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ORIGINAL PAPER

Effects of temperature on growth of *Vallisneria americana* in a sub-tropical estuarine environment

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Abstract The submersed aquatic vegetation (SAV) species *Vallisneria americana* Michx. (tape grass) is a valuable resource in the Caloosahatchee estuary and in many other aquatic systems. Given the variable nature of freshwater inflows and environmental conditions in the Caloosahatchee, it is necessary to understand how tape grass will respond to high and low salinity conditions at different light and temperature levels. Specifically, quantitative information is needed as input to modeling tools that can be applied to predict growth and survival of tape grass under a range of environmental conditions present in the estuary. We determined growth rates for small and medium sized tape grass plants obtained from the Caloosahatchee estuary, southwest coastal Florida, USA in freshwater (0.5 psu) under high (331 μE m^{-2} s^{-1}) and low light (42 μE m^{-2} s^{-1}) and at 10 psu under high light conditions. We ran six treatments at five temperatures spanning 13–32 °C for 8–9 weeks. The optimum temperature for growth was roughly 28 °C, with a minimum threshold temperature of 13 °C and a maximum threshold temperature of 38 °C. Plants grew fastest in freshwater, at high light and temperatures greater than 20 °C. The slowest growth rates were observed at 13 °C regardless of salinity, light or plant size. Our results suggest that tape grass growth is strongly influenced by water temperature and that additional stressors such as low light and elevated salinity can reduce the range of temperature tolerance, especially at colder water temperatures.

Keywords *Vallisneria americana* Michx · Temperature · Light · Salinity · Submersed macrophyte · Tape grass

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Introduction

Submersed aquatic vegetation (SAV) can be a valuable resource to aquatic ecosystems. SAV can be major contributors to system primary productivity (e.g., Penhale 1977), provide habitat for young fish (Orth et al. 1984; Edgar and Shaw 1995), and improve water quality by increasing sedimentation rates (Fonseca and Fisher 1986), and removing dissolved nutrients (see review in Beck et al. 2003). SAV has been used as an indicator of anthropogenic effects from nutrient loading and storm water runoff (Johansson and Greening 2000). Environmental requirements based on SAV can be used to develop water quality goals (e.g. Dennison et al. 1993; Stevenson et al. 1993). Given their ecological significance, SAV are often included in monitoring programs (Tomasko et al. 1996) and have been selected as a sentinel resource in

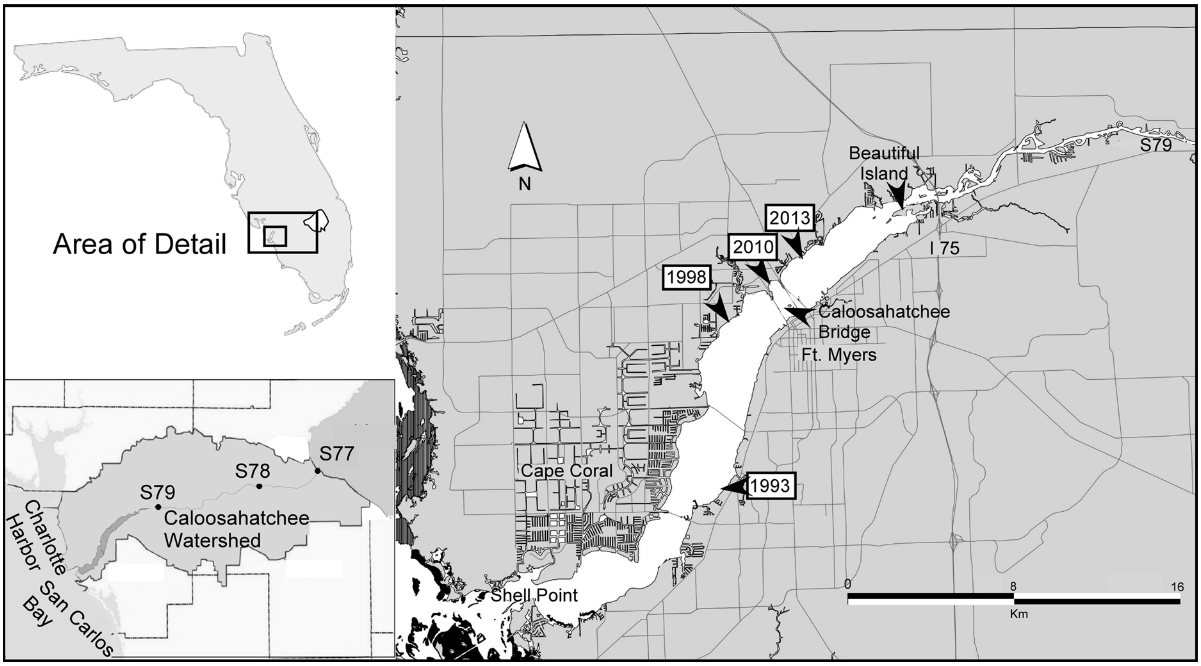

Fig. 1 Map of the Caloosahatchee Estuary. Observations recorded in 1993 indicated the presence of tape grass as far downstream as indicated (Hoffacker 1994). Since then the observed range has decreased (Bartleson, unpublished data). In 1998 tape grass was observed downstream of the Caloosahatchee Bridge (Bortone and Turpin 2000), however since 2000 it has been observed only upstream of this location

management efforts (Johansson and Greening 2000). SAV habitat requirements are used increasingly for the development of coastal water management criteria such as minimum freshwater inflow requirements (SFWMD 2003; SFWMD 2006; Mattson 2002; Doering et al. 2002) and conservation habitat targets.

The Caloosahatchee River and estuary, located on the southwest coast of Florida (Fig. 1), is part of the larger Charlotte Harbor estuary. The Caloosahatchee River runs 67 km from Lake Okeechobee to the Franklin Lock and Dam (Water control structure S-79). S-79 was constructed in 1966 to act, in part, as a salinity barrier (Flaig and Capece 1998). S-79 is one of three control structures along the river and separates the freshwater river from the estuary; which extends from the S-79 structure approximately 42 km downstream to Shell Point (Fig. 1). The River, its watershed, and the estuary have been extensively modified. The River has been straightened, deepened and artificially connected to Lake Okeechobee. A navigation channel has been dredged in the estuary, and in the 1960s a causeway was built across the mouth of San Carlos Bay. Land use and drainage patterns within the watershed have been altered to allow agriculture and urban development.

Because of these changes, large seasonal freshwater inflow fluctuations can occur, resulting in highly variable salinity and light levels within the estuary. During the wet season (June through October), freshwater inflows can freshen the system to <1 psu down to Shell Point and water color can account for a major portion of water column light attenuation in the region (McPherson and Miller 1987). Conversely, freshwater inflow to the estuary can be absent for periods within the dry season (November through May) when salt water intrusion combined with the S-79 structure, may result in a compression or elimination of oligohaline habitat.

Tape grass (*Vallisneria americana* Michx.) is a salt tolerant, freshwater angiosperm that often occurs in the lower salinity reaches (<10 psu) of estuaries in the Northeastern and Southeastern United States (Bourne 1932; Lowden 1982); providing critical habitat for fish and shellfish (Rozas and Minello 2006). Tape grass can form extensive beds in the upper Caloosahatchee estuary and its salinity tolerances have been used to help develop freshwater inflow requirements for this system (Doering et al. 2002; SFWMD 2003).

In the Caloosahatchee, tape grass exhibits a seasonal pattern of growth, with highest biomass achieved in late summer, flowering in late summer to early fall, followed by a winter decline in biomass (Bortone and Turpin 2000). As in other Florida populations (Dawes and Lawrence 1989), plants grow year-round. Vegetative tubers, typically produced to over-winter in less moderate climates have not been observed in the Caloosahatchee estuary (Doering et al. 1999). Thus, the response of plants to both winter temperatures and summer temperatures typical of South Florida's subtropical climate may have important implications for population dynamics.

The occurrence of tape grass in the Caloosahatchee is variable (Hunt and Doering 2005). In some years, growth can be extensive and lush in both wet and dry seasons, while in other years only sparse populations consisting of small plants are found. Tape grass beds were depleted following a drought in 2000–2001 in the Caloosahatchee estuary and despite periods of ample fresh water, only limited re-establishment of small rosettes has occurred and large plants (>50 cm average blade length) have not been observed since 2000. Studies have indicated that water clarity may be an important determinant for recovery or growth of tape grass in the Caloosahatchee estuary (Hunt 2007; Kraemer et al. 1999). Although the climate is semi-tropical, seasonal temperature ranges especially with respect to light and salinity conditions may also be an important consideration (Hunt and Doering 2005).

A numerical model was constructed to integrate both field and laboratory data and provide a tool that could be used to predict the effect of environmental variables on tape grass in the Caloosahatchee estuary (SFWMD 2003; Hunt and Doering 2005). Field and lab experiments with tape grass from the Caloosahatchee were conducted and used to quantify salinity tolerance (Kraemer et al. 1999; Doering et al. 1999, 2001) and light requirements (Hunt and Doering 2005; Hunt 2007) in the model formulation and specific growth relationships were developed for these two variables. A general temperature–growth curve was formulated using the equation of O'Neill et al. (1972), but more information specific to the effects of salinity, light, temperature and plant size are needed to enable improved predictions.

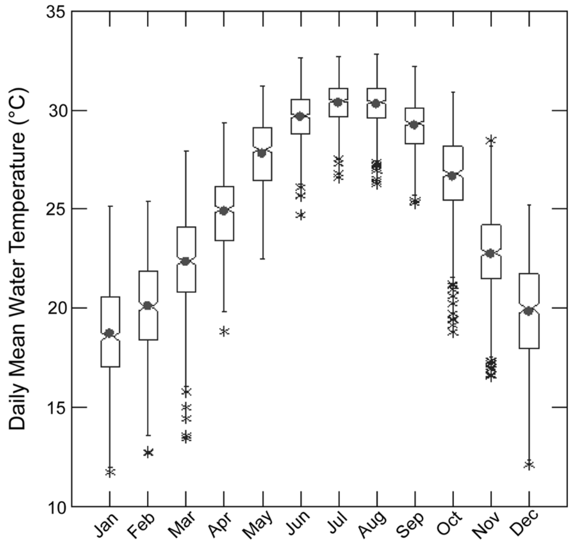

Fig. 2 Average daily water temperatures for the period January 1992 through January 2011 near Ft. Myers bridges. During this period, minimum water temperatures 13 °C or below were recorded during the months of December, January, February and March. Boxes denote 25th to 75th percentile. Whiskers and asterisks denote data range and outliers

The objective of this work was to experimentally determine tape grass growth rates within subtropical growth temperature ranges and develop experimentally based temperature–growth curves which could be used in a simulation model. The experimental design included varying salinity/light levels, and medium and small (i.e. rosette) plants to account for a range of potential responses within the subtropical estuarine environment.

Methods

Experimental setup and overall design

We grew medium (M) and small (S) plants in mesocosm tanks at five temperatures (13, 20, 25, 30, and 32 °C), under three combinations of light and salinity (low light and low salinity (0LS, 0LM), high light and low salinity (0HS, 0HM), and high light and high salinity (10HS and 10HM). A salinity of 10 psu is considered to be the upper limit for a sustainable population of tape grass (French and Moore 2003) and was used as the high salinity condition in this experiment. We maintained a saturating light level of 331 μE m^{-2} s^{-1} (mean at sediment depth) in the

Table 1 Experimental periods

Temperature treatment					
Conditions	13 °C	20 °C	25 °C	30 °C	32 °C
Low light 0 psu (0LM, 0LS)	03/28/08–06/03/08	03/13/07–05/07/07	06/06/07–08/09/07	09/13/07–11/07/07	01/24/08–03/21/08
High light 0 psu (0HM, 0HS)	03/28/08–06/02/08	03/13/07–05/04/07	06/06/07–08/08/07	11/01/08–12/29/08	01/24/08–03/19/08
High light 10 psu (10HM, 10HS)	03/28/08–06/03/08	03/13/07–05/08/07	6/06/07–08/10/07	09/13/07–11/09/07	01/24/08–03/20/08

0L, 0H are 0 psu, low and high light treatments. 10H is the 10 psu high light treatment. S and M denote small and medium plants

high light treatments and 42 $uEm^{-2}\ s^{-1}$ (mean at sediment depth) in the low light treatments for 12 h d^{-1}. In previous mesocosm experiments with tape grass from the Caloosahatchee estuary, growth was not observed in similar conditions of low light (40 $uEm^{-2}\ s^{-1}$, tank bottom mean) (Hunt, unpublished data), thus we did not include a treatment to represent these conditions (e.g. "10L"). The temperatures selected for the experimental treatments span the observed seasonal range in the Caloosahatchee estuary (Fig. 2). Experimental periods lasted for 8–9 weeks (Table 1). The maximum temperature tolerance was determined separately by slowly raising the water temperature from 25 °C to 39 °C as described below.

Plants were initially collected from identical water depths (80 cm) in the upper Caloosahatchee estuary using a PVC coring device. They were then placed in shallow containers and transported to outdoor holding tanks. Plants were allowed to grow in the outdoor tanks until they could be harvested for use in the temperature experiments. For the 10H treatments, we increased the salinity of an outdoor tank gradually to 10 psu over a 5 day period using water from an adjacent estuarine creek. We then determined wet weight, separated shoots by size and planted; three into each of 10 rectangular (30 by 18 by 15 cm) plastic tubs per tank containing sediment collected from the freshwater portion of the Caloosahatchee River. We then placed five replicate containers of each shoot size (small shoots were less than 0.5 grams wet weight, medium shoots were greater than one gram wet weight) in each of the three large indoor treatment tanks maintained at one of the five temperatures. We kept plants clean of epiphytes by gently wiping the affected leaves between fingers and thumb. After 8–9 weeks, we harvested the plants, washed off the sand, made final wet weight measurements of above and belowground biomass and dried the plant material in a drying oven at 60 °C to constant weight before measuring final dry weights. This procedure was repeated for each temperature.

Growth rate for each treatment combination was determined by subtracting initial from final plant dry weights in each tub (n = 5), dividing by the elapsed time in days and calculating the daily rate of increase (gdw $gdw^{-1}\ d^{-1}$). The dry to wet weight ratio for estimating the initial dry weight of the plants was determined using plants (15 of each size group) from the same source as the experimental plants. This ratio ranged between 0.05 and 0.08.

Mesocosm operations

We filled mesocosm tanks (vol = 1 m^3) with reverse osmosis treated city (Sanibel, Florida) water, which was filtered through a carbon block filter to remove chlorine and adjusted to the correct salinity with Instant Ocean®. We kept water levels in the tanks constant through addition of carbon filtered fresh water and maintained the salinity within 10 % of 10 psu in the 10H treatment by adding fresh water to compensate for evaporative loss. We reduced N and P concentrations and phytoplankton by circulating the water through a Red Sea Ocean Clear canister containing poly strand bio media biofilter and Phos-zorb (Mars Fishcare, Chalfont, PA, USA). Prior to each experiment, we tested water hardness, pH and Ca^{2+} levels and adjusted each as necessary to levels appropriate for submersed vascular plant growth (see Smart and Barko 1985). Water was circulated continuously between freshwater and salt water tank groups and within tanks to minimize environmental differences between treatments. We kept temperatures constant by using thermostatically controlled water chillers or 1,000 watt water heaters, and by adjusting room A/C settings.

We used fluorescent lights with an optimal spectral output for plant photosynthesis (Phillips ADV850), operating on a 12 h on/12 h off cycle. This 12 h light period ensures light-saturated growth in the high light treatments, but is a higher level than plants would receive in the estuary where light levels are only maximal at mid-day and low tide. Fewer bulbs and a layer of 50 % shade cloth (Shade-Rite, Green-Tek Inc., Janesville WI, USA) were used to adjust the light level for the 0L treatment.

Plant containers in each tank were shuffled twice weekly to minimize dependence of location within tanks. Initial, intermediate and final water column nutrient samples were taken, filtered, analyzed or frozen and subsequently analyzed using standard methods (APHA 1992). Water quality parameters (temperature and conductivity) were recorded at 30 min intervals with a Campbell Scientific CR10X datalogger and CS557A probes (Campbell Scientific, Logan UT, USA). We also measured conductivity, pH, dissolved oxygen (DO) and temperature weekly with a Hydrolab Quanta multiparameter probe (Hydrolab, Loveland UT, USA). Photosynthetically active radiation (PAR) measurements were taken weekly at the sediment surface in the center of each tank using a calibrated LI-COR spherical quantum sensor (LI-193) and LI-1400 datalogger (LI-COR Inc., Lincoln NB, USA).

Maximum temperature tolerance experiment

To determine the maximum temperature tolerance of tape grass, three shoots were placed in sandy sediment in each of six 30 L containers and kept under 331 $\mu E\ m^{-2}\ s^{-1}$ light conditions at 26–28 °C for two weeks. Water temperatures in three containers were increased by 1 °C every two days up to 35 °C, and then by 1 °C every 4 days until 39 °C was reached. The other three containers remained at 26–28 °C. Plants were examined for signs of senescence (loss of color) daily at the higher temperatures.

Analysis of growth data

Four factors are examined in this experiment: temperature, plant size, salinity, and light (Table 1). Because the 10 psu-low light combination was not tested, salinity and light in the design are not completely crossed with respect to the other factors. However, the three salinity-light combinations are completely crossed with respect to temperature and plant size. Therefore, a three factor ANOVA was conducted on growth rates (gdw $gdw^{-1}\ d^{-1}$) testing for the effects of temperature, plant size and SL treatment (S = salinity, L = light), and their interactions. In this analysis, temperature has five levels, plant size has two levels and SL treatment has three levels (0L, 0H, and 10H). The ANOVA was conducted using the GLM Procedure, SAS version 9 (SAS Institute Inc., Cary NC, USA). The three-way interaction between main effects was evaluated by conducting a one-way ANOVA in which each temperature, plant, and SL combination comprised a treatment. Differences between these treatments were examined using the Least Significant Difference (LSD) test.

In order to further examine treatment effects, additional evaluations were performed on the growth data. A two factor ANOVA was conducted on growth data testing for the effects of temperature, PLS treatment (P = plant size, L = light, and S = salinity), and their interaction. In this analysis, temperature has five levels and PLS has six levels. A two tailed Bonferroni post test (following Neter et al. 1990) was then conducted to determine significance of differences between each treatment and to test effect of plant size and growth. An additional statistical evaluation was performed on the growth data (using ANOVA) to determine whether the effects of treatments (plant size, light, and salinity) were significant at each temperature. These tests were conducted using Graphpad Prism (Graphpad Software, LaJolla CA, USA).

Growth/Temperature curves

The O'Neill equation was used with GraphPad Prism to fit curves to the growth vs. temperature information. O'Neill et al. equation:

$$k_t = k_{\max} U^x e^{(XV)} \tag{1}$$

where.

$$U = (T_{\max} - T)/(T_{\max} - T_{\mathrm{opt}}) \tag{1.1}$$

$$V = (T - T_{\mathrm{opt}})/T_{\max} - T_{\mathrm{opt}}) \tag{1.2}$$

$$X = W^2\left(1 + \left(\sqrt{1 + 40/W}\right)\right)^2 \Big/ 400 \qquad (1.3)$$

$$W = (Q_{10} - 1)(T_{\max} - T_{\mathrm{opt}}) \qquad (1.4)$$

In this formula, k_t is the growth rate at the temperature T, and $k_{\max}$ is the growth rate at the optimum temperature (T_{opt}). U, V, X and W are all unitless temperature effect variables. In the model, $T_{\max}$ was 38 °C as determined by the results of the maximum temperature tolerance experiment, and $k_{\max}$, Q_{10} and T_{opt} were calculated by the curve fit program. Q_{10} is a unitless factor by which a rate (e.g. growth) increases for every 10 °C rise in temperature. Q_{10} is k_2/k_1 in the Arrhenius equation ($\ln(k_2/k_1) = (E_a/R)(1/T_1–1/T_2)$). Where E_a is energy of activation, T is temperature, and k is the rate constant.

Results

Mesocosm conditions

Water temperatures ranged within 0.7–1.0 °C of the desired setting in each tank during each experiment. Salinities of the 0H and 0L treatments were less than 0.6 psu, and the 10H treatment ranged from 9.8 to 10.1 psu. The pH ranged from 7.7 to 8.2 in the freshwater tanks and from 7.9 to 8.4 in the 10 psu tank. Dissolved orthophosphate concentrations were below detection limits (0.15 mg/l) at the beginning and end of each experiment. Nitrate-nitrite concentrations were consistently below limits of detection (0.01 mg/l) and ammonium concentrations were less than 0.10 mg/l at the beginning and end of each experiment.

Differences between treatments

The ANOVA results show that treatments (light, salinity, plant size) had significant effects at each temperature (Table 2). The three-way ANOVA revealed significant effects of temperature (F = 352.6; df = 4,120; $p < 0.0001$), plant size (F = 10.3; df = 1,120; $p < 0.0001$) and salinity–light (SL) treatment (F = 288.7; df = 2,120; $p < 0.0001$). The three-way interaction between these factors was also statistically significant (F = 3.82; df = 8,120; $p < 0.0006$).

The one-way ANOVA treating each temperature-plant size-salinity–light combination as a separate treatment was also significant (F = 78.1; df = 29,120; $p < 0.0001$). Post-hoc comparisons of temperature-plant size-salinity-light means indicated 4 major logical groupings (Table 3). Plants grew fastest in freshwater (0 psu), at high light, with temperatures greater than 20 °C. Slowest growth was observed at 13 °C regardless of salinity, light or plant size. There were two intermediate groups. The 'next to slowest' group was comprised of the 10 psu, high light treatments grown at 20, 25 and 32 °C. The 'next to fastest' group contained all the freshwater, low light treatment combinations. Also included were the freshwater–high light-20 °C treatments and the high salinity-high light-30 °C treatments. A two-way ANOVA showed significant effects of plant treatment (size, light, and salinity levels), temperature and their interaction on growth rate with temperature having the most influence (Table 4).

Plant size did not have a pervasive effect on growth, although LSD evaluations indicate plants of different sizes grew differently under specific combinations of temperature, light and salinity. Within the fastest growing freshwater–high light group, small plants grown at 32 °C outpaced larger plants grown at the same temperature. In the freshwater–low light group, plant size had no measurable effect, with plants grown at the same temperature growing at similar rates regardless of size. Within the high salinity-high light group, small plants grew faster than medium plants at 20 and 30 °C but slower than medium plants at 13 °C. Bonferroni post tests showed that medium (M) and small (S) plants usually grew at statistically similar rates at the same temperature (Table 5).

Table 2 ANOVA results comparing growth rates of each treatment at each temperature.

Treatment	F
13 °C	15.2***
20 °C	41.1****
25 °C	130.7****
30 °C	5.4**
32 °C	56.2***

The results show that treatments (plant size, light, salinity) had significant effects at each temperature. $N = 5$. Determined by subtracting initial from final dry weights of plants in each container and determining daily growth in units of gdw gdw^{-1} d^{-1}

**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$

Table 3 Results of one-way ANOVA on growth rates (g dw/g dw/d) to evaluate the interaction between salinity and light combination (0H, 0L, 10H), plant size (S, M) and temperature (13, 20, 25, 30, and 32)

	LSD group		Mean	Treatment
	A		0.052	0HS32
	B		0.044	0HS25
C	B		0.040	0HM32
C	B		0.039	0HS30
C			0.037	0HM25
C			0.036	0HM30
	D		0.030	10HS30
E	D		0.028	0LM30
E	D		0.028	0LS25
E	D		0.027	0LS30
E	D	F	0.026	0LM25
E		F	0.024	0HS20
H		F	0.022	10HM30
H			0.021	0LM32
H			0.020	0LS32
H		I	0.020	0HM20
H		I	0.020	0LS20
H		I	0.018	0LM20
	J	I	0.015	10HM32
	J		0.012	10HS25
	J		0.011	10HS20
	J		0.011	10HS32
	J		0.011	10HM25
	K		0.006	10HM20
	L		0.000	0LM13
	L		0.000	0LS13
	L		−0.001	0HS13
	L		−0.001	0HM13
	L		−0.005	10HM13
	M		−0.013	10HS13

Means were compared using the LSD test. The bold horizontal lines in the table delineate the four logical groupings (see text)

Table 4 Two-way ANOVA (F values) conducted on growth data for the medium and small plants

Treatment	Plant growth
Temperature	332.5****
PLS	113.9****
Temperature and PLS	11.9****

There was significant interaction between temperature and all other treatment variables. $N = 5$. Determined by subtracting initial from final dry weights of plants in each container and calculating daily growth in units of gdw gdw^{-1} d^{-1}. PLS represents plant size, light level, and salinity level

**** $p < 0.0001$

Table 5 Bonferroni post-test results (t values) for test of effect of plant size on growth

Temperature	0H	0L	10H
13 °C	0.19 NS	0.10 NS	2.66*
20 °C	1.75 NS	0.56 NS	1.49 NS
25 °C	2.70*	0.91 NS	0.28 NS
30 °C	0.71 NS	0.56 NS	3.26**
32 °C	4.96***	0.20 NS	1.75 NS

In the low light treatment, no significant effect of plant size on growth was detected at any level of temperature. For the high light treatments there was an effect at two of the five temperatures tested in both low and high salinity treatments, although the effect is at different temperatures. The effect of these results on the shape of the temperature curves are illustrated in Fig. 4. $N = 5$. Determined by subtracting initial from final dry weights of plants in each container and calculating daily growth in units of gdw gdw^{-1} d^{-1}

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, NS = non-significant ($p > 0.05$)

Table 6 Growth-temperature curve values determined by O'Neill equation curve fit

		Coefficient	
Treatment	k_{max}	T_{opt}	Q_{10}
0HS	0.0497	28.22	2.35
0HM	0.0465	27.78	2.45
0LS	0.0316	26.98	2.58
0LM	0.0291	27.68	2.43
10HS	0.0351	28.64	2.94
10HM	0.0194	29.47	2.23

0L, 0H are 0 psu, low and high light treatments. 10H is the 10 psu high light treatment. S and M denote small and medium plants. k_{max} is the growth rate (gdw gdw^{-1} day^{-1}) at the optimum temperature (T_{opt}). T_{opt} is in units of °C. Q_{10} is unitless

Growth–temperature curves

Specific attributes of the growth–temperature curves such as slopes or curve parameters, can be used to describe general growth characteristics. For example, the increase in rate of growth per 10 °C rise in temperature is represented by the Q_{10} parameter

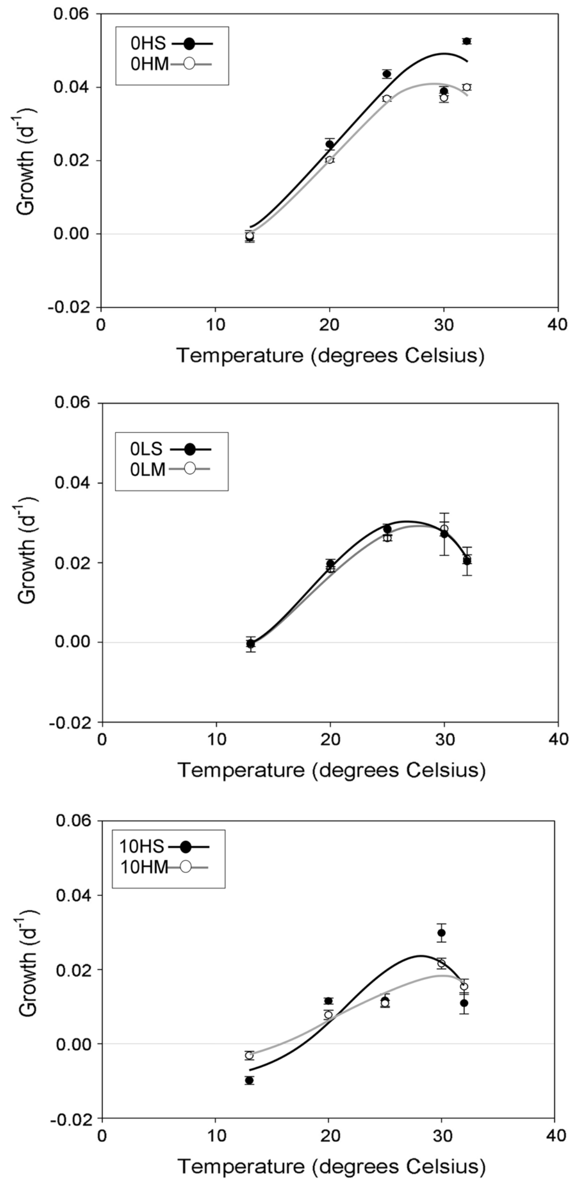

Fig. 3 Growth-temperature curves (mean and SE), for freshwater-high light (upper panel), freshwater-low light conditions (middle panel) and the 10 psu–high light treatments (lower panel). Growth was calculated from the change in dry weight over the course of the experiment, divided by the elapsed time. Curves were fit using the equation of O'Neill et al. (1972). The threshold maximum temperature (T_{max}) was set at 38 °C, and k_{max}, Q_{10} and T_{opt} were calculated by the curve fit program. S and M denote small and medium plant treatments, respectively. In freshwater conditions the plants tolerated exposure to 13 °C but did not grow indicating the low temperature threshold. In contrast, leaf senescence resulted at 13 °C in the 10 psu treatments and a low temperature survival threshold can be estimated at approximately 17 °C

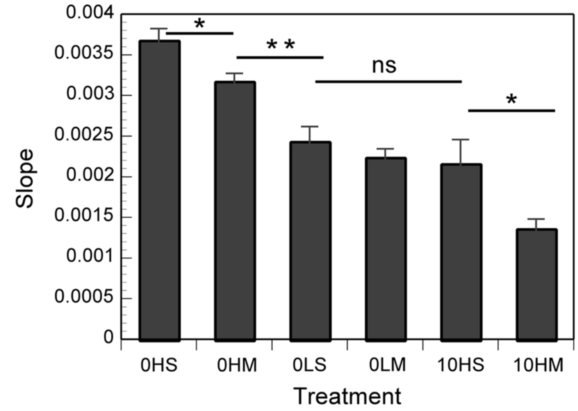

Fig. 4 Initial slopes of growth-temperature curves for different treatments. Slopes were calculated by regression of growth rates vs. temperature at 13–25 °C and units are gdw gdw^{-1} day^{-1}. Standard error bars are shown. Linear regression was used to compare the initial slopes of the growth–temperature curves. Significant differences (T test with Welch correction) are shown by $*p < 0.05$, $**p < 0.01$, while "ns" denotes no significant difference. 0L, 0H are 0 psu, low and high light treatments. 10H is the 10 psu high light treatment. S and M denote small and medium plants. Results show slopes are lower for the low light and high salinity treatments relative to more ideal conditions (0H treatments)

values in the O'Neill equation (Table 6). These values were fairly uniform (approximately 2.5) for the fresh water conditions irrespective of light treatment or plant size. However, for the elevated salinity conditions, Q_{10} appeared higher for small than for medium plants. This difference is reflected in the different shapes of the growth–temperature curves (Fig. 3).

Initial slopes of growth–temperature curves (Fig. 4) can also be compared to evaluate general growth characteristics. This comparison indicates the steepest slopes for the 0H treatments, followed by the 0L treatments, then the10H treatments. Small but significant differences in initial slopes were apparent between small and medium plants within 0 and 10H treatments.

This study indicates optimum temperatures of Caloosahatchee tape grass to be in the range 27–29 °C (Fig. 3, Table 6). The low temperature threshold in fresh water is at the point of zero growth at 13 °C. The low temperature tolerance in the saline 10H treatment is 17 °C as estimated by the x-intercept (Fig. 3).

In the experiment to test the tape grass maximum temperature tolerances, all shoots remained intact and appeared viable at temperatures up to 37 °C. Leaves of seven out of nine plants were damaged (clear and soft) or detached after the temperature had been at 38 °C for two days. After 4 days at 39 °C, the leaves of all plants were damaged or detached, and roots were also damaged. Based on these results, the temperature maximum of 38 °C was used to develop the growth–temperature curves (Fig. 3).

Discussion

In order to better understand the effects of freshwater inflow on tape grass growth and survival, information on the response of tape grass to combinations of salinity, light, and temperature is necessary. Our experiments provided quantitative response curves for temperature under different environmental conditions and information on the interaction of these environmental variables that will help enable improved predictions of tape grass growth and survival.

Observations and differences between treatments

The ANOVA results showing the three-way interaction between treatments (light, salinity, plant size) demonstrate the importance of assessing light and salinity effects with respect to temperature to interpret temporal patterns in the growth of tape grass in the Caloosahatchee estuary. The four logical groupings of treatment combinations identified by the LSD test in Table 3 indicate a hierarchy of factors that may control growth rates of tape grass in the Caloosahatchee. As expected, highest growth rates generally occurred in fresh water with plants grown at high light growing faster than those at low light (e.g. Barko et al. 1982). In general, plants grown at 10 psu and high light grew slower than those in fresh water. Again, this is not an unexpected result based on the literature (e.g. French and Moore 2003). Our results indicate that the salinity effect was at least partially ameliorated at 30 °C. High salinity–high light plants grew as fast as those in freshwater and low light at this temperature; falling in the second grouping (Table 3). This result suggests that in addition to high light (see French and Moore 2003) an optimal temperature for growth may also help alleviate stress due to high salinity. The fact that the slowest growth rates occurred at 13 °C regardless of plant size, salinity or light level indicates that relative to other factors tested, the low temperature condition had an overwhelming effect on growth of tape grass from the Caloosahatchee.

Our results show interactions between all the tested variables (Table 4) and thus highlight the importance of controlled studies assessing multiple variables and stressors for SAV. Though plants in the present study were significantly different in size at the start of the experiment, they became more similar in size by the end. This is not unexpected because of the controlled experimental conditions (i.e. constant depth, constant light, temperature etc.). In the field, larger differences in growth coefficients may be expected within beds where longer leaves can intercept more light than short leaves.

Temperature effects on growth

The temperatures tested were within both the daily and monthly average ranges during the period January 1992 through January 2011 in the Caloosahatchee estuary (Fig. 2). As shown by the growth–temperature curves (Fig. 3), growth in the 0H treatments followed a seasonal pattern with growth increasing with temperature up to an optimal value, followed by a rapid decrease.

Temperature mainly affects growth of plants over predictable seasonal cycles, with more influences at temperature extremes possible at or near the low and high thresholds (Box 1995). Prior to this work the information relating temperature and growth reported for tape grass was derived from studies in freshwater systems; using plants from temperate rather than subtropical climates.

In temperate environments tape grass displays a seasonal growth pattern with biomass peaking in the summer, followed by the production of vegetative tubers in the fall, and dormancy during the winter. Consistent with the southern ecotype of tape grass reported by Smart and Dorman (1993), beds in typical southwest Florida environments are present year-round, and tubers or overwintering buds have not been documented in the Caloosahatchee estuary (Doering et al. 1999). However, assuming conditions are suitable for growth, tape grass growth follows a seasonal pattern in South Florida's estuaries, with small rosette plants during the winter and larger, more densely spaced plants in the summer (e.g. Bortone and Turpin 2000). Interestingly, in Kings Bay, Florida, a spring-fed estuary with constant year-round water temperature ($\sim$25 °C) and salinity, seasonal variation in tape grass biomass was not apparent and values were 5–20 times greater than in the Caloosahatchee (Hauxwell et al. 2007). Thus, the removal of the seasonal temperature variation appears to allow densities to remain high year-round and illustrates the influence of the seasonal temperature variation on the annual pattern of growth for tape grass in coastal Florida.

The optimal growth temperature of submersed macrophytes at maximum light-saturated photosynthesis is considered to increase with latitude (Santamaria and van Vierssen 1997). In the Detroit River, tape grass grew at water temperatures ranging from 19 to 31.5 °C (Hunt 1963). Titus and Adams (1979) reported a temperature optimum for tape grass obtained from University Bay, WI, of 32.6 °C. In contrast, this study of Caloosahatchee tape grass shows optimum temperatures to be in the range 27–29 °C (Table 6, Fig. 3). This temperature range occurs in late spring and early fall.

The maximum growth rate in our experiment ($\sim$5 % d^{-1}) is comparable to that found for *Potamogeton crispus* but is slightly below that of other submersed plant species (see Nielsen and Sand-Jensen 1991). However, in the Caloosahatchee the submersed light levels are rarely ideal for plants and the maximum rate of growth would not be expected over long periods. The treatment levels for high salinity (10H) and low light (0L) were chosen based on previous tape grass studies in the Caloosahatchee estuary where tape grass survived, but signs of stress were observed (Doering et al. 1999, 2001; Hunt et al. 2004). These treatment levels are also within the observed ranges in the estuary.

High temperature affected the survival of tape grass in our study. During mid-summer in the Caloosahatchee estuary, combined effects of temperature stress and low light levels could result in decreased plant production. Compared with other submersed plants reported to have temperature maxima near 40–45 °C (e.g. Campbell et al. 2006), tape grass is relatively sensitive to high temperature exposure. Hutorowicz and Hutorowicz (2008) reported reduced biomass and growth of *Vallisneria spiralis* at high summer temperatures (to 31 °C) in a thermally influenced lake in Konin, Poland; a result similar to ours. There are however, some submersed plant species found in temperate environments that have even greater sensitivity to typical summer temperatures. For example, *Zostera marina* is reported to grow drastically slower during the summer (e.g. Moore et al. 1996) and *Posidonia sinuosa* has a reported optimum temperature in the range 18–23 °C (Masini et al. 1995). As global temperatures continue to rise, SAV species that have narrow temperature tolerance ranges may experience greater stress, especially if high temperature anomalies occur. Species of SAV that have relatively low temperature maxima such as tape grass, could also be negatively affected seasonally in areas receiving thermal effluents from power plants.

A zero growth rate was observed at 13 °C in both light treatments in fresh water and thus we estimate the low temperature threshold for Caloosahatchee tape grass in freshwater conditions is near this value. Leaf senescence was observed in the 10H treatments at 13 °C and we estimated the low temperature tolerance for these treatments by the x-intercept to be near 17 °C (Fig. 3). Our results were consistent with those of Barko et al. (1982, 1984) who reported the growth of commercially obtained plants was severely restricted below 20 °C in freshwater.

Combined environmental effects on growth in dry season

An interesting finding was that under elevated salinity, growth at 20 °C was significantly lower than in freshwater with comparable light levels (Table 3). These findings show that tape grass plants exposed to elevated salinity are more sensitive to low temperature exposure and may not survive as long at low temperatures as plants in freshwater conditions.

Within a 19 year period, the average daily water temperature in the Caloosahatchee during the coldest months (December through February) ranged from 12 to 25 °C (Fig. 2). However, daily temperatures of $\leq$13 °C (the observed low temperature threshold) were observed in only six of the 19 years from 1992 to 2011 and persisted for only short periods (<5 days, data not shown). Based on the results of this study, elevated salinity conditions ($\geq$10 psu) in the winter months, in combination with colder temperatures ($\leq$20 °C), would be detrimental to tape grass survival.

Our findings indicate that growth at 10 psu was reduced relative to conditions of fresh water except at 13 and 30 °C (Table 3). The reduced growth at 10 psu is consistent with previous tape grass studies in the

Caloosahatchee estuary (Kraemer et al. 1999; Doering et al. 1999, 2001). In an outdoor experiment in Virginia, tape grass flowering was reduced and light requirements were higher at elevated salinities of 5–15 psu (French and Moore 2003). Similarly, in a field based study conducted in the St. John's River, Florida (Dobberfuhl 2007), plants from oligohaline and mesohaline sites (0.5 > 6 psu annual mean) had a higher mean light threshold compared to plants from low salinity sites (<0.5 psu annual mean). Plants from the St. Johns River survived for five months in mesocosms at 8 psu (Boustany et al. 2010). Salinities are normally elevated in the upper Caloosahatchee estuary during the dry season and occasionally during the wet season in drought years.

Combined environmental effects on growth in wet season

Quantifying the effects of low light at low salinities (0L treatments) is relevant to tape grass survival in the Caloosahatchee because highly colored water reduces light levels to well below 25 % of surface irradiance at 1 m depth in the upper estuary during the wet season (Doering and Chamberlain 1999; Bartleson 2010). The growth rates in the low light treatments (Fig. 3) indicate that tape grass can grow under conditions of relatively low light when temperature and salinity are suitable. During wet season, water temperatures in the Caloosahatchee are similar to our 30 °C 0L treatments (e.g. Bortone and Turpin 2000). Decreasing salinities in the estuary during late spring to early summer, concurrent with a temperature increase, would be conducive to high growth rates needed to increase leaf lengths of the small, overwintering rosettes if light levels were not concurrently falling because of increasing levels of color. While tape grass can survive at low light levels, the finding that significantly higher growth occurs at higher light levels indicates that periods of relatively high water transparencies may benefit the plants in the estuary. Higher growth rates during the warm months would allow leaves to extend closer to the surface, capture more light, and increase energy storage for vegetative growth and sexual reproduction. Higher water transparencies would also increase the depth range that plants could colonize and thus increase acreage covered and wildlife habitat value.

Conclusions

Though seasonal differences in tape grass growth have been reported for salinities below 10 psu, except for a few studies (French and Moore 2003; Dobberfuhl 2007, Hunt 2007), there has been little information on the interactive effects of both light and salinity on tape grass physiology (see discussion in Doering et al. 1999). None could be found that quantitatively examined salinity, light and temperature in one study.

Our results show that tape grass growth is influenced by water temperature and that additional stressors such as low light and elevated salinity can reduce the range of temperature tolerance, especially at colder water temperatures. In the Caloosahatchee estuary, the oscillation between conditions of low light- low salinity in the warm wet season and high light- high salinity in the colder dry season, may influence the year-round survival of tape grass beds. Given the expected water temperature range, a seasonal pattern of growth would be expected with higher biomass and longer leaves in the warm, wet season and smaller, sparser beds of rosettes in the colder, dry season. However, a period of ideal conditions consisting of both relatively fresh water and high water transparency may be necessary in the early summer months when temperatures are near 25 °C to achieve dense beds. The temperature–growth curves developed here, which span the range of tolerable light and salinity conditions, will allow improved predictions of tape grass populations under varied environmental conditions within the Caloosahatchee estuary. The temperature tolerance values obtained from the experiments as well as the optimum temperature and Q_{10} curve parameter (Table 6) values could also be used to develop site specific temperature curves in other systems. The durations of our experimental periods were relatively long compared to most other laboratory studies (e.g. Barko et al. 1982; Korschgen et al. 1997) and thus may provide information useful in the development and application of tools to allow predictions consistent with this timescale. This time period is also consistent with the frequency of measurements included in typical SAV monitoring programs and may aid in interpreting changes in biomass or densities detected over the course of the year.

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