, $p<0.05$).


25
20
15
10
5
0
Length*Width (cm2)
0 psu = a
5 psu = b
10 psu = c
15 psu = c
A
A
A
28
8
2
Surface Irradiance (%)

Reproductive Structures

Flowering was observed in August and September and was observed in only 6 aquariums, all in 28 or 8% light and 0 or 5 psu treatments (App. 4). Most flowering plants produced seeds later in the season.

Tubers were harvested on December 13, several weeks after winter dieback. Only firm tubers with no evidence of decomposition were counted. Tuber production by treatment ranged from 66 to 500 tubers m^{-2} (Fig. 23). Tuber production was variable within treatments and so was not significantly related to light ($p=0.2127$, Table 7) or salinity ($p=0.1822$). However, there is a clear trend of increasing tubers with increasing light and also with decreasing salinity. Salinity effects were most apparent at 28 and 8% light, and light effects were most prominent at 0 psu. The ratio of end-of-season tubers to maximum seasonal rosettes (Fig. 23) ranged from 0 to 2.2 tubers/rosette by aquarium (mean 0.65, SE $\pm$ 0.096) and did not differ by treatment ($p>0.05$, Table 7).

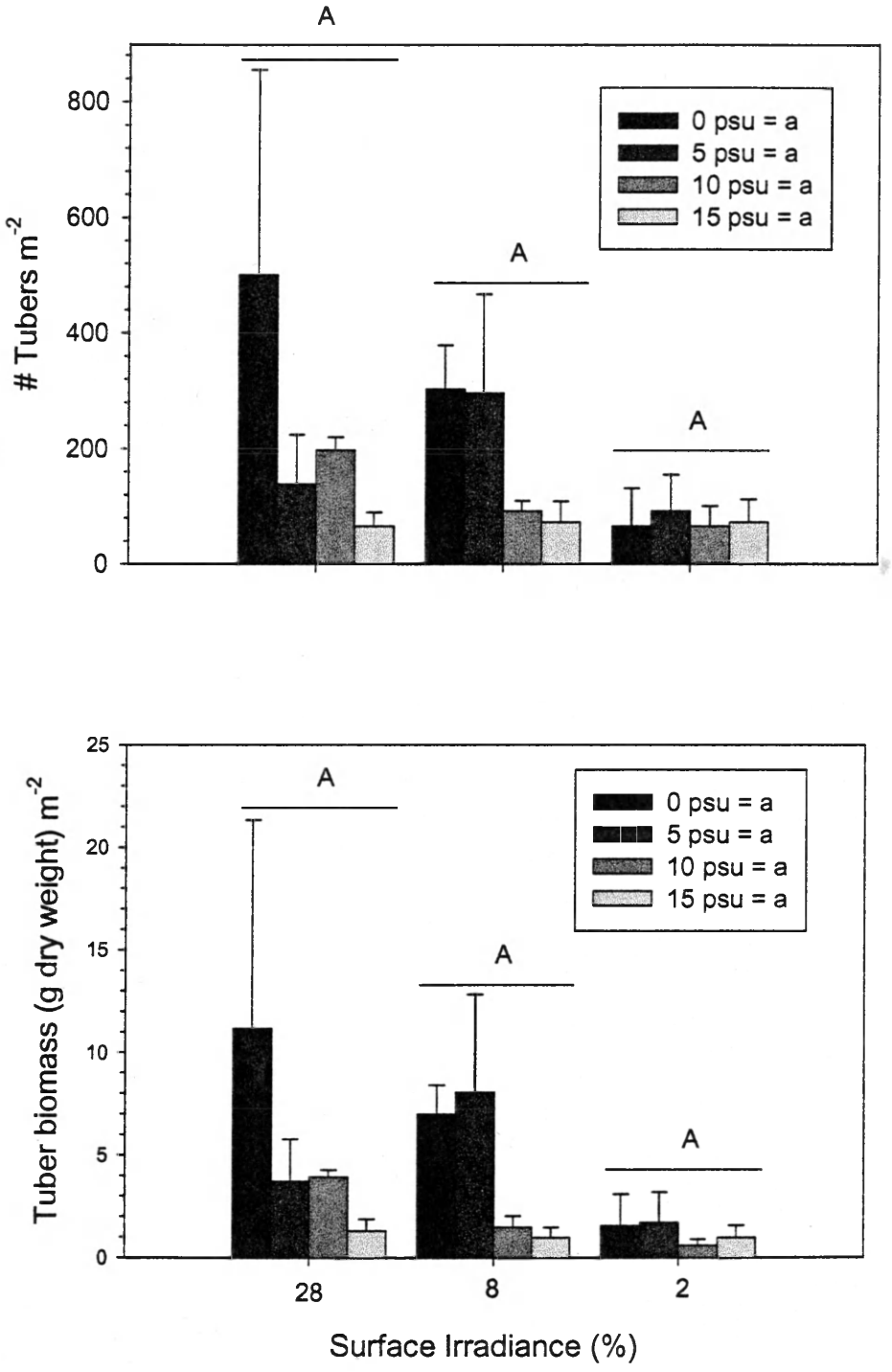
Individual tuber biomass was 0.019 gdw (SE $\pm$ 0.002) and was not related to light or salinity ($p>0.05$). Total tuber biomass m^{-2} also was not significantly related to light ($p=0.2558$, Table 7, Fig. 23) or salinity ($p=0.2140$), but displayed a similar trend to that of number of tubers produced.

Photosynthesis

Chlorophyll

Leaves were collected for chlorophyll *a* and *b* analysis on July 6, August 2, September 1 and September 29. Statistical analyses of July 6 and August 2 data are

Figure 23. Mean number and biomass of tubers (g dry weight) 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, $p>0.05$).


A
A
A
0 psu = a
5 psu = a
10 psu = a
15 psu = a
Tubers m-2
0
200
400
600
800
Tuber biomass (g dry weight) m-2
0
5
10
15
20
25
28
8
2
Surface Irradiance (%)

presented below. Data obtained from September 1 samples were lost due to analytical problems. Due to plant dieback, September 29 sample size was too small for ANOVA analysis (n=21).

For July 6 samples, chlorophyll *a* concentrations ranged from 0.60 to 1.93 mg/dm^2 and decreased with increasing light ($p<0.0001$, Table 8, Fig. 24). Chlorophyll *a* concentrations were also significantly related to salinity ($p=0.0321$), although the only significant difference was between 0 psu and 10 psu treatments, and there was only a trend of increasing concentrations with increasing salinity. Chlorophyll b concentrations ranged from 0.46 to 1.11 mg/dm^2 and also decreased with increasing light ($p=0.0001$, Table 8, Fig. 24), with concentration of plants in the 2% light treatment significantly greater than those in the 8% and 28% light treatments (SNK, $p<0.05$). Salinity did not have a consistent effect ($p=0.1041$). The ratio of chlorophyll *a* to chlorophyll *b* concentration was also inversely related to light ($p=0.0021$, Table 8, Fig. 24), with chlorophyll *a:b* of plants in the 2 and 8% light treatments (1.75 to 2.11) greater than that in the 28% light treatment (1.20 to 1.54) (SNK, $p<0.05$). Salinity did not have a consistent effect on the ratio of chlorophyll *a* to *b* ($p=0.4712$).

August 2 samples displayed similar chlorophyll patterns. Chlorophyll *a* concentration ranged from 0.49 to 2.04 mg/dm^2 and decreased with increasing light ($p=0.0024$, Table 8, Fig. 25), with the chlorophyll concentration of plants in the 2% light treatment greater than that in the 8 or 28% light treatment (SNK, $p<0.05$). Chlorophyll *a* concentration was not, however, related to salinity ($p=0.2746$). Chlorophyll *b* concentration ranged from 0.39 to 1.16 mg/dm^2 and followed the same pattern as that of

Table 8. 2-way ANOVA results for light and salinity effects on chlorophyll concentrations and chlorophyll a/b ratios. 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.
Chlorophyll a, July 6	Light	2	16.435	**<.0001**	46.5
	Salinity	3	3.46	**0.0321**	14.7
	Light * Salinity	6	0.57	0.7502	4.8
	Error	24			34.0
Chlorophyll b, July 6	Light	2	13.1	**0.0001**	42.5
	Salinity	3	2.289	0.1041	11.1
	Light * Salinity	6	0.762	0.6065	7.4
	Error	24			38.9
Chlorophyll a/b, July 6	Light	2	8.079	**0.0021**	36.2
	Salinity	3	0.868	0.4712	5.8
	Light * Salinity	6	0.307	0.927	4.1
	Error	24			53.8
Chlorophyll a, August 2	Light	2	7.906	**0.0024**	31.8
	Salinity	3	1.378	0.2746	8.3
	Light * Salinity	6	1.137	0.3729	13.7
	Error	23			46.2
Chlorophyll b, August 2	Light	2	9.979	**0.0008**	35.6
	Salinity	3	1.919	0.1546	10.3
	Light * Salinity	6	1.228	0.3283	13.2
	Error	23			41.0

Figure 24. Mean chlorophyll *a* and *b* (mg/dm^2) and chlorophyll *a/b* ratio of leaf tissue harvested on July 6. One leaf was collected per aquarium (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, $p<0.05$).

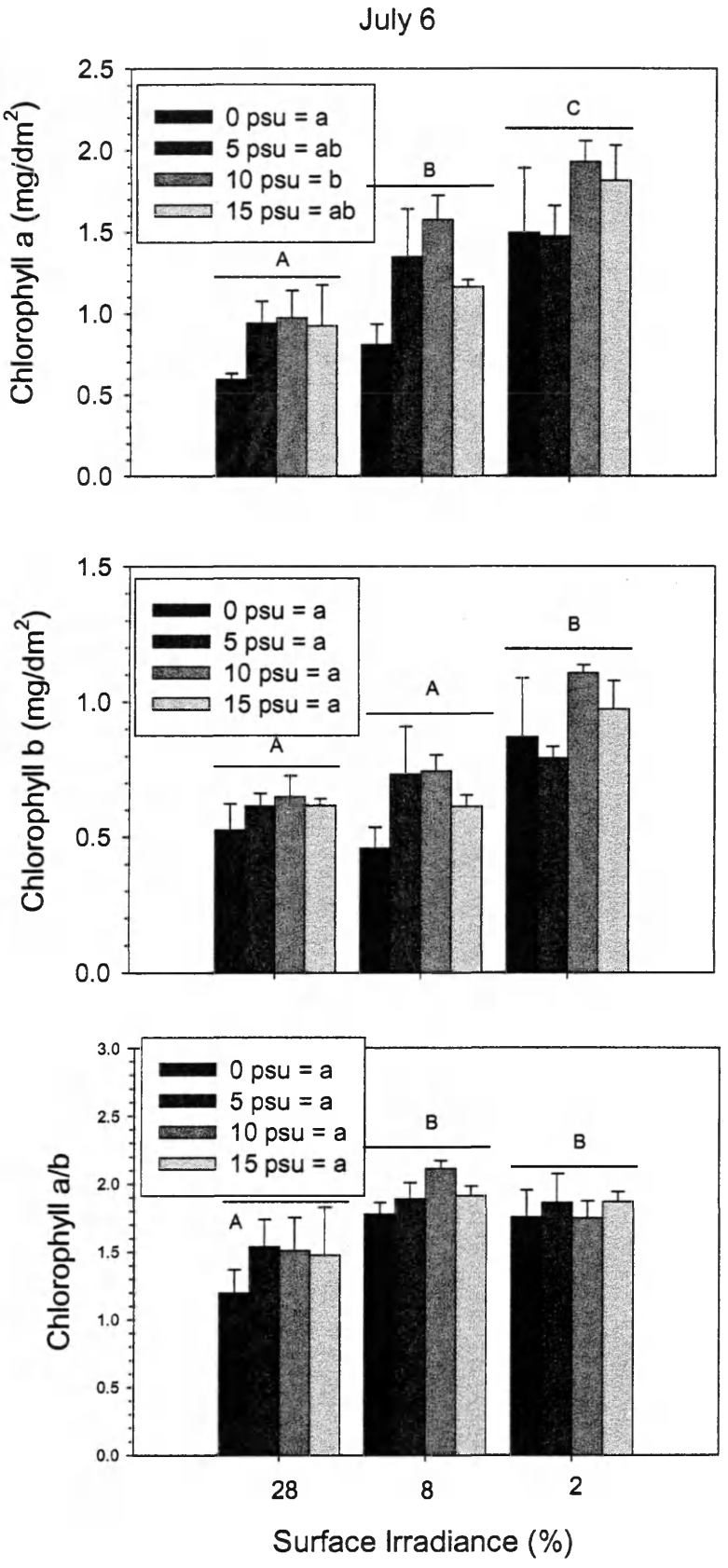
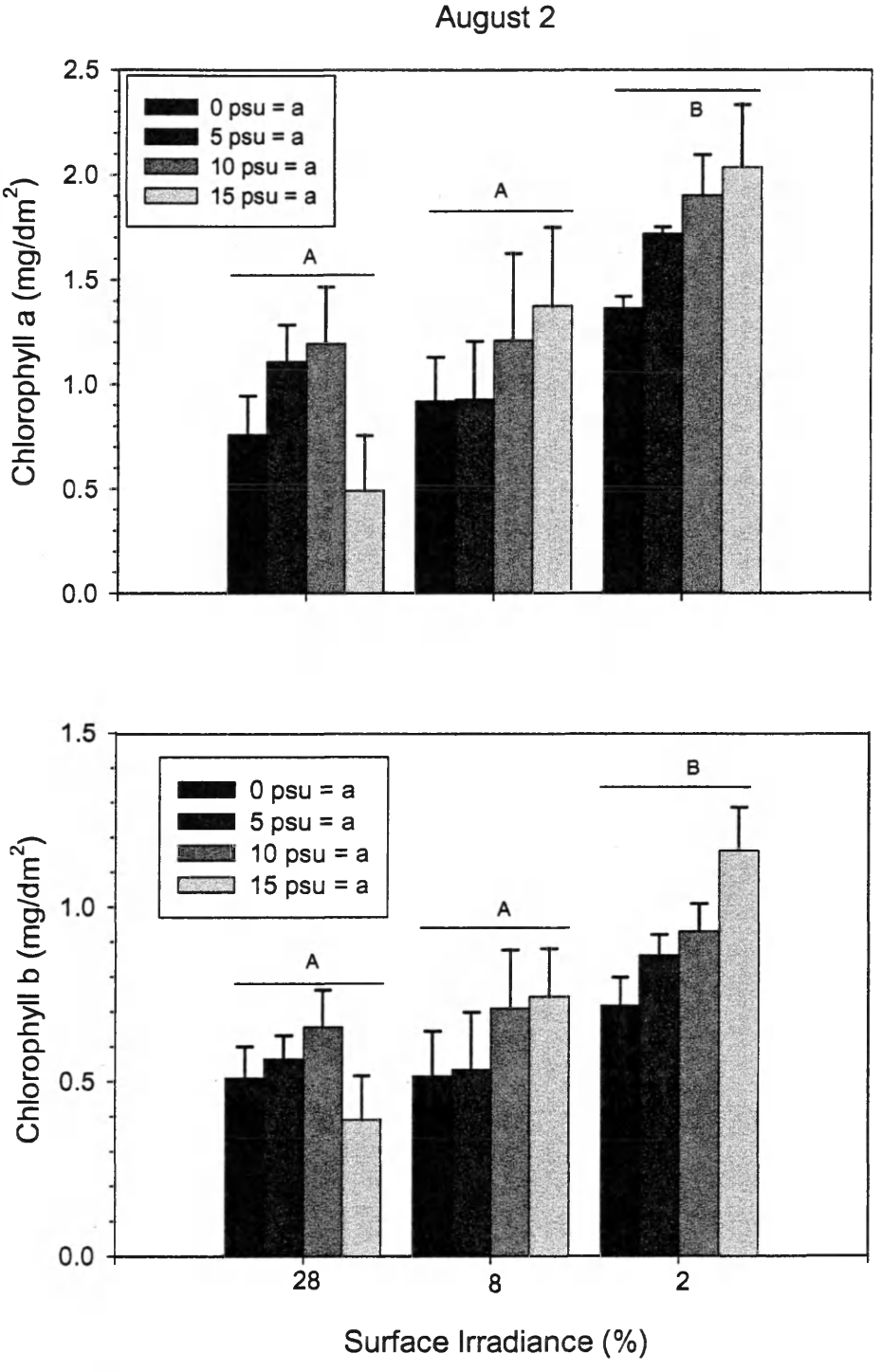

July 6
Chlorophyll a (mg/dm^2)
2.5
2.0
1.5
1.0
0.5
0.0
0 psu = a
5 psu = ab
10 psu = b
15 psu = ab
A
B
C
Chlorophyll b (mg/dm^2)
1.5
1.0
0.5
0.0
0 psu = a
5 psu = a
10 psu = a
15 psu = a
A
A
B
Chlorophyll a/b
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 psu = a
5 psu = a
10 psu = a
15 psu = a
A
B
B
28
8
2
Surface Irradiance (%)

Figure 25. Mean chlorophyll *a* and *b* concentrations (mg/dm^2) of leaf tissue harvested on August 2. One leaf was collected per aquarium (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, $p<0.05$).


August 2
Chlorophyll a (mg/dm^2)
2.5
2.0
1.5
1.0
0.5
0.0
0 psu = a
5 psu = a
10 psu = a
15 psu = a
A
A
B
Chlorophyll b (mg/dm^2)
1.5
1.0
0.5
0.0
0 psu = a
5 psu = a
10 psu = a
15 psu = a
A
A
B
28
8
2
Surface Irradiance (%)

chlorophyll *a*, with concentration in the 2% light treatment greater than that in the 8 or 28% light treatment (Table 8, Fig. 25, SNK, $p<0.05$). Again, chlorophyll b concentration was not related to salinity. The ratio of chlorophyll *a* to *b* concentrations was not related to light ($p=0.155$) or salinity ($p=0.4741$).

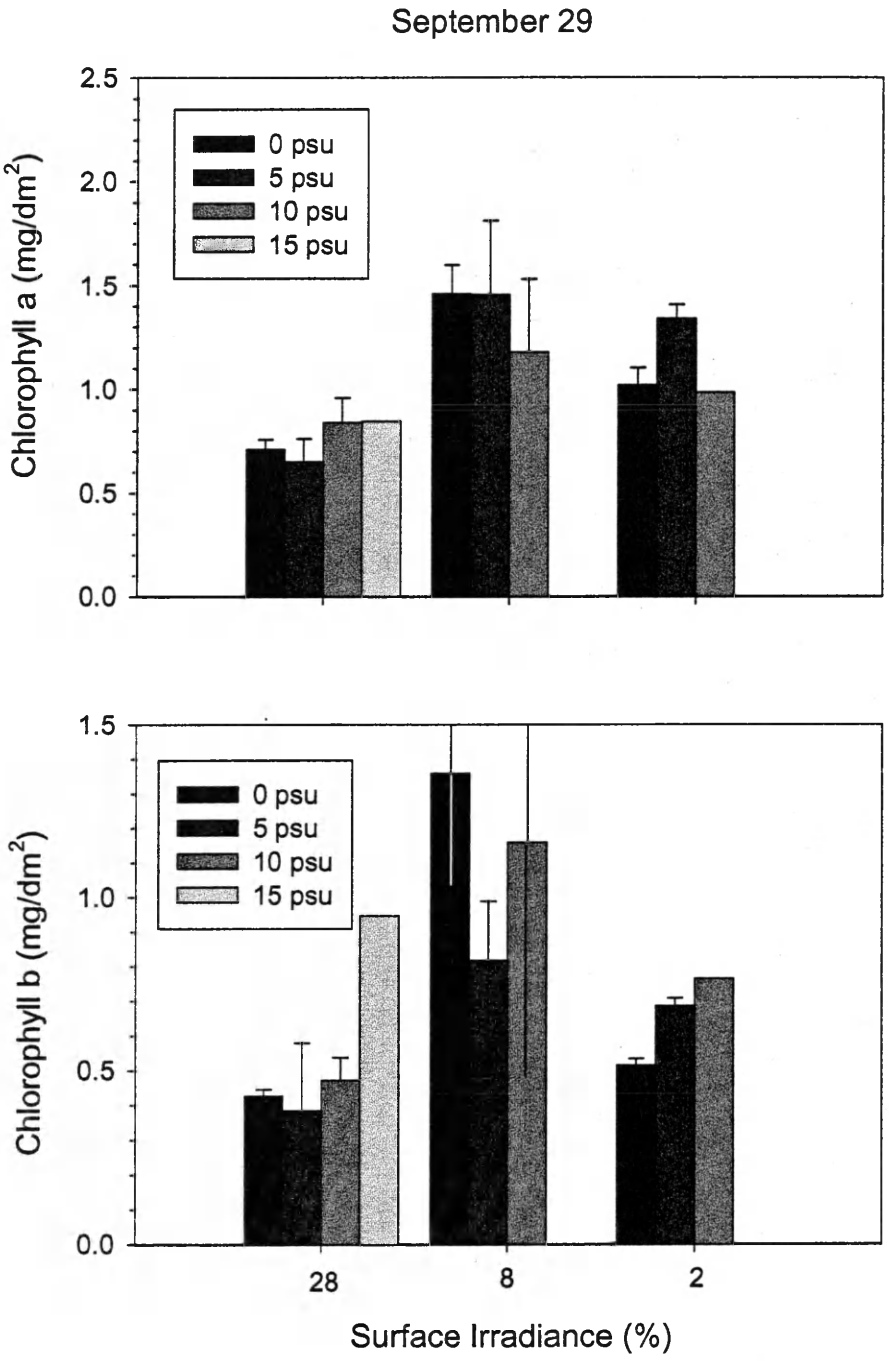
September 29 samples exhibited a trend of higher chlorophyll *a* and *b* concentrations in the 8% light treatment (Fig. 26). This pattern was driven primarily by a decrease in chlorophyll *a* concentration from July 6 and August 2 levels in the 2% light treatment and an increase in chlorophyll b concentration from earlier levels in the 8% light treatment.

PAM Fluorometry

Quantum Yield

Due to plant dieback throughout the season, there were too many missing quantum yield data (App. 5) to conduct repeated measures ANOVA, even when employing the general linear model for imbalanced design. There were still insufficient data when the last two dates were eliminated from analysis, at which time half the aquariums no longer had plants (The SAS® System for Windows, SAS Institute Inc.). Three-way ANOVA was conducted instead, with date as a factor, in addition to light and salinity, to quantify treatment effects. Due to small sample size on September 29 ($n=16$), this sampling date was eliminated from the effective and maximal quantum yield analyses.

Figure 26. Chlorophyll a and b concentrations (mg/dm^2) of leaf tissue harvested on September 29. One leaf was collected per aquarium (n=3). Sample size is less than three for some treatments due to plant dieback. Error bars represent standard error.


September 29
Chlorophyll a (mg/dm^2)
2.5
2.0
1.5
1.0
0.5
0.0
0 psu
5 psu
10 psu
15 psu
Chlorophyll b (mg/dm^2)
1.5
1.0
0.5
0.0
0 psu
5 psu
10 psu
15 psu
28
8
2
Surface Irradiance (%)

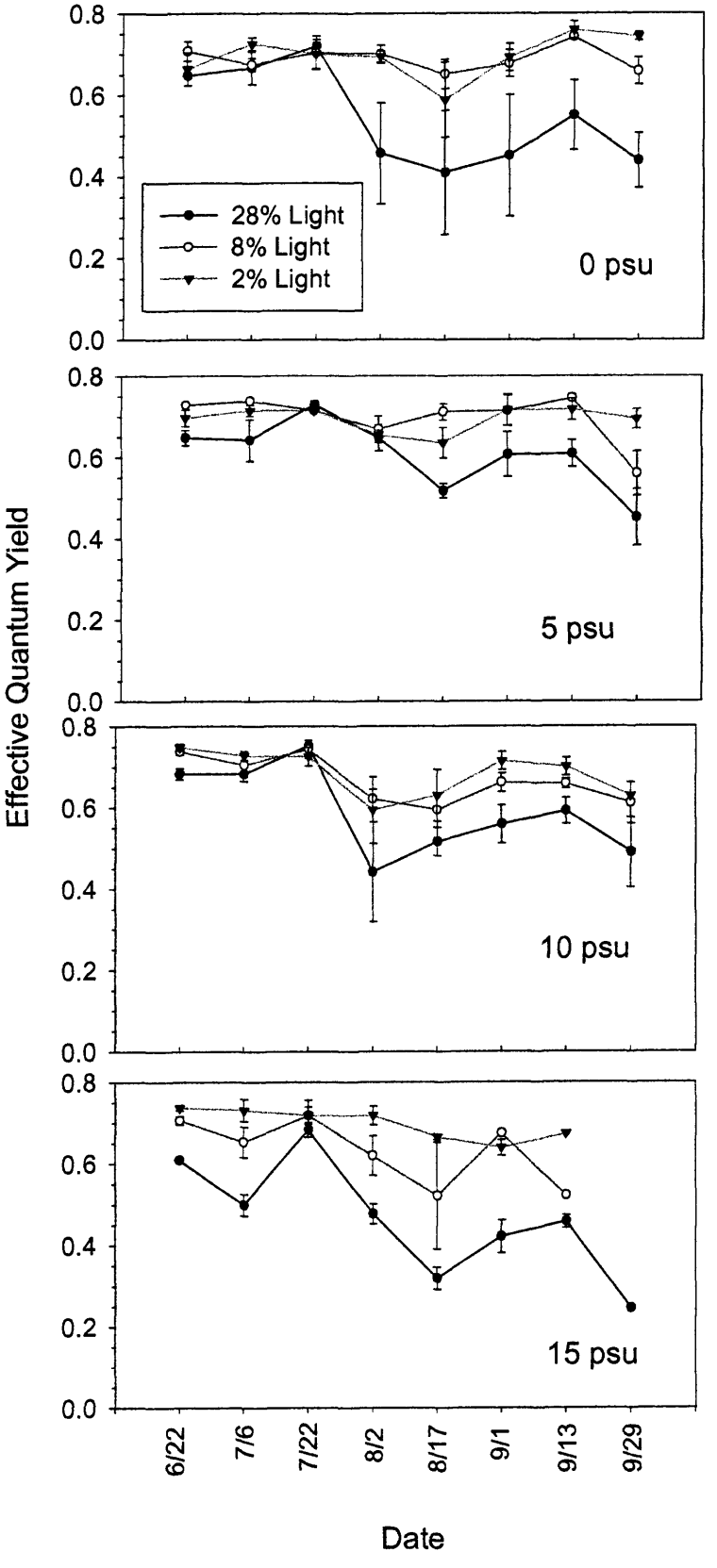
Effective Quantum Yield

Effective quantum yield ranged from 0.61 to 0.75 at the start of the sampling season. Light had a strong negative impact on effective quantum yield throughout the season ($p<0.0001$, Table 9, Fig. 27), and explained 23% of variance, more than salinity or any interactive effects. Plants in the 28% light treatment had the lowest effective quantum yield (SNK, $p<0.05$), 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 the 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 varied significantly over time ($p<0.0001$). 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 start of seasonal dieback. Effective quantum yields of the 8% and 2% light, 0 and 5 psu plants largely recovered by the end of August (0.56 to 0.74), while effective quantum yields of plants in the other treatments recovered only partially, if at all (minimum of 0.25). The effects of salinity and light interacted ($p=0.0128$), although no clear pattern emerged. The effects of light and date also interacted ($p=0.0019$), with light effects generally greatest in August and September. A large portion (33%) of variance was unexplained by single or interactive effects of light, salinity, or date.

Table 9. 3-way ANOVA for light and salinity effects on photosynthetic yield characteristics. n=3 for most light/salinity combinations. % Variance is the percent variation attributed to each parameter, as determined by partitioning of variance.

		DF	F	P	% Var.
Effective Quantum Yield	Salinity	3	6.835	**0.0002**	4.5
	Light	2	52.216	**<.0001**	22.9
	Date	6	14.711	**<.0001**	19.4
	Salinity * Light	6	2.807	**0.0128**	3.7
	Salinity * Date	18	1.208	0.2616	4.8
	Light * Date	12	2.778	**0.0019**	7.3
	Salinity * Light * Date	36	0.575	0.9731	4.5
	Error	150			32.9
Maximum Quantum Yield	Salinity	3	12.055	**<.0001**	7.6
	Light	2	53.363	**<.0001**	22.3
	Date	6	15.515	**<.0001**	19.5
	Salinity * Light	6	2.095	0.057	2.6
	Salinity * Date	18	1.305	0.1923	4.9
	Light level * Date	12	1.325	0.21	3.3
	Salinity * Light * Date	36	1.109	0.3259	8.4
	Error	150			31.4
Minimum Fluorescence	Salinity	3	6.03	**0.0007**	6.1
	Light	2	1.69	0.188	1.1
	Date	6	5.882	**<.0001**	11.9
	Salinity * Light	6	3.227	**0.0052**	6.5
	Salinity * Date	18	0.598	0.8966	3.6
	Light level * Date	12	1.994	**0.0284**	8.1
	Salinity * Light * Date	36	0.968	0.5274	11.8
	Error	150			50.7
Maximum Fluorescence	Salinity	3	2.589	0.0551	2.8
	Light	2	2.592	0.0782	1.9
	Date	6	7.215	**<.0001**	15.5
	Salinity * Light	6	3.006	**0.0084**	6.5
	Salinity * Date	18	0.623	0.8775	4.0
	Light level * Date	12	1.374	0.1843	5.9
	Salinity * Light * Date	36	0.741	0.8527	9.6
	Error	150			53.8

Figure 27. 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.

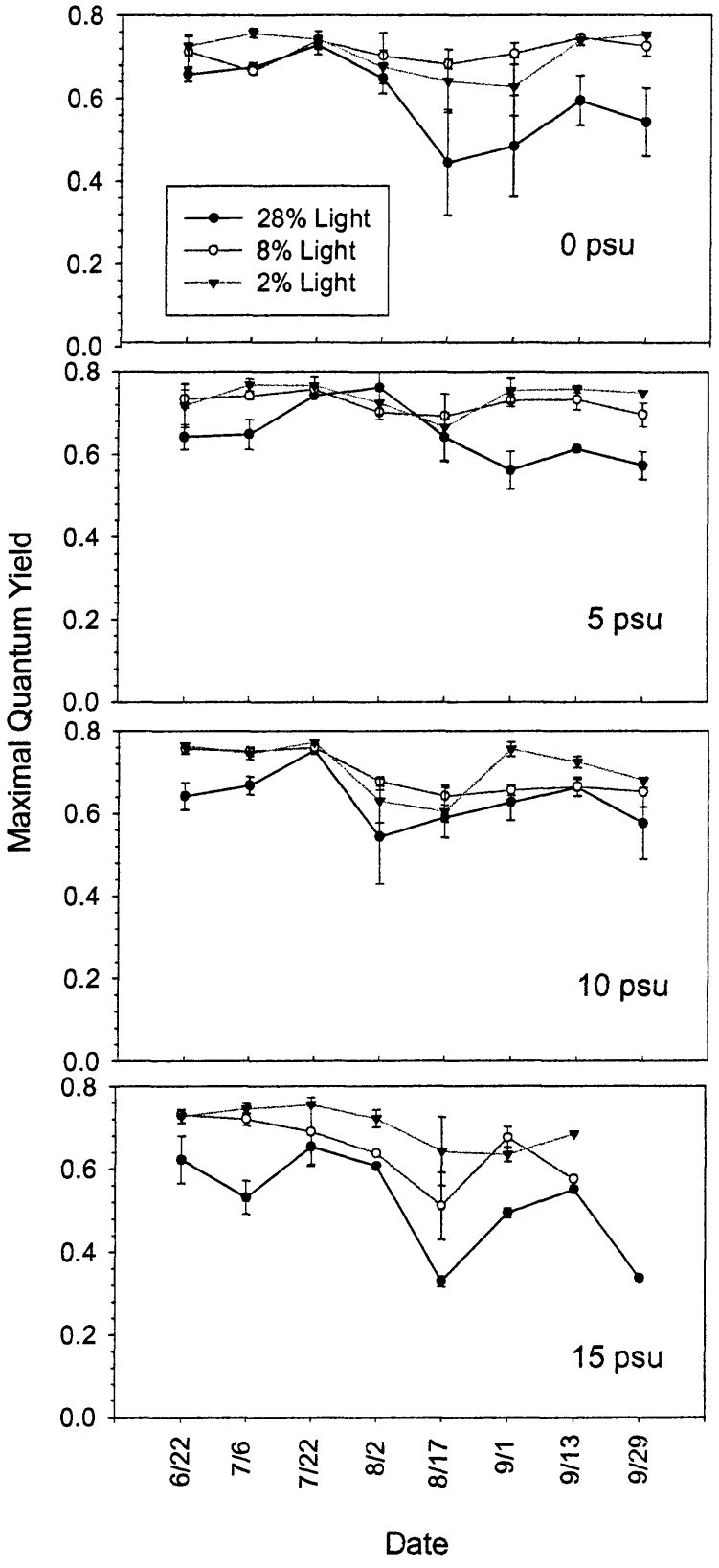

0.8
0.6
0.4
0.2
0.0
28% Light
8% Light
2% Light
0 psu
5 psu
10 psu
15 psu
Effective Quantum Yield
6/22
7/6
7/22
8/2
8/17
9/1
9/13
9/29
Date

Effective quantum yield of plants in the 28% light, 0 psu treatment was low by the Aug 2 sampling date (0.45, Fig. 27) due to very low effective quantum yields of plants in two of the three aquariums. Leaves of these plants had become very thin, pale, and brown by this time.

Maximal Quantum Yield

Initial maximal quantum yield ranged from 0.62 to 0.77. Similar to effective quantum yield, light had a strong negative effect on maximal quantum yield ($p<0.0001$, Table 9, Fig. 28) and explained 22% of variance. Maximal quantum yield of plants in the 28% light treatment was significantly lower than that of the other two light treatments (SNK, $p<0.05$). Maximal quantum yield in the 8% light treatment was generally lower than that in the 2% treatment, although not significantly so. Salinity also had a significant effect on maximal quantum yield ($p<0.0001$), although it only explained 8% of variance. The maximal quantum yield of plants in the 15 psu treatment (minimum of 0.33) was significantly lower than that of any of the other three treatments (SNK, $p<0.05$), particularly at high light. Maximal quantum yield was next lowest in the 0 psu treatment, followed by the 10 psu treatment. Maximal quantum yield was highest in the 5 psu treatment, although it was not significantly greater than in the 10 psu treatment. Maximal quantum yield varied significantly over time ($p<0.0001$). Date explained 19% of variance. Although the pattern was not as great as for effective quantum yield, maximal quantum yield tended to decrease in August. This parameter partially or fully recovered by the end of September for plants in the 2 and 8% light, 0 and 5 psu treatments. None of

Figure 28. Mean maximal quantum 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.


Maximal Quantum Yield
0.0
0.2
0.4
0.6
0.8
28% Light
8% Light
2% Light
0 psu
5 psu
10 psu
15 psu
6/22
7/6
7/22
8/2
8/17
9/1
9/13
9/29
Date

the interactions between factors was significant ($p>0.05$). A large portion of variance (33%) was unexplained by any of the factors or their interactions.

Minimum Fluorescence

Initial minimum fluorescence (F_0), determined from dark-adapted leaves, ranged from 175 to 483 mV. Salinity had a significant effect on F_0 ($p=0.0007$, Table 9, Fig. 29). It followed roughly the opposite pattern as that of effective and maximal quantum yield, with F_0 highest in the 15 psu treatment, followed by the 10, 0, and 5 psu treatments. F_0 in the 15 and 10 psu treatments was significantly greater than that in the 5 and 0 psu treatments (SNK, $p<0.05$). Salinity, however, accounted for only 6% of total variance. Light did not have a significant effect on F_0 ($p>0.05$). Date was significantly related to F_0 ($p>0.0001$) and accounted for the most variance of the three factors (12%). F_0 generally increased throughout the season. The effects of salinity and light ($p=0.0052$), as well as salinity, light, and date ($p=0.0284$), interacted. The majority of variance (51%), however, was unexplained by any of the three factors or their interactions.

Maximum Fluorescence

Initial maximum fluorescence (F_m) ranged from 641 to 1461 mV. Salinity did not have a significant effect on F_m ($p=0.0551$, Table 9, Fig. 30), although there was a trend of F_m in the 10 and 15 psu treatments exceeding F_m in the 0 and 5 psu treatments. Light did not significantly affect F_m ($p=0.0782$). Date did, however, have a highly significant

Figure 29. Mean minimum fluorescence for light and salinity treatment 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.

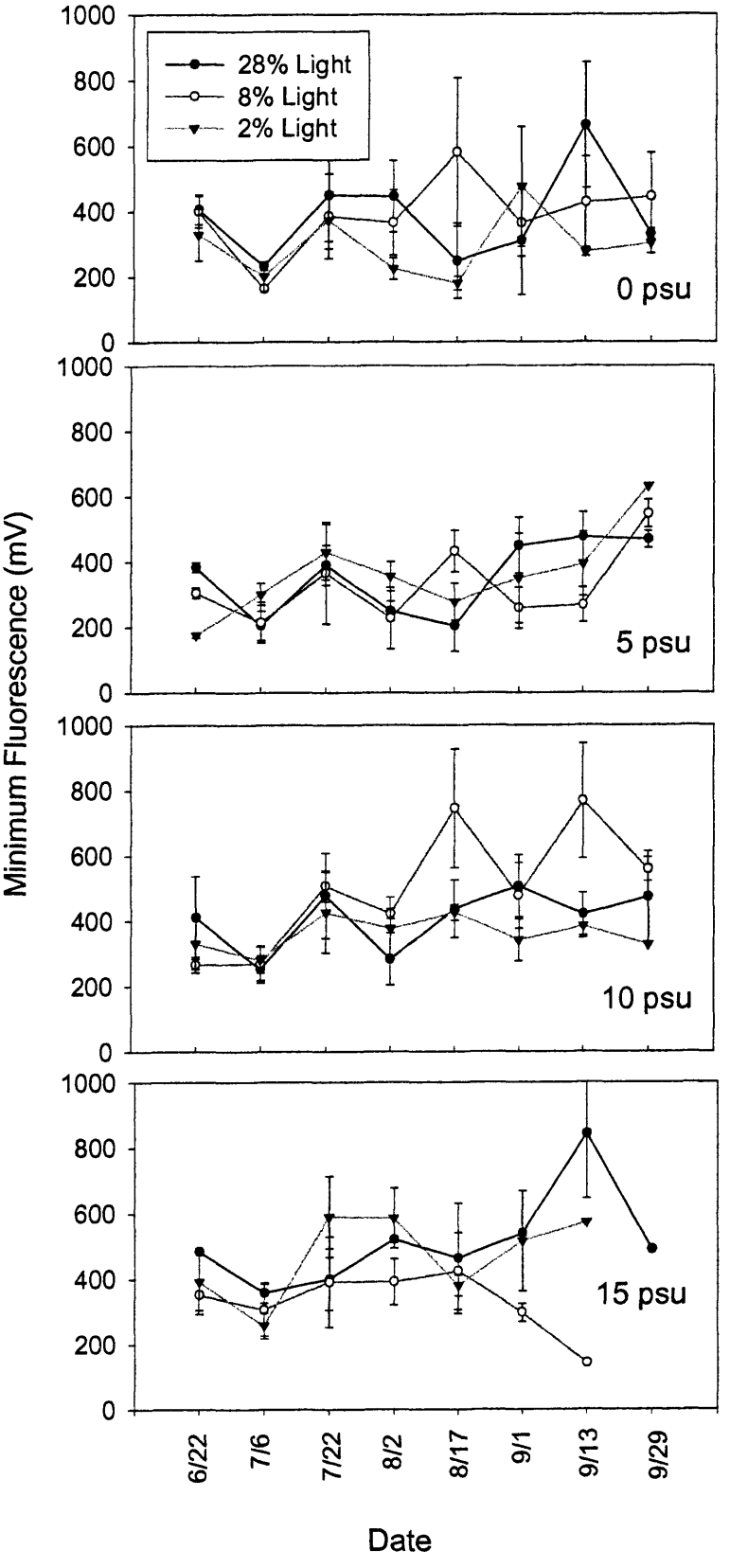
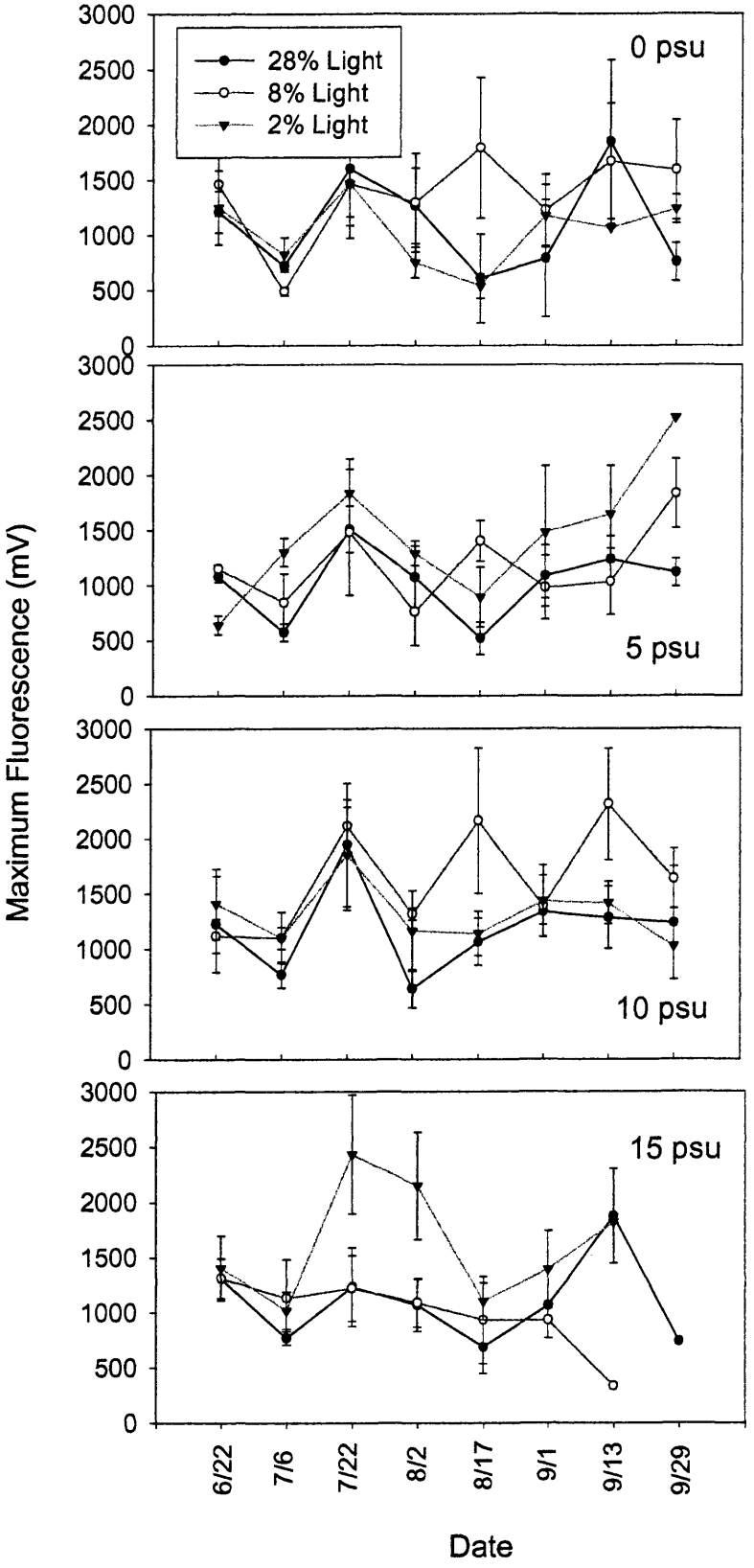

28% Light
8% Light
2% Light
0 psu
5 psu
10 psu
15 psu
Minimum Fluorescence (mV)
0
200
400
600
800
1000
6/22
7/6
7/22
8/2
8/17
9/1
9/13
9/29
Date

Figure 30. Mean maximum fluorescence 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.


28% Light
8% Light
2% Light
0 psu
5 psu
10 psu
15 psu
Maximum Fluorescence (mV)
3000
2500
2000
1500
1000
500
0
6/22
7/6
7/22
8/2
8/17
9/1
9/13
9/29
Date

effect ($p<0.0001$) and explained 16% of variance. The effects of salinity and light interacted ($p=0.0084$), but the others did not. As with F_0, the three treatment factors and their interactions accounted for little of the total variance, with 53% of variance unexplained.

Rapid Light Curves

Rapid light curves were generated on August 20 (Fig. 31), September 12 (Fig. 32), October 3, and Oct 20. One light curve was conducted per aquarium that had plants (App. 5). Because of limited sample size on October 3 ($n=18$) and October 20 ($n=13$), these dates were excluded from analysis.

Maximum ETR (determined here as the average of the three highest consecutive ETRs per light curve) is an indication of photosynthetic capacity. ETR_{max} was directly related to light availability on August 20 ($p=0.0017$, Table 10, Fig. 33). ETR_{max} in the 28% light treatment was significantly higher than that in the 8% or 2% light treatment (SNK, $p<0.05$, Fig. 33). 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 these factors.

The minimum saturating irradiance (I_k) varied by light treatment. I_k in the 28% light treatment was significantly greater than I_k in the 8 or 2% light treatments ($p=0.0024$, Table 10, Fig. 34). Light explained 39% of variance. Salinity did not have a consistent effect on I_k. A large portion (41%) of variance was unexplained by light or salinity or their interaction.

Figure 31. Rapid light curves on August 20. Sample size is 3 for most light/salinity combinations. It is less than 3 for several treatments due to insufficient leaf material. Error bars represent standard error.

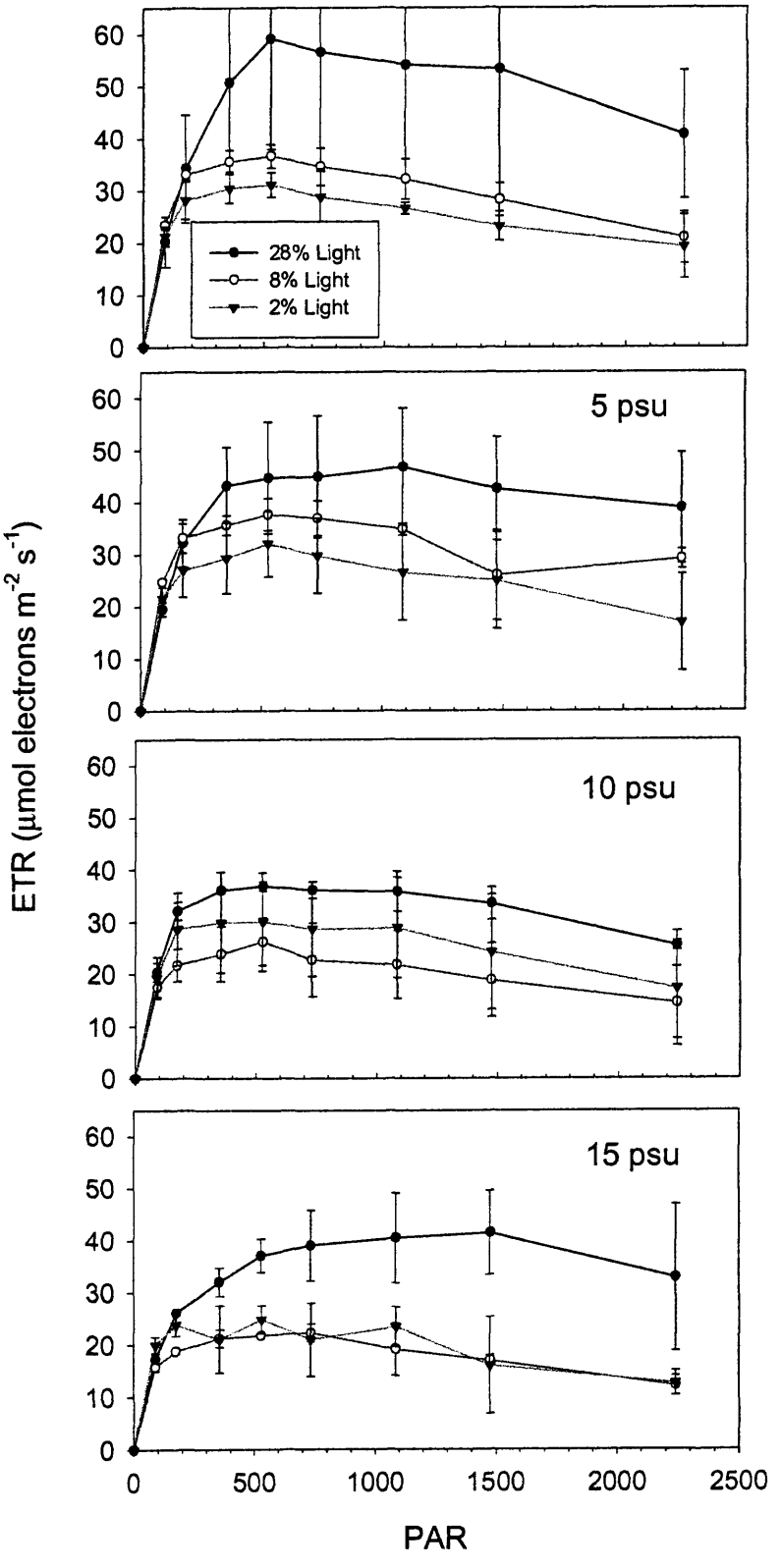

28% Light
8% Light
2% Light
5 psu
10 psu
15 psu
ETR (µmol electrons m-2 s-1)
PAR
0
10
20
30
40
50
60
0
500
1000
1500
2000
2500

Figure 32. Rapid light curves for light and salinity treatments on September 12. Sample size is 3 for most light/salinity combinations. It is less than 3 for several treatments due to insufficient leaf material. Error bars represent standard error.

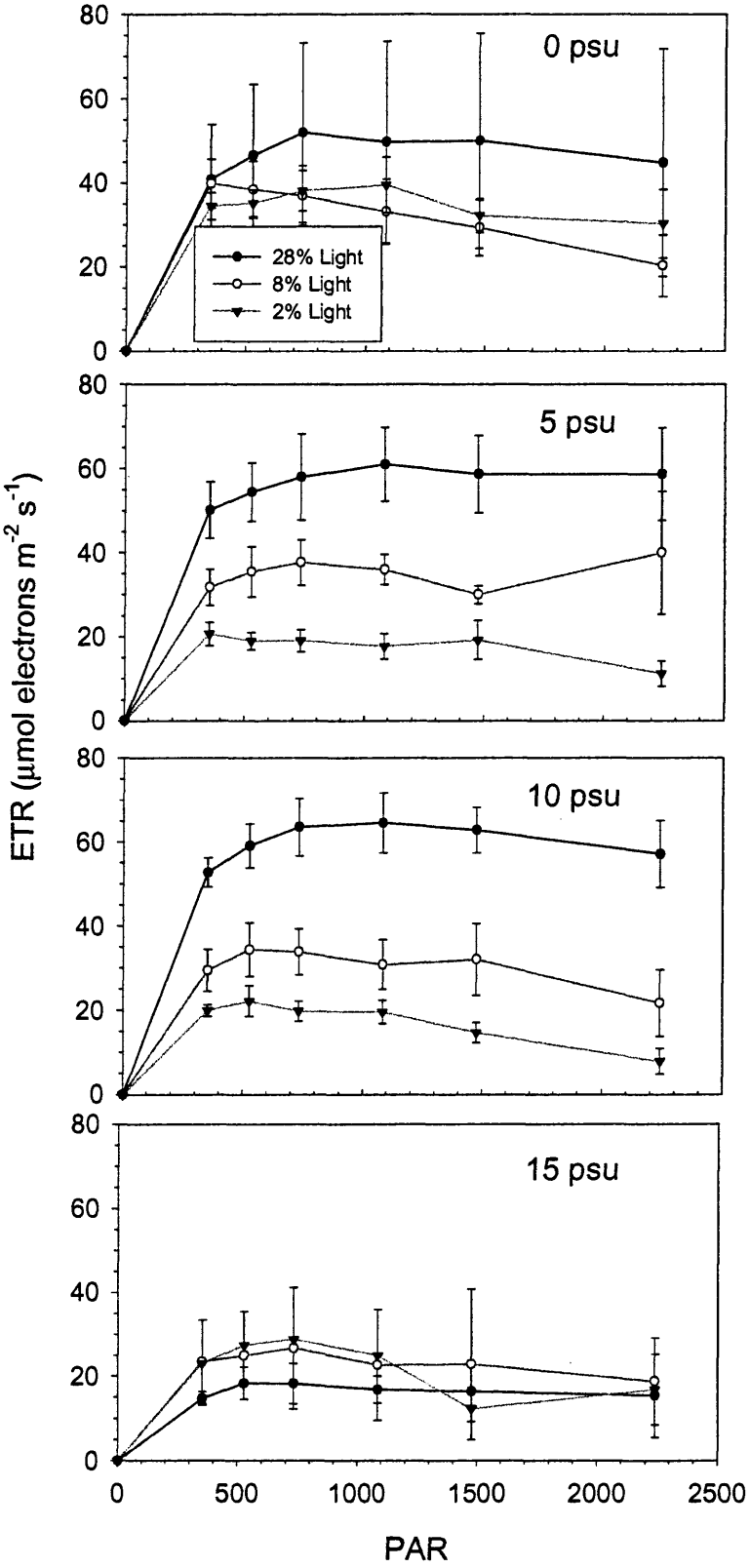

0 psu
5 psu
10 psu
15 psu
28% Light
8% Light
2% Light
ETR (µmol electrons m-2 s-1)
PAR
0
20
40
60
80
0
500
1000
1500
2000
2500

Table 10. 2-way ANOVA of light and salinity effects on ETRmax and I_k. n=3 for most light/salinity combinations. % Variance is the percent variation attributed to each parameter, as determined by partitioning of variance.

		DF	F	P	% Var.
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
ETR_{max}, September 12	Light	2	4.36	**0.0276**	22.2
	Salinity	3	1.413	0.2697	10.8
	Light * Salinity	6	1.235	0.3325	18.8
	Error	19			48.3

Figure 33. Mean maximum electron transport rate (ETR_{max}) for each treatment on August 20 and September 12. Maximum ETR is the average of the three highest points on a rapid light curve. 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 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, $p<0.05$).

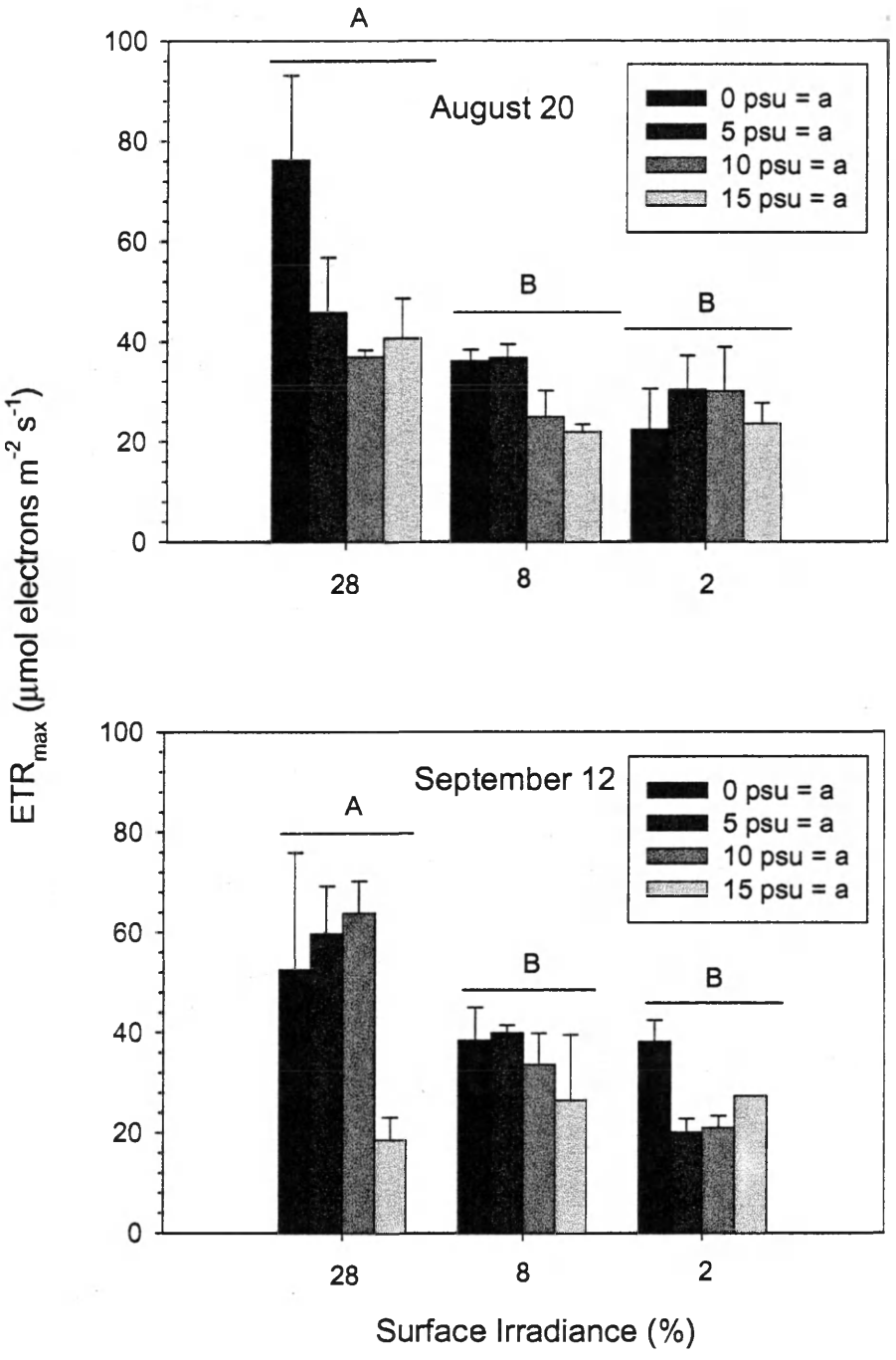
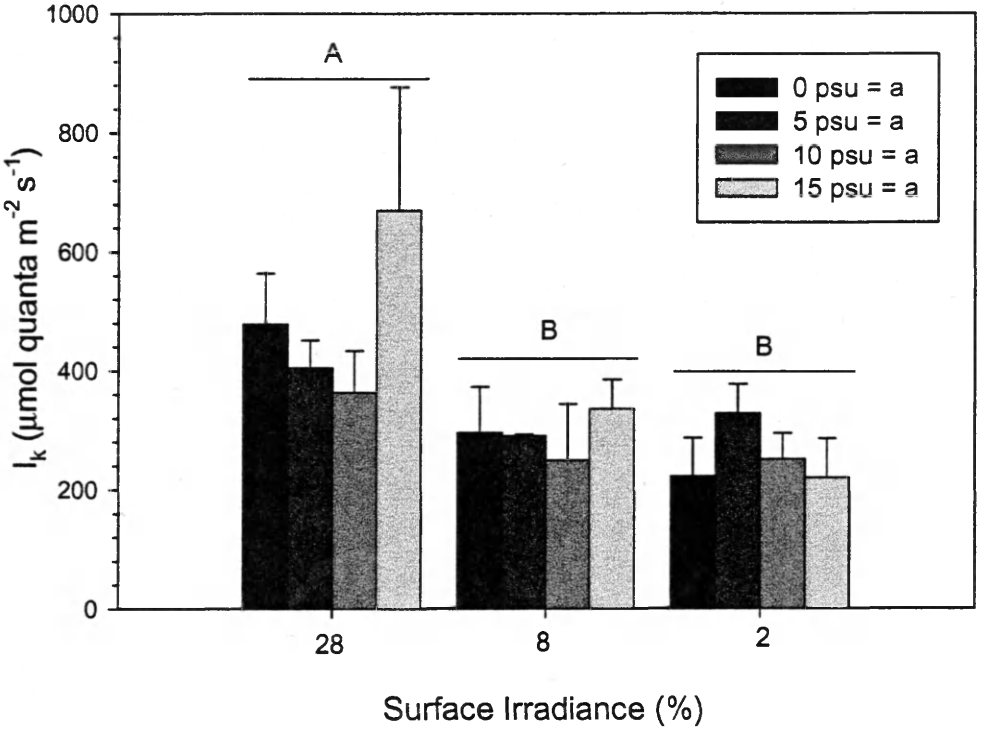

A
100
80
60
40
20
0
August 20
0 psu = a
5 psu = a
10 psu = a
15 psu = a
B
B
28
8
2
ETR$_{max}$ (µmol electrons m^{-2} s^{-1})
September 12
A
B
B
Surface Irradiance (%)

Figure 34. Mean minimum saturating irradiance (I_k) for each treatment on August 20. Sample size is 3 for most light/salinity combinations. It is less than 3 for several treatments due to insufficient leaf material. 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, $p<0.05$).


1000
800
600
400
200
0
I_k (µmol quanta m^{-2} s^{-1})
A
B
B
0 psu = a
5 psu = a
10 psu = a
15 psu = a
28
8
2
Surface Irradiance (%)

The initial slope of the rapid light curve (α) was not significantly affected by light or salinity ($p>0.05$).

Maximum ETR was also directly related to light on September 12 ($p=0.0276$, Table 10, Fig. 32). Again, ETR_{max} in the 28% light treatment was significantly higher than that in the 8% or 2% light treatment (SNK, $p<0.05$, Fig. 32). Salinity did not have a significant effect on ETR_{max} ($p=0.2697$), although in the 28% light treatment ETR_{max} was much lower at 15 psu than at the other three salinity levels. Light explained 22% of variance, and 48% was not explained by light, salinity, or their interaction.

Summary

The effects of light and salinity and their interaction on major plant characteristics are summarized in Table 11.

Table 11. Summary of the effects of light and salinity and their interaction on major plant characteristics. +, factor was positively related to characteristic; -, factor was negatively related; X, no relationship, or no interactive effect; I, light and salinity effects interacted; *, no significant relationship, but strong trend in direction indicated.

	Light	Salinity	Interaction
Morphology			
Length	-	-	X
Width	+	-	X
# Leaves	X	X	X
Production			
Vegetative Growth	+	-	I
Aboveground Biomass	+	-	I
Belowground Biomass	+	-	I
Leaf Area	+	-	I
Photosynthesis			
Chlorophyll	-	X	X
Quantum Yield	-	-	X
ETRmax	+	X	X
Reproduction			
Flowering	+*	-*	X
Tuber Production	+*	-*	X

DISCUSSION

Morphology and Production

Salinity and Light Stress

Although both light and salinity markedly influenced *V. americana* production, salinity exerted a stronger control for the ranges considered. This suggests that salinity may control distribution of *V. americana* more than light availability. Upper salinity tolerance was between 5 and 10 psu, while light tolerance was between 28 and 8% of surface irradiance. The relatively small effect of light is likely due in large part to 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 salinity stress.

Production (as defined by vegetative reproduction, above- and belowground biomass, and leaf area index) decreased dramatically at higher salinity levels (Fig. 13, 14, 15, 17, 19). For instance, at 28% light aboveground biomass, leaf area index, and maximum seasonal rosette production at 15 psu were 4%, 2%, and 17%, respectively, of that at 0 psu. Overall, production was generally greatest in the 0 psu treatment, moderate at 5 psu, and minimal at 10 and 15 psu.

The deleterious effects of elevated salinity on the production of *V. americana* are well known. Although measurements of the morphology and production of naturally existing populations of *V. americana* in different light and salinity regimes are generally

lacking, many mesocosm and transplanting studies have examined these characteristics. For instance, Staver (1986) found that aboveground biomass of transplanted adults at 6 psu was 10% of that at 0 psu, while belowground biomass at 6 psu was 12% of that at 0 psu. Haller et al. (1974) found death at 13.32 and 16.65 psu and survival but no net growth at 6.66 and 10.00 psu. In a transplanting field study Kraemer et al. (1999) observed smaller plants at two sites with salinities ranging seasonally from 7 to 20 psu, as compared to lower salinity sites, and death at another site with salinity exceeding 20 psu. In a field study of the Pamlico River estuary, Davis and Brinson (1976, in Twilley and Barko 1990) found *V. americana* present in 78.1% of quadrats sampled in a region of mean salinity 5.3 psu. No *V. americana* was present in a region with a mean salinity of 7.6 psu. These findings support the upper salinity tolerance of 5 to 10 psu determined the current study.

Contrary to these observed patterns, Twilley and Barko (1990) found no difference in total biomass or vegetative reproduction between plants transplanted for 5 weeks into salinities ranging from 0 to 12 psu. They attribute this high salinity tolerance to their methodology of gradually raising salinity levels, thus enabling some osmoregulatory mechanism to operate. It is also likely that these plants could flourish in higher salinities in the short term only, particularly because these adult plants likely had stored C reserves. The results of their study (i.e., salinity tolerance greater than 12 psu), then, are more applicable to short-term salinity changes on established populations, whereas the results of the present study are more applicable to recruitment, colonization, and longer-term survival.

Reduced irradiance also stunted production. For example, in the 0 psu treatment aboveground biomass, leaf area index, and maximum seasonal rosette density at 2% light were 9%, 21%, and 17%, respectively, of that at 28% light. There was generally a trend of lower production in the 2% light treatment than in the 8% light treatment, but there was no significant difference between 2 and 8% light treatments for any of these characteristics. The reduction in production at lower light levels indicates that light was limiting at the 2 and 8% levels.

Reduced irradiance has been found to decrease *V. americana* production in several other studies. Biomass and rosette production decreased with decreasing light at 1500, 600, and 100 μmol m^{-2} s^{-1} (approximately equivalent to 75, 30, and 5% light) (Barko et al. 1982) and 550 and 125 μmol m^{-2} s^{-1} (50% and 8% light) (Barko et al. 1991). Blanch et al. (1998) found a decrease in total number of leaves and leaf area index with decreasing light over a wide range of light levels for plants transplanted to a pond. Similarly, biomass and rosette production increased dramatically for transplants in embayments when the areas were artificially illuminated (Carter et al. 1996). Twilley and Barko (1990), however, again found no differences between biomass or rosette production at 8% versus 50% light, likely due to the short duration of the study and to the relatively high irradiance of even the lower light level.

Based on the results of the present study alone, it is unknown whether the 28% light level was saturating. However, the results of several other studies suggest that this light level was saturating. For instance, aboveground biomass increased approximately 43% from 8 to 28% light (at 0 psu) in the present study, whereas in the study by Barko et al. (1991) it increased to a similar degree (44 to 53%, depending on nutrient and DIC

levels) from 8 to the higher level of 50% light. In the study by Barko et al. (1982) aboveground biomass was not consistently higher at 75 versus 30% light. In fact, it was lower at 75% light when grown at 24 and 28°C and only increased with increasing light at 32°C. Rosette density was higher at 75 versus 30% light only at 28 and 32°C. The maximum temperature achieved in the present study was 28°C; thus, it is unlikely that growth here would have increased substantially at higher light levels.

Light and salinity levels strongly influenced not only plant production but also morphology, which in turn altered the plants' ability to capture available light. As with production, salinity impacted morphology (as defined by length, width, and leaf production per rosette) to a larger degree than did light. Leaves of high salinity plants were shorter and narrower than those in lower salinity (Fig. 7, 8, 10, 11) and thus had less leaf area over which to capture light (Fig. 21, 22). For instance, at 28% light surface area per leaf in the 15 psu treatment was only 13% of that in the 0 psu treatment. Staver (1986) also found a lower surface area per leaf at higher salinity: leaf area at 6 psu was 28% of that at 0 psu. The reduced ability to capture light due to less leaf area was compounded by the shorter canopy height of the higher salinity treatments. Similar to the pattern for production, leaf length and width were greatest at 0 psu, moderate at 5 psu, and minimal at 10 and 15 psu.

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, Barko et al. 1991), as well as other SAV species (Stross 1979, Barko and Smart 1981, Barko et al. 1982). The elongation capacity of *V.*

americana may confer a competitive advantage over certain other freshwater macrophyte species with more limited elongation ability, such as *Potamogeton nodosus* (Barko et al. 1982). However, it is not a canopy-former like *Hydrilla verticillata* or *Myriophyllum spicatum* and so generally cannot successfully compete with these species (Haller and Sutton 1975, Titus and Adams 1979).

Although leaf lengths in the 2 and 8% light treatments were not statistically different, length under 8% light was longer at mid-season than that 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. This reduced leaf elongation effectively increased the optical depth, or distance between the water surface and leaf surface, thereby compounding the effect of turbidity, especially in shallow water areas.

Leaf width increased with increasing light, particularly mid-season (Fig. 10, 11). The contrary responses of length and width to light resulted in approximately the same surface area per leaf (measured as length * width) under reduced light, but a greater proportion of leaf surface area near the water's surface. Barko et al. (1982) also found no difference in surface area per leaf between 5 and 30% light treatments. However, surface area per leaf at 75% light was reduced. Lower light plants in the present study appeared to shift resources away from vegetative reproduction, biomass, and total leaf area to elongation. Thus, light stress, unlike salinity stress, elicited a response that maximized light capture per unit of production. Therefore, the phenotypic plasticity in response to light, in contrast to salinity, may be considered adaptive, as it appears to facilitate *V.*

americana survival in a variety of environments. In addition, it may contribute to the species' wider tolerance to light, relative to salinity.

Unlike leaf length or width, leaf production per rosette was not affected by light or salinity (Fig. 12), indicating that it was neither negatively impacted by higher salinity, nor used by low light plants as a means to increase light capture. Because this characteristic is not generally studied, it is unknown whether its lack of plasticity to stressors is typical. It appears to be primarily under seasonal control, with a mid-season peak of 6.5 leaves per rosette and an end-of-season minimum of 2.9 leaves per rosette.

The impact of light on aboveground biomass was primarily through new rosette formation, as aboveground biomass per rosette was not strongly affected by light availability (Fig. 16). The absence of strong light effects on aboveground biomass per rosette, coupled with the lack of effects on surface area per leaf (Fig. 22) and leaf production per rosette (Fig. 12) suggests that leaf thickness also was not substantially affected by light. This conclusion is supported by the lack of relationship between light and the ratio of aboveground biomass to leaf area (Fig. 20). However, rosette size (leaf surface area and biomass per rosette) may have been constrained by nutrients or pot size in this study.

Contrary to these results, Blanch et al. (1998) found leaf thickness to decrease with decreasing light. The authors suggested that the resulting increase in surface area to volume ratio may have allowed lower light plants to intercept more light. However, without measurements of chlorophyll concentrations, the accuracy of this statement is unknown.

Although length * width per leaf (Fig. 22) and leaf area per rosette (Fig. 21) both increased with decreasing salinity, aboveground biomass per rosette only displayed a nonsignificant trend of higher values at lower salinity. The absence of a significant relationship between aboveground biomass per rosette and salinity may be partially due to a counteracting trend of leaf thickness increasing with increasing salinity (Fig. 20). Thicker leaves minimize the surface area to volume ratio, and, thus, ion exchange, and so may be an adaptation to salinity stress.

Belowground biomass was the only morphological or production characteristic more controlled by light than by salinity (Fig. 17). As with aboveground biomass, salinity induced changes to belowground biomass primarily through rosette production. Light, however, induced changes through both rosette production and through increased growth per rosette (Fig. 18). The decreasing belowground biomass per rosette with decreasing light suggests that low light plants minimized belowground material in order to maximize photosynthetic material. However, the pattern of decreasing belowground biomass per rosette with decreasing light was primarily driven by the 28% light, 5 psu treatment, and the ratio of aboveground to total biomass was not significantly different across light or salinity levels. Barko et al. (1982, 1991) found no relationship between light and the ratio of aboveground to total biomass. Twilley and Barko, however, found this ratio to increase with increasing salinity, especially when light was not limiting. The results of the present study are also in conflict with those of Blanch et al. (1998), who found that under low light *V. americana* maintained belowground material in preference to leaves, thus providing a reserve for rapid leaf production when light availability increases. These

conflicting results suggest that factors other than light and salinity may be important in determining relative above- and belowground appropriations.

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. There were strong seasonal patterns for all growth characteristics measured repetitively, including vegetative reproduction, leaf elongation, leaf production per rosette, and width. Growth generally increased over time until it peaked in mid-July through early August, after which it decreased, rapidly in the more prolific treatments, and gradually in the other treatments. Peak growth corresponded approximately with the time of maximum seasonal temperature and occurred several weeks after the time of maximum seasonal irradiance. Elongation and colonization were most rapid May through July, when the greatest treatment effects were observed. Thus, long-term survival may be particularly sensitive to stress at this time of year. Further, restoration may be more successful when planting occurs in these early months, as vegetative spreading will be greatest then.

The seasonal pattern of vegetative reproduction was very rapid colonization (as high as 17% d^{-1}) during the first half of the year, followed by net loss. The linear, instead of exponential, growth during the first part of the season was likely the result of the linear means of spreading of the species, whereby a rosette sends out one stolon to produce one new rosette (personal observation). The decrease in rosette density in the second half of the season was the result of a greater rate of leaf senescence and uprooting than of rosette production. The treatment effects of rate of increase indicate that low salinity, high light

treatments colonize faster, which may confer the added advantage of early modification of the local environment toward conditions more favorable for *V. americana* survival.

Leaf length, like vegetative reproduction, also peaked in July. 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 tuber formation. Any stress preventing plants from achieving maximum height during this time may retard growth or tuber formation later in the season. 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, and thus conferring an additional benefit to plants in these treatments.

Water temperature likely limited relative growth rates throughout the growing season. *V. americana* generally sprouts from tubers when temperatures reach 10 to 14°C (Korschgen and Green 1988). At the start of the experiment tubers were transferred from water 4°C to water at least 20°C. Tubers began to sprout within the first week of planting. This rapid temperature change, while not typical of field conditions, did not appear to inflict any lasting damage, as mid-season morphology and production were in the range reported for other studies (Barko et al. 1982, Staver 1986, Barko et al. 1991). Temperature throughout the season mirrored that of the adjacent York River. *V. americana* growth typically is not pronounced until temperatures reach 19 to 20°C (Barko et al. 1982, Korschgen and Green, 1988) and is most vigorous at temperatures of 28 to 36°C (Barko et al. 1982, Korschgen and Green 1988). Temperature in the present study was 23°C at midday at the first sampling period (June 10) and remained in the range of 25 to 28°C throughout July and August. Thus, temperature throughout the summer was in the range conducive to moderate, but not maximum growth. Temperature

did not drop below 20°C until the end of September, whence it declined steadily throughout the fall. Growth measurements, particularly leaf elongation and vegetative reproduction, roughly followed the peak and downward decline of the temperature pattern.

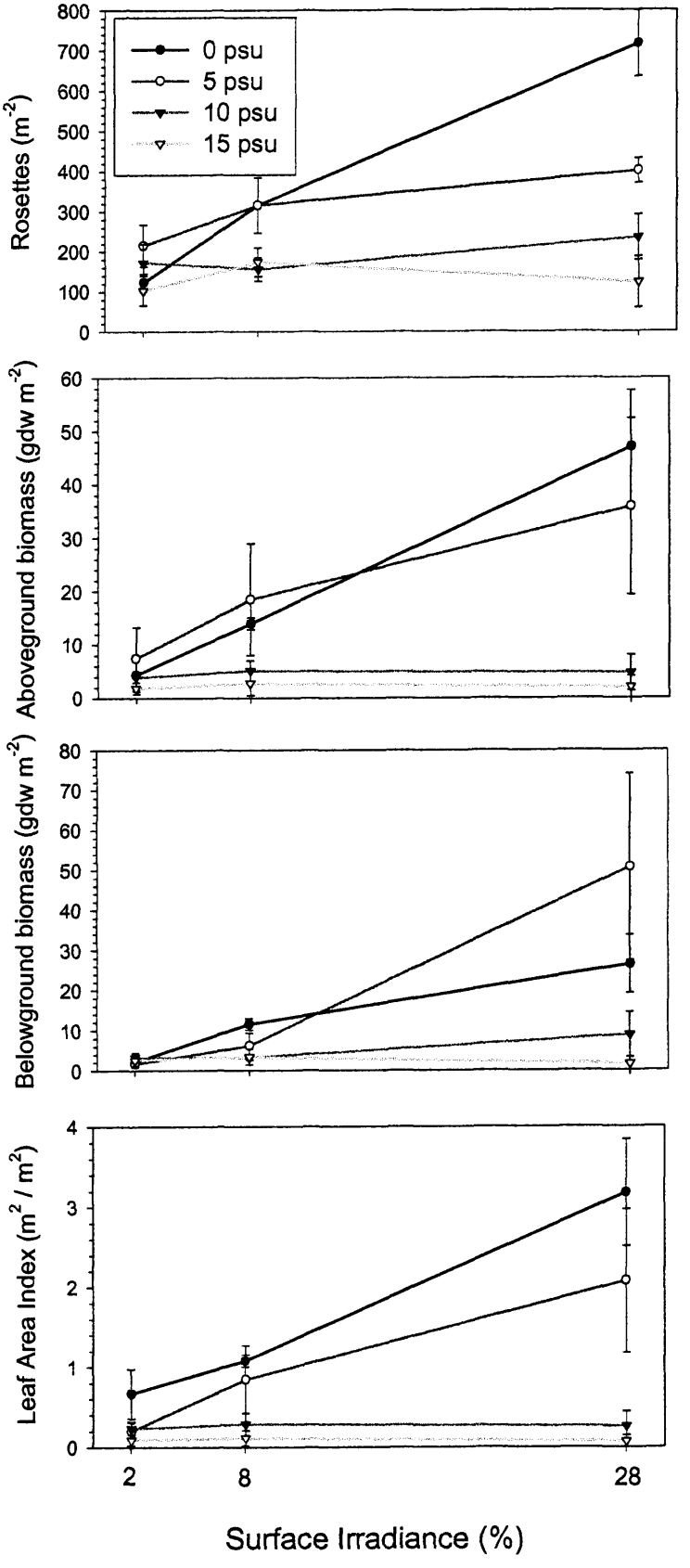
Total daily irradiance and maximum irradiance also contributed to seasonal growth patterns. Leaf elongation and vegetative reproduction generally followed seasonal light changes, in additional to temperature patterns, and light and temperature effects on most growth characteristics likely paralleled each other. However, for leaf elongation the effects of irradiance may have been partially counteracted by those of temperature. Partitioning of variance reveals that light had a very small effect on elongation relative to salinity (2.6 and 5.2 versus 64.3 and 73.4%) (Table 6, 7; Fig. 8, 9). Temperature and irradiance were both highest in the summer, although irradiance peaked several weeks before temperature. However, the factors elicit opposing responses on leaf elongation. Temperature can have a very strong positive influence on elongation. Barko et al. (1982) determined its influence to be stronger than that of light for the ranges considered (12 to 32°C and 8 to 75% light). The greater light and salinity effects mid-season on leaf length, as well as rosette production (Fig. 7, 13), may have been the result of temperature limitation early and late season. Barko et al. (1982) also found that *V. americana* was most responsive to light at optimal temperature. Because light and temperature effects were not tested in the present experiment, it is unknown which had a stronger effect. However, maximum leaf length occurred sometime between maximum seasonal temperature and irradiance, suggesting the importance of both of these factors.

Interactive Effects

The effects of light and salinity interacted for all production measures (vegetative reproduction, above- and belowground biomass, and leaf area) (Fig. 35). 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. Thus, salinity tolerance is contingent on light availability in the 0 to 5 psu range. However, production at 10 and 15 psu did not respond to additional 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 with significant light and salinity effects: length and width. Light and salinity effects for these characteristics were instead additive, in large part due to better performance for leaf length and width relative to production measures at higher salinity and lower light levels. This difference between responses of production and morphological measures may be due to the effects of light and salinity stress on a morphological characteristic compounding effects on another morphological characteristic or on photosynthesis, thus disproportionately decreasing production. For instance, it has been determined for other SAV species that environmental stressors can increase light requirements through increasing P_{max}, I_c, and/or I_k (Kerr and Strother 1985, Perez and Romero 1992, Goodman et al. 1995, Masini et al. 1995). Salinity can also

Figure 35. Interaction plots for maximum seasonal rosette density (m^{-2}), and above- and belowground biomass (gdw m^{-2}), and leaf area index at mid-season harvest.


0 psu
5 psu
10 psu
15 psu
Rosettes (m-2)
Aboveground biomass (gdw m-2)
Belowground biomass (gdw m-2)
Leaf Area Index (m2 / m2)
2
8
28
Surface Irradiance (%)

decrease maximal and effective quantum yield and damage the photosystem (Ralph 1998a , Ralph 1999c). Thus, salinity stress induces photosystem changes that may result in decreased biomass under low light. Effects of multiple stressors (such as light and salinity) on photosynthetic functioning alone (including maximal and effective quantum yield, I_c, P_{max}, and α) appear to be additive (Goodman et al. 1995, Ralph 1999c). However, salinity and light stress also elicit morphological changes, primarily reduced leaf area index (caused by light and salinity stress) and reduced canopy height (caused by salinity stress only), that result in reduced light capture, compounding photosystem stress and, consequently, further reducing production.

Plant production responded synergistically to lower stresses across the range of light and salinity levels (Fig. 35). That is, production was much higher with higher light and lower salinities than would be predicted if the effects of the stressors were additive. Although generally not explicitly studied in SAV, this response is common in terrestrial plants (Chapin et al. 1987) and may be explained by the compounding effects of stress on morphology and photosynthesis, as described above.

Plants also appeared to be more tolerant of mid-range salinity when under higher irradiance. Kraemer et al. (1999) observed this same phenomenon of increased salinity tolerance at higher light levels and proposed that greater light may moderate salinity stress by providing additional energy for osmotic regulation. However, the greater salinity tolerance found in that study (upwards of 15 psu) compared to the present study (5 to 10 psu) cannot be explained by greater light availability in the former, as the highest light level in the Kraemer et al. study was approximately the same as the highest level in

the present study. Thus, discrepancy between the salinity tolerances found in the two studies is more likely due to the shorter duration of the Kraemer et al. study.

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, Ralph 1999b) and can result in greater light capture. Chlorophyll has rarely been measured in *V. americana* specifically. Twilley and Barko (1990) found a trend of increasing chlorophyll *a* from 50 to 8% light but no signficant difference. These results are consistent with the general lack of differences between treatments in that study. Their measurement of chlorophyll in mg/g afdm precludes direct comparison to measurements in the present study. Even as production changed seasonally, chlorophyll concentrations remained approximately the same from July 7 to September 29 sampling dates, suggesting that seasonal light changes are not strong enough to cause discernible changes in chlorophyll production.

Chlorophyll *a* and *b* concentrations on August 2 and chlorophyll *b* concentrations on July 7 were not significantly different between the 8 and 28% light treatments, but concentrations in the 2% light treatment were signficantly 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 different. 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 these two 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. Similarly, Dunton (1996) found no change in chlorophyll concentrations in *Halodule wrightii* growing along a salinity gradient. However, chlorophyll concentrations have been found to increase with increasing salinity for *H. verticillata, Naja indica,* and *Najas gramenia* (Rout and Shaw 2001), *H. ovalis* (Ralph 1998a) and for many terrestrial plants, including tomatoes (Romero-Aranda et al. 2001), soybeans (Wang et al. 2001), and rice (Asch et al. 2000). A trend of increasing chlorophyll *a* concentration with increasing salinity, however, was noted in the present study on July 6. The reduced canopy height and thus reduced light availability in the higher salinity treatments may have stimulated increased chlorophyll production. Alternatively, reduced chlorophyll in the low salinity, vigorous growth treatments may have been the result of nutrient limitation. Water column DIN and PO_4^{-3} concentrations were lowest in these treatments (Fig. 4, 5). NH_4^+ porewater concentrations were not related light or salinity treatments. However, it is possible that NO_3^{-2} or PO_4^{-3}, which were not measured, were limiting growth in the high light, low salinity treatments.

The chlorophyll *a/b* ratio was observed to increase with decreasing light on July 6, but was not related to light on August 2. In contrast, many other studies have found the

ratio to decrease with decreasing light (Dring 1986, Hale and Orcutt 1987, Ralph 1999b). Although Ralph (1999b) found higher chlorophyll *a/b* under high light (200, 400 μmol m^{-2} s^{-1}) compared to controls (120 μmol m^{-2} s^{-1}) for *H. ovalis*, he found no difference in *a/b* between controls and low light treatments (25, 50, and 75 μmol m^{-2} s^{-1}). Dunton (1996) also found no change in chlorophyll *a/b* with light or salinity for *H. wrightii*. Further complicating conclusions about the relationship between *a/b* and light availability, Ralph (1999c) found a decrease in chlorophyll *a/b* under high light for *H. ovalis*. Differences in results may partly be attributed to the different experimental light levels. Chlorophyll *b* tends to be degraded faster than chlorophyll *a* at high light levels (Ralph 1999b). Light intensity in the 28% light treatment here may not have been great enough to cause chlorophyll *b* degradation. Further, changes in chlorophyll *a/b* with light availability are often due to changes in attenuation of certain wavelengths at depth (Kirk 1994). Because neutral density shade cloth was used in this study, the relative attenuation of different wavelenghts was the same in the three light treatments, perhaps reducing the benefits of the chlorophyll *b* accessory pigment.

Other adaptive responses to low light were increases in both photosynthetic capacity (effective quantum yield) and efficiency (maximal quantum yield). Photosynthetic response to salinity stress, however, was not adaptive, as high salinity decreased both photosynthetic capacity and efficiency. This salinity-induced stress to the photosynthetic apparatus may have been the primary cause of stunted growth at high salinities. However, like chlorophyll concentrations, and unlike growth measurements, light had a stronger effect on quantum yield than salinity did.

Effective quantum yield and maximal quantum yield decreased with increasing light availability (Fig. 27, 28). This pattern has been demonstrated for seagrass species, as well. Ralph (1999b) found effective and maximal quantum yields to increase from control to low light conditions for *Halophila ovalis*. This may be an adaptive mechanism to sustain adequate photosynthesis. Similarly, Ralph and Burchett (1995) found quantum yield to decrease for *H. ovalis* from control to high light conditions. This decrease in efficiency under high light, or photoinhibition, appears to be due to a combination of photoprotection and photodamage (Ralph and Burchett 1995). In the present study the difference in quantum yield between light levels is 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 ETR_{max}, which decreases with photoinhibition (White and Critchley 1999), was highest in the 28% light treatment (Fig. 33). The apparent increase in efficiency under low light, along with greater leaf elongation and chlorophyll concentrations, may facilitate plant growth under low light conditions.

V. americana has a remarkable ability to physiologically adapt to low light conditions compared to other SAV species (Meyer et al. 1943, Titus and Adams 1979, Korschgen and Green 1988). Although the exact mechanism behind this adaptability is unknown, it is likely to involve an increase in photochemical efficiency, as suggested in this study. Because PAM fluorometry studies have not been conducted on other freshwater SAV species, it is unknown whether they are capable of the same degree of plasticity in photochemical efficiency. Comparisons of PAM fluorometry measurements of seagrass species to those of this study are also difficult, as most seagrass PAM

fluorometry studies examined only the effects of short-term (less than one week) stress. Future studies should compare the long-term photosynthetic response of *V. americana* to that of other SAV species to determine whether the photochemical efficiency of *V. americana* is relatively more responsive to changes in light.

Although light was the primary factor controlling photochemical efficiency (as determined by partitioning of variance, Table 9), 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 stress (Havaux 1992, Schreiber et al. 1994, Ralph 1998a, Ralph and Burchett 1998a, Ralph 1999c, 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 1998a). PAM fluorometry studies on freshwater SAV are rare, but hypo- and hypersaline stress in the seagrasses *H. ovalis* (Ralph 1998a, 1999c) and *Zostera marina* (Kamermans et al. 1999) has also been found to decrease quantum yield. The quantum yield data in the present study indicate that at higher salinities *V. americana* may not be as efficient at fixing carbon. This decrease in photochemical efficiency could be one, if not the primary, mechanism leading to reduced growth at elevated salinity.

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 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. The August 2 chlorophyll data also reveal lower chlorophyll *a* and *b* concentrations at 0 psu than at 5 or 10 psu in the 28% light treatment, indicating chlorosis. Thus,

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. Ralph (1999c) found that elevated temperature had a more negative effect on photochemical efficiency than did light for the levels examined. High temperatures appear to primarily 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.

The effects of light and salinity stress were generally additive. Few PAM fluorometry studies have examined the effects of multiple stressors. However, Ralph (1999c) also found the effects of multiple stressors on *H. ovalis* yield to be additive. Although quantum yield measurements were correlated with physical signs of salinity stress, they did not predict physical decline: that is, yield did not decrease prior to plant die-back. Vegetative reproduction, 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), for instance, 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 0_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.

Maximal quantum yield in this study ranged from 0.642 to 0.768. These values were lower than those of terrestrial plants under no stress (0.832 ± 0.004, Bjorkman and Demmig 1987) but similar to those found for seagrass (0.73) (Ralph and Burchett 1995). Ralph and Burchett (1995) suggested that the lower yield in seagrass compared to terrestrial plants indicates that their ecological growth conditions are physiologically sub-optimal, which may also be the case in the present study.

The pattern of F_0 response argues against damage to the photosystem as plant growth declined. A decrease in F_0 is generally a sign of membrane dysfunction of the chloroplast and thylakoid (Ralph and Burchett 1995, Ralph 1999c). Here, F_0 tended to increase over time (Fig. 29), even as plants were starting to die. It also tended to increase with increasing salinity. These results suggest photoprotection, not photodamage, over time and with increasing salinity (Ralph and Burchett 1995). However, F_0 data were highly variable, with trends difficult to discern, and literature on interpretation of F_0 patterns is scarce, thus obviating clear conclusions.

There was considerable variability of PAM fluorometry data within treatments in the present study compared to other studies, as evidenced by high standard error and a

large portion of variance unattributable to the three factors (light, salinity, and date). This variability may be due to the use of different leaves for each sampling period and use of only one leaf per aquarium (three per treatment) per sampling date. Short-term studies generally use the same leaves throughout the experiment and use more replicates. Yield measurements may vary considerably by leaf within a treatment. Due to the relatively short life span of a leaf (such as two months for *Z. marina*, Sand-Jensen and Borum 1983), it is not generally possible to use the same leaves throughout a long-term study of most SAV species. However, increasing sample size is advisable to better detect differences between treatments and temporal patterns. It may be particularly useful in discerning any treatment differences in F_0 and F_m, which were generally not found in this study.

Plants displayed photoadaptation, as evidenced by a higher maximum rate of electron transfer (ETR_{max}) (Fig. 33) and higher minimum saturating irradiance (I_k) (Fig. 34) in the 28% light treatment. A higher ETR_{max} indicates a greater photosynthetic capacity. ETR is generally directly related to O_2 evolution, particularly at low light intensities (Beer et al. 2000, Beer and Bjork 2000), although the relationship can be curvilinear, likely due to photorespiration (Kromkamp et al. 1998, Beer and Bjork 2000). The theoretical molar O_2/ETR ratio is 0.25 (4 mol electrons transported per mol O_2 evolved or CO_2 fixed) (Beer and Bjork 2000). Approximately this ratio has been found for the macroalgae *Ulva lactuca* (0.238) and *Ulva fasciata* (0.261) (Beer et al. 2000) and the seagrasses *H. ovalis* (0.28) (Beer and Bjork 2000) and *Cymodoca nodosa* (0.3) (Beer et al. 1998b), although ratios of 0.12 and 0.5 have been found for *Halophila stipulacea* and *Zostera marina*, respectively (Beer et al. 1998b). Thus, the O_2/ETR relationship can

vary by a factor of 4, and the utility of ETR as an estimate of photosynthetic rates may be quite specific.

An increase in ETR_{max} under high light conditions is a common response found in photosymbionts of coral (Beer et al. 1998a) and sponges (Beer and Ilan 1998, Steindler et al. 2001), and microphytobenthos (Kromkamp et al. 1998). It allows plants to take better advantage of greater light ability. Thus, 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. An increase in I_k (as determined by rapid light curves) under high light is another common adaptive response (Beer and Ilan 1998, Steindler et al. 2001).

Salinity stress did not appear to reduce photosynthetic capacity. Studies on the effects of stress on ETR_{max} are rare. However, one might expect salinity to damage the photosystem and thus ETR. The direct relationship between F_0 and salinity stress in the present study, though, argues against salinity-induced damage to the photosystem. The lack of consistent relationship between salinity and ETR_{max} is further supported by Kraemer et al. (1999), who found no relationship between salinity level and O_2 evolution rates. Thus, while ETR 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 ETR. However, 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 photosynthetic capacity.

Ralph et al. (1998) found that ETR_{max} varied widely among seagrass species *in situ*, ranging from approximately 20 to 53 μmol electrons m^{-2} s^{-1}. ETR_{max} in the present

study was within this span, ranging from 18 to 63 μmol electrons m^{-2} s^{-1}, indicating similarities between seagrasses and this freshwater species of SAV.

Reproduction

Sexual reproduction can be important in maintaining genetic diversity and in long-distance dispersal (Howe and Smallwood 1982, Orth et al. 1994, Philbrick and Les 1996, Inglis 2000). Flowering in this study was minimal and occurred only in 28 and 8% light and 0 and 5 psu treatments. Thus, the lack of flowering in the 2% light and 10 and 15 psu treatments may hamper long-term survival and dispersal.

The year-to-year maintenance and local spreading of existing populations is strongly tied to tuber production. Although the trends were nonsignificant due to several outliers, light and salinity stress here generally appeared to impede tuber production (Fig. 23), which may diminish long-term survival. Although little has been known of the effects of salinity on *V. americana* tuber production, the negative effects of reduced light have been demonstrated. For instance, Korschgen et al. (1997) found that tuber density increased with increasing seasonal irradiance. Similarly, Kimber et al. (1995) found that plants in artificial ponds produced tubers at 9 and 25% light levels but not at 2 and 5% light levels. Conversely, Twilley and Barko (1990) found no effect of light or salinity on number of tubers produced. However, the duration of the Twilley and Barko (1990) study was likely too short (5 weeks) for light or salinity stress to affect tuber production. In addition, both of the light treatments in that study (8 and 50% of light) may have been above a threshold level for tuber production, which is supported by the threshold level between 5 and 9% determined by Korschgen et al. (1997). Similarly, a marked decrease

in the number of the tubers was observed in the current study at 2% compared to 8 and 28% light levels. Although the effects of light availability on *V. americana* tuber density has been examined in several studies, its effects on total tuber biomass or individual tuber weight are rarely studied. However, Korschgen et al. (1997) found that total tuber biomass was greater under higher light levels. *Potamogeton pectinatus* has also been found to exhibit the same response (Hootsmans and Vermaat 1991, van Dijk 1991,). The results of this and other studies indicate that environmental conditions can strongly influence SAV tuber production.

Light and salinity effects may have been partially obscured by other factors. For instance, stressed plants appeared to devote a higher proportion of resources to tuber production, and less to seasonal production, compared to less stressed plants. This plasticity in allocation of resources may be an adaptive strategy to promote long-term survival. In addition, the upper range of end-of-season tuber density was at least twice that found in several field studies (Korschgen and Green 1988). Consequently, tuber production in the most favorable environmental conditions may have been constrained by the size of the pots, nutrient limitation, or some other factor.

Although more tubers may be produced in less stressful conditions, this study indicates that tubers will not be larger under unstressed conditions, and thus may not confer any additional benefit per tuber. The importance of tuber size has been illustrated in several studies. Early season growth is particularly dependent on tuber C reserves (Batiuk et al. 1992) and can help plants elongate in order to intercept adequate light. Van Dijk (1991) found that larger tubers of *P. pectinatus* had higher sprouting rates than smaller ones and also produced larger plants early in the season. The production of larger

plants from larger tubers has also been found for *V. americana* (Korschgen, unpublished, in Korschgen et al. 1997) and for other SAV species (Batiuk et al. 1992).

Applicability of Results to Field Conditions

The capacity of results of mesocosm studies to be applied to field conditions is dependent on the significance of the constraints imposed on the design (c.f. Giesy and Odum 1980). The mesocosms in this study appeared be good analogues of natural systems. Environmental conditions in the mesocosms generally mirrored those in the field. Water temperature tracked that of the adjacent York River, due to the water bath of continuously circulating river water. The use of ambient light ensured natural seasonal changes. The glass tops attenuated UV light; however, this attenuation was not likely to have substantially affected photosynthesis, as UV light is generally absorbed by the dissolved O and organic matter of surface waters (Kirk 1994). Sediment and water column nutrient concentrations, DIC, pH, grain size, and sediment organic carbon content were all in the range of field values for *V. americana* habitat reported by others (Korschgen and Green 1988). The mesocosms may have been better replicates of nontidal, lentic systems than tidal, lotic systems, due to the absence of tidal cycle or current.

Water column nutrient concentrations varied by treatment on some dates (Fig. 4, 5), but these differences were not likely to have differentially affected plant growth. Although initially well above the growth requirements of 0.65 μM (Batiuk et al. 1992), PO_4^{-3} concentrations remained low from July through October, presumably due to uptake by *V. americana* and microalgae. The high DIN concentrations at 2% light, compared to

28 and 8% light treatments, were likely due to greatly reduced uptake by microalgae, as well as *V. americana*. Over the course of the experiment, DIN levels were consistently lower in the lower salinity treatments at 2% light. As there was little difference in the production and biomass of the *V. americana* among the different salinity levels at 2% light, the difference in DIN concentrations may have been due to greater uptake by bacteria and microalgae in low salinity conditions, or greater release from the sediments in the higher salinity treatments.

One difference of potential importance between mesocosms in this study and natural conditions was the absence of grazers in the mesocosms. Grazers, such as carp, turtles, and waterfowl, can have substantial effects on *V. americana* populations. Generally, *V. americana* transplanted into the field does not survival unless it is protected from grazers by exclosures (Carter and Rybicki 1985, Moore et al. 2000b). Grazer effects on large, well-established populations can be considerable (Korschgen and Green 1988), but are probably less severe than effects on small populations. Therefore, grazers may have a disproportionate negative effect on the less robust populations in lower light, higher salinity conditions.

Both maximum seasonal rosette density and end-of-season tuber density were greater than those under natural field conditions. Maximum seasonal rosette density in the 0 psu, 28% light treatment was more than twice the maximum density reported in the field (Korschgen and Green 1988). The upper density of tubers was likewise at least twice the density found in several field studies (Korschgen and Green 1988). These high densities may have been due to more favorable conditions, such as lack of grazers or high light availability. However, high density may have resulted in more intraspecies

competition for light or nutrients than in the other treatments or than in natural conditions. Production in the more prolific treatments may also have been limited by pot boundaries. Few measurements of plant biomass in natural field conditions exist. However, biomass (m^{-2}) in the 28% light, 0 psu treatment was similar to that of maximum seasonal biomass in Chenango Lake, New York (Titus and Stephens 1983).

Most controlled studies on *V. americana* have not been conducted throughout an entire growing season (Haller et al. 1974, Barko et al. 1982, Staver 1986, Twilley and Barko 1990, Barko et al. 1991, 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. Sprouting tubers and young plants may have different growth requirements than their adult counterparts: they may be either more sensitive or more resilient. Further, stress on newly sprouted plants may have lasting effects on adult plants. Following plant growth throughout the entire growing season would also reveal at which points of the season the effects of continuous stress are most apparent, although it would not reveal critical growth periods, as visible responses to stress may be delayed. Studying the entire growing season also facilitates an understanding of sprouting behavior and tuber production under stress. For these reasons, following growth from sprouting to tuber production enables a more accurate assessment of growth requirements. Further study is needed to determine critical periods of growth and to elucidate the effects of short-term stress, which can be considerable (Moore et al. 1997).

Epiphytic growth was not explicitly studied. Although epiphytic material on SAV can be extreme and can considerably reduce light reaching the leaves, it was minimal in the present study in all treatments and so did not appreciably reduce light exposure.

Epiphytic growth is generally low at low salinities (0.02 mgdw cm-2 for *V. americana* in the field, Moore et al. 2000b; less than 1 gdm/gafdm for *V. americana* in mesocosms, Twilley and Barko 1990), although very little information is available on the specific effects of epiphytic material on light availability for freshwater or oligohaline species (Batiuk et al. 1992). Also largely unknown is the relationship between grazers, epiphytes, and *V. americana.*

Determination of Light and Salinity Tolerance Limits

A variety of criteria may be used to define light requirements and salinity tolerance limits. Potential criteria may include survival throughout the first growing season, survival into subsequent years, or achieving a certain level of production adequate to provide important ecological services, such as food source, sediment stability, and oxygen production. Another criteria may be the point at which production sharply declines. The criterion expressly measured in this study was survival throughout the growing season. Thus, this criterion formed the basis of conclusions on light requirements and salinity tolerance limits. However, although not expressly studied, the other criteria were considered, as well.

Production was greatly reduced at lower light levels. Production did not significantly differ between 2 and 8% surface irradiance, suggesting some threshold level between 8 and 28% light. However, under 2 and 8% light environments plants were moderately productive, and even produced tubers. Thus, although growth was more vigorous at 28% light, growth at 2 and 8% light may have been adequate to ensure long-term survival and to provide ecosystem functions. 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. However, light requirements may be greater in the field, where more organic carbon may be lost to herbivory, leaf sloughing and fragmentation. Natural variability and tidal or riverine currents may further stress plants (Batiuk et al. 1992).

Salinity tolerance appears to be 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 production characteristics were greatly reduced at these higher levels, and populations thus would not be likely to adequately provide important ecosystem functions. This salinity tolerance is in concordance with that determined by most other studies (Haller 1974, Staver 1986; Bourn 1932, Bourn 1934, Davis and Brinson 1976, all in Twilley and Barko 1990). However, it is lower than the tolerance to 12 psu or more suggested by Twilley and Barko (1990) and Kraemer et al. (1999). As discussed above, the higher tolerance in these two studies compared to the present study were likely due in large part to their shorter timescale and use of adult plants, which likely would have had stored reserves to help fight 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. However, results of this study indicate that 15 psu should not be considered an upper tolerance level, as plants growing from tubers in salinities 10 and 15 psu remained very small. Kraemer et al. (1999) stated that "[i]t is clear that the salinity tolerances of *V. americana* need to be revised."

However, they admit "[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, at least not robustly, in the long term.

The tolerance of *V. americana* to moderate salinity stress was dependent on the concurrent light regime. Light and salinity effects interacted for all production characteristics (Fig. 35). In the 5 psu treatment production was considerably higher under 28% light than at the lower light levels. It appears that high light ameliorated salinity stress at the 5 psu level. For instance, assuming that growth is a linear function of irradiance, aboveground biomass in the 5 psu treatment at 28% light may be equivalent to biomass at approximately 20% light in the 0 psu treatment. Similarly, leaf area index of 5 psu plants at 28% light was comparable to that at approximately 17% light at 0 psu. Thus, within the 0 to 5 psu range salinity tolerance was greater at higher light. This implies that *V. americana* may colonize more saline regions up to a point 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 growing season salinities are 10 to 15 psu.

Similarly, *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, Lazar and Dawes 1991, Perez and Romero 1992, Goodman et al. 1995, Masini 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). *V. americana* light

requirements have been generally defined as approximately 9% light (Batiuk et al. 1992), regardless of salinity or other environmental conditions. The results of the current study support a light requirement somewhere between 8 and 28% light in 0 to 5 psu. However, light requirements for *V. americana* growing at 5 psu may be approximately 50% higher than for plants growing at 0 psu.

The relationship 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 1999c) and are largely ignored in the development of growth requirements. Different combinations of stressors should be studied for a variety of SAV species in order to develop more accurate growth requirements and to better manage this valuable resource.

APPENDIX 1

Salinity (psu) of aquarium water throughout the season. The letter "a" after a date indicates salinity before adjustment, whereas the letter "b" indicates salinity after adjustment.

Aquarium	Light	Salinity	6/5/00	6/8/00	6/10/00	6/10/00	6/13/00a	6/13/00b	6/23/00	7/27/00a
1	M	2	5	5.2	5	5.1	5.41		5.4	5.4
2	M	3	11	8.1	10	8.6	9	10.7	10.1	9
3	M	4	15	11.5	15	12.4	12.83	16.04	14.9	14.2
4	M	1	3	0.1	2	0.1			0.1	0.5
5	M	1	3	0.1	2	0.1			0.1	0.4
6	M	2	6	4.5	6	4.4	4.64	5.59	5.8	4.8
7	H	3	11	9.2	10	9.4	9.78	10.97	11	10.5
8	H	1	2	0.2	0	0.1			0.2	0.4
9	H	2	8	4.6	5	4.4	4.65	5.56	6	5
10	H	4	16	12.4	14	13.6	14.13	15.94	14.8	12.9
11	H	3	13	8.1	9	8.3	8.61	10.49	10.2	9.4
12	H	2	8	4.4	5	4.1	4.35	5.57	5.8	5.3
13	L	4	15	12.4	14	12.6	13.01	16.13	14.8	14
14	L	1	4	1.1	2	0.9			1.1	1.9
15	L	1	5	0.8	1	0.6			0.8	1
16	L	3	11	8.3	10	8.5	8.75	10.51	10.2	10
17	L	3	11	8.6	10	8.2	8.54	10.54	10.1	10
18	L	2	8	4.5	5	4			5.6	5.7
19	H	2	8	4.5	5	4.2	4.56	5.42	5.7	8
20	H	4	17	12.4	15	13.6	14.07	15.93	15.1	14.1
21	H	1	4	0.2	1	0.2			0.1	0.3
22	H	3	13	8.4	10	8.7	9.02	10.71	10.6	9.5
23	H	4	17	13	15	13.8	4.34	15.98	0.2	14
24	H	1	3	0.2	0	0.1			15	0.3
25	M	1	2	0.2	0	0.1			1.5	2.1
26	M	4	17	12.3	15	13	13.52	15.98	15.1	16.2
27	M	2	8	4.7	5	4.4	4.62	5.62	5.8	5.5
28	M	3	11	8.1	10	8.2	8.55	10.5	10.4	10.6
29	M	3	11	8.5	10	8.8	9.1	10.52	9.4	10.2
30	M	4	16	12.4	15	13.2	13.56	15.9	15.2	14.1
31	L	3	11	8.7	10	7.8	9.18	10.66	12.8	12.1
32	L	2	8	4.2	5	3.9	4.54	5.54	5.9	6.6
33	L	2	9	5.2	5	4.9	5.13	5.56	5.9	5.8
34	L	4	16	11.8	14	12.5	13	16.06	14.9	14.8
35	L	4	17	12.3	14	13.1	13.54	15.98	14	14.8
36	L	1	3	0.2	0	0.2			0.1	0.2

Aquarium	Light	Salinity	7/27/00b	8/21/00a	8/21/00b	9/11/00	9/16/00a	9/16/00b	10/15/00a	10/15/00b
1	M	2	5.2	5	5	8	6.09	5.15	5.18	
2	M	3	10.2	10.3	8.5	10.9	9.19	10.6	10.16	9.88
3	M	4	15.1	17	13	16	14.55		13.92	
4	M	1		0.4		2.5	0.41		0.42	
5	M	1		0.5		2.5	0.45		0.44	
6	M	2		5.1	4.1	5.5	4.26	5.16	5.11	4.8
7	H	3		10.2	8.6	8.8	8.69	9.99	10.61	
8	H	1		0.3		1.5	1.46		2.19	1.58
9	H	2		4.8	4.2	4.6	3.99	4.98	5.23	
10	H	4	15.1	14.3	13	13	13.23	14.99	15.26	14.94
11	H	3	10.2	10.2	8.5	8.9	8.64	10.04	10.48	10.26
12	H	2		5.3	4	4.7	3.87	5.12	5.35	5.07
13	L	4	15.1	15.1	13	13.5	14.38	15.05	14.76	
14	L	1		2.1		2.2	2.23	1.63	1.82	1.71
15	L	1		1		1	0.9		0.87	
16	L	3		10.5	8.5	9.1	9.26	10.6	11.3	10.63
17	L	3		10	8.7	8	8.83	10.12	10.28	9.53
18	L	2	5.4	5.5	4.1	4.1	3.8	5.16	5.61	5.43
19	H	2	5.5	6	4		4.61	5.25	5.64	5.46
20	H	4	15	15	13		13.29	15.04	15.41	14.18
21	H	1		0.4			0.53		0.57	
22	H	3	10.2	10.3	8.5		7.84	10.19	9.55	
23	H	4	15.2	14.8	13		12.66	14.88	15.47	14.9
24	H	1		0.5			0.43		0.49	
25	M	1		2.9	2		2.06	1.52	2.26	1.53
26	M	4	15.3	15.2	13		13.49	15	15.16	14.93
27	M	2		5.9	4.1		3.9	5.67	6.25	5.43
28	M	3		10.2	8.5		8.68	10.06	10.59	9.74
29	M	3		10.3	8.6		8.91	9.99	10.26	9.56
30	M	4	15.1	15.4	13		13.28	15.03	15.56	14.87
31	L	3	10.5	12.2	8.5		7.95	9.84	10.87	10.39
32	L	2		6	4		4	4.97	5.94	5.71
33	L	2	5.3	5.9	4		4.02	5.58	5.62	5.23
34	L	4	15.1	15.6	12.9		12.93	15.05	15.07	
35	L	4	15.3	16	13		13.45	15.08	14.34	
36	L	1		0.5			0.4		0.4	

APPENDIX 2

Fraction organic carbon of pot sediment. On June 7, 4 pots were selected randomly for sampling. Cores were analyzed at 3 depth intervals. On August 15 and November 28, one core was taken per aquarium and was homogenized for analysis.

sample	depth (cm)	organic carbon content
		June 7
1	0-2	0.0157
	2-5	0.0148
	5-10	0.0143
2	0-2	lost sample
	2-5	0.0165
	5-10	0.0157
3	0-2	0.0178
	2-5	0.0175
	5-10	0.0170
4	0-2	0.0189
	2-5	0.0182
	5-10	0.0178
mean		0.0167
SE		0.00044

APPENDIX 2 (continued)

	Organic Carbon Content	
Aquarium	**August 15**	**November 28**
1	0.0146	0.0167
2	0.0129	0.0219
3	0.0125	0.0156
4	0.0118	0.0137
5	0.0112	0.0144
6	0.0140	0.0138
7	0.0143	0.0123
8	0.0126	0.0128
9	0.0120	0.0119
10	0.0146	0.0161
11	0.0139	0.0119
12	0.0112	0.0144
13	0.0122	0.0144
14	0.0123	0.0122
15	0.0108	0.0130
16	0.0116	0.0149
17	0.0152	0.0137
18	0.0132	0.0112
19	0.0125	0.0120
20	0.0149	0.0128
21	0.0117	0.0120
22	0.0129	0.0130
23	0.0154	0.0144
24	0.0132	0.0128
25	0.0122	0.0111
26	0.0146	0.0143
27	0.0130	0.0120
28	0.0155	0.0141
29	0.0135	0.0146
30	0.0119	0.0141
31	0.0144	0.0149
32	0.0103	0.0135
33	0.0123	0.0145
34	0.0124	0.0133
35	0.0140	0.0138
36	0.0125	0.0133
mean	0.0130	0.0138
SE	0.00136	0.00032

APPENDIX 3

pH of aquarium water throughout the season.

Aquarium	Light (%)	Salinity (psu)	7/27	8/23	9/16	10/15	Average
4	8	0	7.93	8.24	7.77	8.41	8.09
5	8	0	7.86	8.16	7.1	8.22	7.84
8	28	0	8.48	8.55	7.56	7.84	8.11
14	2	0	7.2	7.73	7.55	7.83	7.58
15	2	0	7.83	8.01	7.64	8.01	7.87
21	28	0	8.25	8.23	8.18	8.16	8.21
24	28	0	8.66	7.91	7.75	8.16	8.12
25	8	0	8.01	8.1	7.83	7.94	7.97
36	2	0	8.11	8.26	7.95	8.26	8.15
1	8	5	7.52	8.09	7.54	7.93	7.77
6	8	5	7.17	7.3	7.15	7.2	7.21
9	28	5	7.84	8	7.32	7.52	7.67
12	28	5	7.82	8.07	7.45	7.6	7.74
18	2	5	7.3	7.72	7.61	7.72	7.59
19	28	5	8.03	8.08	8.06	7.84	8.00
27	8	5	8.09	8.03	7.75	7.79	7.92
32	2	5	7.93	8.04	7.76	7.84	7.89
33	2	5	7.81	8.08	7.89	7.91	7.92
2	8	10	6.95	7.6	7	7.5	7.26
7	28	10	7.56	7.63	7.66	7.71	7.64
11	28	10	7.98	7.66	7.33	7.5	7.62
16	2	10	7.02	7.16	6.97	7.51	7.17
17	2	10	6.06	7.03	7.09	7.44	6.91
22	28	10	7.85	7.77	7.64	7.67	7.73
28	8	10	5.04	8.1	7.8	7.9	7.21
29	8	10	7.45	7.52	7.29	7.43	7.42
31	2	10	7.38	7.88	7.56	7.79	7.65
3	8	15	7.18	8.15	7.12	7.71	7.54
10	28	15	7.92	7.68	6.94	7.34	7.47
13	2	15	7.32	7	6.91	7.3	7.13
20	28	15	8.36	7.67	7.12	7.39	7.64
23	28	15	8.23	7.66	7.13	7.46	7.62
26	8	15	7.75	7.62	7.22	7.52	7.53
30	8	15	7.81	8.05	7.53	7.61	7.75
34	2	15	7.67	7.7	7.21	7.54	7.53
35	2	15	7.76	7.79	7.41	7.69	7.66
						average	7.67

APPENDIX 4

Flowering structures observed.

Date	Light (%)	Salinity (psu)	Aquarium	# Flowers
8/2	28	0	24	3
	8	5	27	2
	28	5	19	1
8/16	28	0	24	4
	8	5	27	5
	28	5	9	1
8/29	8	0	5	1
	28	0	8	1
	28	5	9	1
	28	0	24	2
	8	5	27	4
9/14	8	0	5	1
	28	0	8	2
9/27	28	0	8	1

APPENDIX 5

Sample size for quantum yield and ETR_{max} measurements. n=3 unless otherwise noted. n<3 due to leaf senescence.

Quantum Yield

Salinity (psu)	Light (%)	6/22	7/6	7/22	8/2	8/17	9/1	9/13	9/29
0	28								2
	8								
	2		2					2	2
5	28								
	8								
	2								2
10	28								
	8						2		2
	2								1
15	28	2		2	2	2	2	2	1
	8					2	2	1	0
	2				2	2	2	1	0

ETR, August 20

n=2 for the following:

Salinity (psu)	Light (%)
0	28
5	8
10	28
15	28
	8
	2

ETR, September 12

n=2 for the following:

Salinity (psu)	Light (%)
5	8
15	28
	8

n=1 for the following:

Salinity (psu)	Light (%)
15	2

LITERATURE CITED

Adair, S.E., J.L. Moore, and C.P. Onuf. 1994. Distribution and Status of submerged vegetation in estuaries of the upper Texas coast. *Wetlands* **14**(2): 110-121.

Adams, J. B. and G. C. Bate. 1994. The ecological implications of tolerance to salinity by *Ruppia cirrhosa* (Petagna) Grande and *Zostera capensis* Setchell. *Botanica Marina* **37**(5): 449-456.

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**(1-2): 1-10.

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., 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., 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**(2): 199-207.

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., B. Vilenkin, A. Weil, M. Veste, L. Susel, and A. Eshel. 1998. Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry. *Marine Ecology Progress Series* **174**: 293-300.

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., 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**(1): 69-74.

Beer, S., M. Ilan, A. Eshel, A. Weil, and I. Brickner. 1998a. Use of pulse amplitude modulated (PAM) fluorometry for in situ measurements of photosynthesis in two Red Sea faviid corals. *Marine Biology* **131**(4): 607-612.

Beer, S., B. Vilenkin, A. Weil, M. Veste, L. Susel, and A. Eshel. 1998b. Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry. *Marine Ecology Progress Series* **174**: 293-300.

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**(4): 613-617.

Bjork, M., J.N. Uku, A Weil and S. Beer. 1999. Photosynthetic tolerances to dessication of tropical intertidal seagrasses. *Marine Ecology Progress Series* **191**: 121-126.

Bjorkman, O. and B. Demmig. 1987. Photon yield of O2-evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta* **170**: 489-504.

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.

Bloom, A.J., F.S. Chapin, III, and H.A. Mooney. 1985. Resource limitation in plants—an economic analogy. *Annual Review of Ecology and Systematics* **16**: 363-392.

Brenchley, J. L. and R. J. Probert. 1998. Seed germination responses to some environmental factors in the seagrass *Zostera capricorni* from eastern Australia. *Aquatic Botany* **62**(3): 177-188.

Carter, V. and N.B. Rybicki. 1985. Effects of grazers and light penetration on the survival of transplants of *Vallisneria americana* Michx in the tidal Potomac River, Maryland. *Aquatic Botany* **23**(3): 197-213.

Carter, V. and N.B. Rybicki. 1986. Resurgence of submersed aquatic macrophytes in the tidal Potomac River, Maryland, Virginia, and the District of Columbia. *Estuaries* **9**(4B): 368-375.

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**(4): 337-345.

Carter, V., N.B. Rybicki, J.M. Landwehr, and M. Naylor. 2000. Light requirements for SAV survival and growth. *in* Chesapeake Bay SAV water quality and habitat-based requirements and restoration targets: A second technical synthesis. U.S. EPA. Chesapeake Bay Program.

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. In: Kennedy, V.S. (Ed.), Estuarine Perspectives, Academic Press, New York, pp. 263-277.

Chapin, F.S. III, A.J. Bloom, C.B. Field, and R.H. Waring. 1987. Plant responses to multiple environmental factors: Physiological ecology provides tools for studying how interacting environmental resources control plant growth. *BioScience* **37**(1): 49-57.

Conacher, C. A., I. R. Poiner, J. Bulter, S. Pun, and D. J. Tree. 1994. Germination, storage, and viability testing of seeds of *Zostera capricorni* Aschers from a tropical bay in Australia. *Aquatic Botany* **62**(3): 177-188.

Dennison, W.C. 1990. Chlorophyll content. *In* Seagrass Research Methods. R.C. Phillips and C.P. McRoy (eds.). Unesco, Paris.

Dennison, W.C., R.J. Orth, K.A. Moore, J.C. Stevenson, V. Carter, S. Kollar, P.W. Bergstrom, and R.A. Batiuk. 1993. Assessing water quality with submersed aquatic vegetation: Habitat requirements as barometers of Chesapeake Bay health. *BioScience* **43**(2): 86-94.

Dionisio-Sese, M.L. and S. Tobita. 2000. Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance. *Journal of Plant Physiology* **157**(1): 54-58.

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

Dunton, K.H. 1996. Photosynthetic production and biomass of the subtropical seagrass *Halodule wrightii* along an estuarine gradient. *Estuaries* **19**(2): 436-447.

Genty, B. J.M. Briantais, and N.R. Baker. 1989. The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990**: 87-92.

Giesy, J.P. Jr. and E.P. Odum. 1980. Microcosmology: introductory comments. In: Giesy J.P. (ed) Microcosms in ecological research. Technical Information Center, U.S. Department of Energy, Symposium Series No. 52, pp. 1-13.

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.

Grise, D., J.E. Titus, and D.J. Wagner. 1986. Environmental pH influences growth and tissue chemistry of the submersed macrophyte *Vallisneria americana*. Canadian Journal of Botany **64**: 306-310.

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.

Haller, W.T. and D.L. Sutton. 1975. Community structure and competition between *Hydrilla* and *Vallisneria*. *Hyacinth Control Journal* **13**: 48-50.

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.

Hootsmans M.J.M. and J.E. Vermaat. 1991. Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems. International Institute for Hydraulic and Environmental Engineering Report Series 21, The Netherlands.

Hopkinson, C.S. Jr., A.E. Giblin, J. Tucker, and R.H. Garritt. 1999. Benthic metabolism and nutrient cycling along an estuarine salinity gradient. *Estuaries* **22**(4): 863-881.

Howe, H.F. and J. Smallwood. 1982. Ecology of seed dispersal. *Annual Review of Ecology and Systematics* **13**: 201-228.

Inglis, G.J. 2000. Variation in the recruitment behavior of seagrass seeds: implications for population dynamics and resource management. *Pacific Conservation Biology* **5**: 251-259.

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**(3): 527-539.

Kaul, R.B. 1978. Morphology of germination and establishment of aquatic seedlings in Alismataceae and Hydrocharitaceae. *Aquatic Botany* **5**: 139-147.

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**(2): 78-89.

Kerr, E. A. and S. Strother. 1985. Effects of irradiance, temperature and salinity on photosynthesis of *Zostera muelleri*. *Aquatic Botany* **23**(2): 177-184.

Khan, M. A. and I. A. Ungar. 1999. Effect of salinity on seed germination of *Triglochin maritima* under various temperature regimes. *Great Basin Naturalist* **58**(2): 144-150.

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**(12): 1966-1973.

Kirk, J.T.O. 1994. Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge. 509 pp.

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. 1988. American wild celery (*Vallisneria americana*): Ecological considertions for restoration. Technical Report Series U. S. Fish and Wildlife Service, 31 pp.

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): 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**(1): 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 constitutents and tolerances to salinity and temperature. *Botanica marina* **34**: 265-269.

Longstaff, B.J., N.R. Loneragan, M.J. O'Donohue, and W.C. Dennison. 1999. Effects of light deprivation on the survival and recovery of the seagrass *Halophila ovalis* (R.Br.) Hook. *Journal of Experimental Marine Biology and Ecology* **234**(1): 1-27.

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 responses 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**(345): 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., R.J. Orth, and J.F. Nowak. 1993. Environmental regulation of seed germination in *Zostera marina* L. (eelgrass) in Chesapeake Bay: Effects of light, oxygen and sediment burial. *Aquatic Botany* **45**(1): 79-91.

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. 2000a. Analysis of abundance of submersed aquatic vegetation communities in the Chesapeake Bay. *Estuaries* **23**: 115-127.

Moore, K.A., R.J. Orth, and J. Fishman. 2000b. Restoration of submerged aquatic vegetation (SAV) in the tidal freshwater James River: 1999 pilot study. Special Report No. 365 in Applied Marine Science and Ocean Engineering.

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**(4B): 531-540.

Orth, R.J., M. Luckenbach, and K.A. Moore. 1994. Seed dispersal in a marine macrophyte: implications for colonization and restoration. *Ecology* **75**(7): 1927-1939.

Orth, R.J., R.A. Batiuk, and J.F. Nowak. 1994. *Trends in the Distribution, Abundance, and Habitat Quality of Submerged Aquatic Vegetation in Chesapeake Bay and its Tidal Tributaries: 1971-1991.* Chesapeake Bay Program, Annapolis, MD. CBP/TRS 137/95. EPA 903-R-95-009. 216 pp.

Orth, R.J., J.F. Nowak, D.J. Wilcox, J.F. Whiting, and L.S. Nagey. 1999. Distribution of submerged aquatic vegetation in the Chesapeake Bay and Coastal Bays – 1998. Virginia Institute of Marine Science. Gloucester Point, VA. 370 pp.

Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. A Manual of Chemcial and Biological Methods for Seawater Analysis, Pergamon Press, New York, 173 pp.

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.

Philbrick, C.T. and D.H. Les. 1996. Evolution of aquatic angiosperm reproductive systems: What is the balance between sexual and asexual reproduction in aquatic angiosperms? *Bioscience* **46**(11).

Poljakoff-Mayber, A., G. F. Somers, E. Werker, and J. L. Gallagher. 1994. Seeds of *Kosteletzkya virginica* (Malvaceae): Their structure, germination, and salt tolerance. 2. Germination and salt tolerance. *American Journal of Botany* **81**(1): 54-59.

Prange, J.A. and W.C. Dennison. 2000. *Marine Pollution Bulletin* **41**(7-12): 327-336.

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. 1996. Diurnal photosynthetic patterns of *Halophila ovalis* (R. Br.) Hook *f.* Seagrass Biology: Proceedings of an International Workshop. Rottnest Island, Western Australia, 25-29 January 1996. 197-202.

Ralph, P.J. and M.D. Burchett. 1998a. Impact of petrochemicals on the photosynthesis of *Halophila ovalis* using chlorophyll fluorescence. *Marine Pollution Bulletin* **36**(6): 429-436.

Ralph, P.J. and M.D. Burchett. 1998b. Photosynthetic response of *Halophila ovalis* to heavy metal stress. *Environmental Pollution* **103**: 91-101.

Ralph, P.J., R. Gademann, and W.C. Dennison. 1998. In situ seagrass photosynthesis measured using a submersible, pulse-amplitude modulated fluorometer. *Marine Biology* **132**: 367-373.

Ralph. P.J. 1998a. 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. 1998b. Photosynthetic response of laboratory-cultured *Halophila ovalis* to thermal stress. *Marine Ecology Progress Series* **171**: 123-130.

Ralph, P.J. 1999a. Herbicide toxicity of *Halophila ovalis* assessed by chlorophyll *a* fluorescence. *Aquatic Botany* **1349**: 1-12.

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

Ralph, P.J. 1999c. Photosynthetic response of *Halophila ovalis* (R. Br.) Hook. *f.* to combined environmental stress. *Aquatic Botany* **1329**: 1-14.

Romero-Aranda, R., T. Soria, and J. Cuartero. 2001. Tomato plant-water uptake and plant-water relationships under saline growth conditions. *Plant Science* **160**(2): 265-272.

Rout, N.P. and B.P. Shaw. 2001. Salt tolerance in aquatic macrophytes: Possible involvement of the antioxidative enzymes. *Plant Science* **160**(3): 415-423.

Rybicki, N.B. and V. Carter. 1986. Effect of sediment depth and sediment type on the survival of *Vallisneria americana* Michx grown from tubers. *Aquatic Botany* **24**: 233-240.

Sand-Jensen, K. and J. Borum. 1983. Regulation of growth of eelgrass (*Zostera marina* L.) in Danish coastal waters. *Marine Technology Society Journal* **17**(2): 15-21.

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.

Sculthorpe, C.D. 1967. The biology of aquatic vascular plants. St. Martin's Press, New York. 610 pp.

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. Thesis, University of 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**(3): 511-515.

Stevenson, J.C., C.B. Piper, and N. Confer. 1979. The decline of submerged aquatic plants in the Chesapeake Bay. U.S. Fish and Wildlife Service, FWS/OBS-79/24. 12 pp.

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**(2): 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.

Titus, J.E. and M.D. Stephens. 1983. Neighbor influences and seasonal growth patterns for *Vallisneria americana* Michx. *Oecologia* **56**: 23-29.

Titus, J.E. and W.H. Stone. 1982. Photosynthetic response of two submersed macrophytes to dissolved inorganic carbon concentration and pH. *Limnology and Oceanography* **27**(1): 151-160.

Twilley, R. R. and J. W. Barko. 1990. The growth of submersed macrophytes under experimental salinity and light conditions. *Estuaries* **13**(3): 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, OH, 460 pp.

Van Dijk, G.M. 1991. Light climate and its impacts on *Potamogeton pectinatus* L. in a shallow eutrophic lake.

Walker, D. I. and A. J. McComb. 1990. Salinity response of the seagrass *Amphibolis antarctica* (Labill.) Sonder et Aschers.: An experimental validation of field results. *Aquatic Botany* **36**(4): 359-366.

Walz, Heinz. 1998. Underwater Fluorometer: Diving-PAM. Submersible Photosynthesis Yield Analyzer. Handbook of Operation. Germany.

Wang, D, M.C. Shannon, and C.M. Grieve. 2001. Salinity reduces radiation absorption and use efficiency in soybean. *Field Crops Research* **69**(3): 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.

VITA

GAIL T. FRENCH

Born in Boston, MA, 31 March 1974. Graduated from Belmont High School in 1992. Earned B.A. in Biology from Williams College in 1996. Entered masters program in College of William and Mary, School of Marine Science in 1999.