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# *Thalassia testudinum* seedling responses to changes in salinity and nitrogen levels

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#### Abstract

The dominant seagrass in Florida Bay, Thalassia testudinum Banks ex König, is a stenohaline species with optimum growth around marine salinity (30-40 PSU). Previous studies have examined the responses of mature short shoots of T. testudinum to environmental stresses. Our goal was to assess responses of seedlings to changes in water chemistry in Florida Bay that might occur as part of the Comprehensive Everglades Restoration Plan (CERP). Specifically, we examined seedling survival, growth, photosynthesis, respiration and osmolality in response to hypo- and hyper-salinity conditions, as well as possible synergistic effects of depleted and elevated ammonium concentrations. The study was conducted in mesocosms on T. testudinum seedlings collected during August 2003 near Florida Bay. Hyper- and hypo-saline conditions were detrimental to the fitness of T. testudinum seedlings. Plants at 0 and 70PSU exhibited 100% mortality and a significant decrease in survival was observed in the 10, 50 and 60PSU treatments. Increased levels of ammonium further decreased growth in the lower salinity treatments. Seedlings in 30 and 40PSU had the greatest growth. Quantum yield and relative electron transport rate, measured using PAM fluorometry, showed a decrease in photosynthetic performance on either side of the 30-40 PSU optimum. Tissue osmolality decreased significantly with decreased salinity but tissue remained consistently hyperosmotic to the media across all salinity treatments. Maintaining negative water potential and allocating more energy to osmoregulation may decrease the productivity of this species in salinity-stress conditions. Our results suggest that the salinity-tolerance limits of this seagrass at the seedling stage are not as broad as those reported for mature plants. Increased fresh water inflow, especially if co-occurring with an increase in water-column ammonium, could negatively affect successful recruitment of T. testudinum seedlings in northern regions of Florida Bay. © 2006 Elsevier B.V. All rights reserved.

Keywords: Ammonium; Osmolarity; Photosynthesis; Salinity; Seedlings; Thalassia testudinum

## 1. Introduction

Declines and die-offs of seagrasses are generally linked with decreased water quality which may be

associated with human influence or naturally occurring variations in the environment (EPA, 1990; Short and Wyllie-Echeverria, 1996; Gunderson, 2001; Rudnick et al., 2005). Die-off and an overall decline in fitness of the seagrass beds in Florida Bay has lead to numerous ecological repercussions (Butler et al., 1995). Seagrass species abundance and distribution in Florida Bay vary based on the fluctuating environmental and hydrological conditions (Fourqurean et al., 2002). Southern Florida

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has undergone many hydrological changes over the course of the last century due to the construction and installation of integrating levees, pumps and canals (Sklar et al., 2002). A new management plan, part of the Comprehensive Everglades Restoration Plan (CERP). proposes to redirect water flow in south Florida in an effort to increase freshwater input to the Everglades and Florida Bay and restore the natural hydrology (CERP. 2001; Rudnick et al., 2005). The patterns of changes in water chemistry upon altering the water flow are difficult to predict due to the complex hydrology of the Bay. Florida Bay is not one large basin, but an intricate series of small, shallow basins surrounded by mud banks and mangrove islands with an average depth of 2m (Lee et al., 2002). Florida Bay undergoes seasonal fluctuations of both hypo- and hyper-salinity periods (Fourgurean and Robblee, 1999), which influence benthic habitat composition (Montague and Ley, 1993; Fourgurean et al., 2003). The planned increase in freshwater inflow will change water quality for portions of Florida Bay by affecting factors such as salinity and total nitrogen as well as other chemical and biological parameters (Brand, 2002).

It is unclear how rapidly seagrasses adapt to changes in environmental conditions or what their tolerance limits are at the seedling stage. Although much research has focused on how biotic and abiotic factors affect mature seagrasses (Zieman, 1975; Carlson et al., 1994; Durako and Kuss, 1994; Doering and Chamberlain, 2000; Koch and Erskine, 2001; Cruz-Palacios and van Tussenbroek, 2005; Koch et al., submitted for publication), there is little information on the effects of these factors on seedling recruitment, growth or survival (Durako and Moffler, 1981, 1987b). Although seagrasses are clonal organisms, sexual reproduction plays a key role in increased genetic variation and recruitment (Laushman, 1993; Schleuter and Guttman, 1998; Williams and Orth, 1998). Changes in the environment can greatly affect sexual reproduction in seagrasses (Orth and Moore, 1983; Durako and Moffler, 1987a; Koch and Dawes, 1991). Thalassia testudinum Banks ex. König was previously thought to have been solely clonal, but it has been found that sexual reproduction and the release and survival of fruits and seedlings is important for the persistence, expansion and recovery of seagrass beds (Kaldy and Dunton, 1999; Whitfield et al., 2004).

This study considered the possible impacts a change in water chemistry in Florida Bay may have on the recruitment of seedlings of the dominant marine seagrass species. *T. testudinum* was chosen for this study due to its present dominance in Florida Bay and marine-salinity optimum, around 30-40PSU (Zieman, 1975; Rudnick et al., 2005). Amplitude and duration of salinity change may affect seed viability and the vitality of young plants based on whether their physiological and anatomical adaptations are developed enough to acclimate to these changes, especially since osmoregulation in T. testudinum is an active process requiring ATP (Jagels, 1973). Ammonium was chosen as the manipulated nutrient due to its expected fluctuations in the water column in response to changing freshwater flow (Rudnick et al., 1999). As submerged macrophytes, seagrasses are susceptible to changes in water ammonium levels because ammonium is readily absorbed through the leaves (Stapel et al., 1996; Lee and Dunton, 1999), but has been found to be toxic at fairly low levels in other submerged macrophytes (Smolders et al., 1996; Katwijk et al., 1997).

Specifically, this study evaluated *T. testudinum* seedling growth and survival under increased and decreased levels of salinity and decreased and increased levels of ammonium. The following questions were addressed: 1) will hyper- and hypo-salinity conditions differentially affect *T. testudinum* seedling survival, growth, respiration, photosynthetic rates, or tissue osmolality, and 2) will the physiological responses of *T. testudinum* seedlings to varying levels of salinity be affected by increased or decreased levels of ammonium?

# 2. Materials and methods

## 2.1. Collection

*T. testudinum* seedlings were collected on August 3, 2003. Seedlings were collected from fruits floating in rack lines along the eastern shoreline of Tavernier Key (25°01.57'N, 80°29.67.00'W), an area that exhibits typically oceanic salinity around 35PSU. Seedlings were kept in ambient seawater and transported to Wilmington, North Carolina. Upon returning to the University of North Carolina Wilmington Center for Marine Science (UNCW/CMS) they were placed in a fiberglass mesocosm located in a seawater-supplied greenhouse for 1 week at a salinity of 30PSU to allow seedlings to acclimatize to the greenhouse light and temperature conditions.

#### 2.2. Experimental design

Following transport to UNCW/CMS, initial measurements of root length and blade length and width were made of all seedlings before they were planted in aragonite shell hash in individual  $5 \times 5 \times 10$  cm<sup>3</sup> plastic pots. Planted seedlings were placed in 30-1 aquaria containing gravel-bed filters and aeration systems. The aquaria were located in a temperature-controlled (23-29°C, variation between nighttime and daytime temperatures) greenhouse with additional overhead lights providing ~10% increased daily PPFD (eight 500W metal-halide lamps, 14:10h L:D, yielding an average mid-day PPFD of  $1267 \mu mol photons cm^{-2} s^{-1}$ ). After 7 days, ten seedlings remained in the 30 PSU treatment and the remaining tank salinities were changed by 2PSU every 3 days. This increment was chosen to emulate field conditions (Baskin and Baskin, 1998; Kahn and Durako, 2005). Instant Ocean<sup>©</sup> salts were used to increase salinity and DI water was added to decrease the salinity. The upward and downward acclimations were performed until ten seedlings were at each of the following salinity treatments: 0, 10, 20, 30, 40, 50, 60 and 70PSU. For each of these salinity treatments, there were three ammonium treatments: 0, 10 and 20µM NH<sub>4</sub><sup>+</sup>. Ammonium was added in the form of NH<sub>4</sub>Cl and levels monitored twice a week using the Koroleff reagent wet chemistry technique (Koroleff, 1969). The control was considered 30PSU with ambient  $(10 \mu M)$ ammonium conditions.

#### 2.3. Morphometric measurements

Morphometric characteristics were measured weekly for the duration of the experiment. Individual shoots were measured and total leaf lengths calculated. Blade width for each leaf was measured just above the sheath and average blade width was multiplied by total blade length to calculate shoot total leaf area. A seedling was considered dead when all blades fell from the sheath or 100% of blades were chlorotic (no green tissue remaining).

# 2.4. Fluorescence

Photosynthetic rates were measured using PAM fluorometry (Walz GmbH Mini-PAM<sup>©</sup>) when all seedlings had reached treatment salinities as well as after 1 month in the treatment salinities. Rapid light curves (RLC) were used to calculate relative electron transport rate (rETR=Yield\*PPFD\*0.5\*0.84), and effective quantum yields of each seedling (Beer et al., 1998; Ralph and Gademann, 2005). The PAM settings were as follows: MI=6, SI=4, SW=0.8, Gain=3 LW=0:10. The rapid light curve program produced eight discrete irradiance levels: 105, 180, 287, 401, 604, 830, 1272 and 1866 µmol photons m<sup>-2</sup> s<sup>-1</sup>, plus an initial quasi-darkness measurement with no artificial actinic

light. Electron transport rate was considered relative due to the instrument-default absorbtance factor of 0.84 being applied. This absorptance factor value has been found to be higher than absorbtances measured in seagrasses (Beer and Björk, 2000; Durako and Kunzelman, 2002; Runcie and Durako, 2005). Relative electron transport rates were generated using WIN Control<sup>©</sup> software (Walz GmbH). rETR vs. irradiance curves were fitted to a hyperbolic tangent function (Platt et al., 1980) using a non-linear curve fitting algorithm (SigmaPlot, SPSS, California), from which  $\alpha$  and rETR<sub>max</sub> (µmole<sup>-</sup>m<sup>-2</sup>s<sup>-1</sup>) were calculated (where  $\alpha$  is the rate of change to rETR<sub>max</sub>). To eliminate diel variation effects among treatments, all measurements were taken between 2 and 4 pm and the plants were chosen arbitrarily.

#### 2.5. Osmolality

At the end of the experiment, osmolality of the leaf tissue was measured using a Wescor VAPRO Vapor Pressure Osmometer Model 5520<sup>©</sup>. The osmometer measured the total concentration of dissolved particles (osmolality) via a measurement of vapor-point depression. When osmolality of solid samples such as leaf tissue is measured, a time delay must be determined to allow equilibrium (vapor and thermal) to be reached which is based on tissue type. To determine the appropriate time-delay, the protocol of Murphy et al. (2003) was used. Fresh leaf-tissue samples from seedlings of the same age grown in ambient seawater were used and osmolality measured every 2min for 30min. After 10min, there were no significant differences between measurements (data not shown). Seedling leaf tissue was cut underwater with a 0.25-in.diameter hole-punch. When the tissue width was too small to cover the osmometer sample holder, two halfcircle pieces were used. To determine the comparative osmolality of leaf tissue relative to the treatment seawater media, osmolality measurements were made on 10µl of media for each salinity treatment.

#### 2.6. Respiration

At the termination of the experiment (1 month after all seedlings had reached treatment salinity) seedling leaf respiration was measured using a Hansatech<sup>©</sup> oxygen electrode. After the final morphometric measurements were obtained, a 1 cm<sup>2</sup> piece of *T. testudinum* seedling blade (cut underwater) was placed within a Hansatech DW1 chamber, filled with 2 ml media of treatment salinity at 25 °C. The chamber was kept in the dark for 20 min and the level of oxygen in the chamber recorded

Average survival in days of Thalassia testudinum seedlings in aquarium tanks at three levels of ammonium and eight treatment salinities over the 116
day experimental period (±S.D.)

Salinity	0	10	20	30	40	50	60	70
0μM NH <sup>+</sup>	85 (±28)	100 (±33)	116 (±0)	116 (±0)	116 (±0)	116 (±0)	91 (±19)	74 (±6)
10μM NH <sup>+</sup>	83 (±30)	114 (±5)	116 (±0)	116 (±0)	106 (±27)	93 (±37)	65 (±27)	76 (±10)
20μM NH <sup>+</sup> 4	76 (±13)	85 (±45)	116 (±0)	115 (±4)	116 (±0)	84 (±31)	97 (±15)	74 (±12)

every 5 min. Respiration rate  $(\mu molO_2 cm^{-2} h^{-1})$  was calculated as the slope of oxygen level versus time for the linear portion of the incubation.

#### 2.7. Statistical analyses

Data were statistically analyzed using SigmaStat  $2.0^{\circ}$  (Jandel Scientific, California) and SAS<sup> $\circ$ </sup> (Cary,

North Carolina) statistical software. The effect of salinity on seedling growth and photosynthesis was assessed by one-way ANOVAs. Tukey's post hoc multiple pair-wise comparison test was applied to determine wherein the differences lay when  $\alpha < 0.050$ . For cases where normality failed, transformations were performed. Were the transformations unsuccessful, a Kruskal– Wallis one-way ANOVA on ranks was run followed by



Fig. 1. Average total leaf area (cm<sup>2</sup>) of *Thalassia testudinum* seedlings over the experimental period for three salinity ranges: high (50–70 PSU, black circles), mid (20–40 PSU, white circles) and low (0–10 PSU, black triangles) for each ammonium treatment: a)  $0\mu$ M, b)  $10\mu$ M, and c)  $20\mu$ M.

Table 1

Dunn's multiple pair-wise comparisons. For repeatedmeasures analysis, linear repeated-measures analysis of covariance (SAS PROC-mixed) was used.

## 3. Results

#### 3.1. Survival and growth

Seedling survival was greatest in the 10, 20, 30, and 40 PSU treatments followed by the 50 PSU treatments at all ammonium levels (Table 1). Mortality was high in both the extreme hypo- and hyper-saline treatments (0, 60 and 70 PSU). To determine the morphological effects of hyper- versus hypo-salinity, plants were grouped into three salinity categories. "Low" salinities (=hyposaline) were 0-10 PSU, "mid" 20–40 PSU, and "high" (=hy-

persaline) 50-70PSU. By week 4, salinity had a significant impact on leaf area, total blade length and leaf width ( $F_{2,231}$ =5.33, 3.58, 10.76; P=0.0054, 0.0294 and <0.0001, respectively). At all ammonium levels, mid-salinity treatment plants had significantly greater total leaf area than the low- or high-salinity plants  $(F_{2,231}=26.66, P < 0.0001)$  (Fig. 1a, b, c). This was due to the combination of significantly greater total blade lengths (F<sub>2.231</sub>=21.28, P<0.0001) (Fig. 2a, b, c) as well as increased blade widths  $(F_{2,231}=3.09, P=0.0473)$  (Fig. 3a, b, c). At mid salinities, blade widths showed no significant differences among the three ammonium levels. There was, however, a decrease in total leaf area in the 0 µM ammonium, mid-salinity treatments due to significant decreases in blade length  $(F_{2,231}=6.67,$ P=0.0015). Blade widths were narrower in the high



Fig. 2. Average total blade length (cm) of *Thalassia testudinum* seedlings over the experimental period for three salinity ranges: high (50–70 PSU, black circles), mid (20–40 PSU, white circles), and low (0–10 PSU, black triangles) for each ammonium treatment: a)  $0\mu$ M, b)  $10\mu$ M, and c)  $20\mu$ M.



Fig. 3. Average blade width (cm) of *Thalassia testudinum* seedlings over the experimental period for three salinity ranges: high (50–70 PSU, black circles), mid (20–40 PSU, white circles) and low (0–10 PSU, black triangles) for each ammonium treatment: a)  $0\mu$ M, b)  $10\mu$ M, and c)  $20\mu$ M.

salinity treatments at all ammonium levels and the low salinity treatments showed a decrease in blade width at all ammonium treatments as well. At low salinities, blade widths and total leaf areas were significantly less in the  $20\mu$ M ammonium treatment than the  $10\mu$ M or  $0\mu$ M treatments ( $F_{2,231}$ =4.14, P=0.0171). Overall, the high ammonium treatment had a negative impact on plants at low salinities and the low ammonium treatment had a negative impact on the plants at mid-range salinities. The overall apparent decrease in leaf growth towards the end of the experimental period is indicative of leaf turnover.

#### 3.2. Fluorescence

 $\alpha$  values were significantly greater ( $F_{1,169}$ =16.083, P<0.001) upon reaching treatment salinity (initial) than

1 month later (Fig. 4). Initial  $\alpha$  values were significantly greater ( $F_{5.118}$ =9.117, P<0.001) in the 40 and 30PSU treatment compared to the 0 and 10PSU treatments indicating higher efficiency of achieving rETR<sub>max</sub> at near-seawater salinities. Initial values for rETR<sub>max</sub> across salinities were significantly greater ( $F_{2.118}$ = 3.081, P=0.05) in the 0  $\mu$ M ammonium than 20  $\mu$ M ammonium treatment (Fig. 5). Values for initial rETR<sub>max</sub> across ammonium treatments for the 30 and 20PSU treatments were significantly greater than the 0, 10 and 50 PSU treatments ( $F_{5,118}$ =11.220, P<0.001). Neither rETR<sub>max</sub>, nor  $\alpha$  values were significantly different among treatments after 1 month at treatment salinity and ammonium (data not shown). After 1 month in the treatments, only plants from 10-50PSU survived for measurement. None of the end-members survived and in



Fig. 4. Alpha values for *Thalassia testudinum* seedlings upon reaching treatment salinity for each ammonium treatment: 0µM (black), 10µM (light grey), and 20µM (dark grey) (±S.D.). Numbers above bars represent number of replicates for each ammonium level.

the remaining hyper- and hypo-saline treatments, only a portion of the plants survived.

#### 3.3. Osmolality

Salinity, with ammonium treatments pooled, had a significant impact ( $F_{4,66}$ =242.025, P<0.001) on osmolality at each treatment salinity level (Fig. 6). Ammonium, with salinity treatments pooled, had little impact on osmolality, but it did have a significant interactive

effect with salinity. At the lower ammonium levels, osmolality in the acclimated tissue was more similar among the 30, 40 and 50 PSU treatment plants than among the same treatments at elevated ammonium levels. In the absence of ammonium (0µM), there was a significant increase in osmolality ( $F_{4,24}$ =105.459, P<0.001) with increasing salinity except between 30 and 40 PSU. For the 10µM ammonium treatment there was a significant increase in osmolality ( $F_{5,24}$ =426.378, P<0.001) with increased salinity except between 40



Fig. 5. rETR<sub>max</sub> for *Thalassia testudinum* seedlings upon reaching treatment salinity for each ammonium treatment: 0µM (black), 10µM (light grey), and 20µM (dark grey) (±S.D.). Numbers above bars represent number of replicates for each ammonium level.



Fig. 6. Osmolality of *Thalassia testudinum* seedling tissue at each treatment salinity for each of the three ammonium treatments:  $0\mu$ M (black),  $10\mu$ M (light grey), and  $20\mu$ M (dark grey) (±S.D.), n=5 unless otherwise noted.

and 50PSU. For plants in the 20 $\mu$ M ammonium treatment there was a significant difference among some treatments ( $F_{4,18}$ =35.255, P<0.001), although not as many as in the other two ammonium treatments. Osmolalities of tissues from the 40PSU treatment were not significantly different than at 50PSU or 30PSU. Osmolality of plants at 50PSU was not significantly different than in plants at 60PSU and osmolality of the 20PSU treatment plants was not significantly greater than that of 10PSU treatment plants. Within salinity treatments, osmolality was significantly different at 30PSU between the 0 and 10 $\mu$ M NH<sup>+</sup><sub>4</sub> treatments.

#### 3.4. Respiration

Plants at the  $10\,\mu\text{M}$  ammonium treatment had significantly greater respiration rates than those at the 0 or  $20\,\mu\text{M}$  ammonium treatments ( $F_{2,68}$ =11.535, P<0.001) when pooled across salinities. Across all ammonium treatments, when examining only the effect of salinity, plants at 20PSU had significantly greater respiration rates than those at 10PSU ( $F_{4,68}$ =3.149, P=0.021). Interactions between salinity and ammonium were also significant ( $F_{8,68}$ =4.433, P<0.001). Within the 10 $\mu$ M ammonium treatments, respiration rates were greater at 20PSU than 10, 30,



Fig. 7. Respiration of *Thalassia testudinum* seedlings at each treatment salinity for each of the three ammonium treatments:  $0\mu M$  (black),  $10\mu M$  (light grey),  $20\mu M$  (dark grey) (±S.D.), n=5 unless otherwise noted.

and 40PSU and greater at 50PSU than 10PSU. Within 40PSU, respiration rates were greater in plants at ammonium levels of 20 than  $0\mu$ M. Plants at both 20 and 50PSU had significantly greater respiration rates at 10 $\mu$ M than at 0 or 20 $\mu$ M ammonium (Fig. 7).

## 4. Discussion

## 4.1. Survival and growth

*T. testudinum* seedlings are not tolerant of extreme changes in salinity to ranges outside their salinity optimum (20–40PSU). The salinity optimum range observed here for seedlings is similar to ranges reported by previous lab and field studies involving mature *T. testudinum* short shoots (Zieman, 1975; Doering and Chamberlain, 2000; Lirman and Cropper, 2003) with decreased growth in hypo- and hypersaline conditions. Hyposaline-limit studies showed a significant decrease in growth of mature short shoots at salinities down to 6 PSU (Doering and Chamberlain, 2000). Field studies also support the negative impact of freshwater on blade width, shoot production and biomass per m<sup>2</sup> (Irlandi et al., 2002).

McMillan and Moseley (1967) and Doering and Chamberlain (2000) incrementally increased and decreased salinity at 0.75 PSU per day and 1.5 PSU per day, respectively. Our results of seedling responses with the incremental changes in salinity were similar to the growth and survival trends of these two previous studies of mature *T. testudinum* short shoots. In this regard, Koch et al. (submitted for publication) found that for mature *T. testudinum* plants, grown in mesocosms and acclimated to incremental changes in salinity, growth did not significantly change across salinities of 28–60 PSU. This suggests that mature *T. testudinum* plants may be better adapted to high salinity conditions than are seedlings.

In our study, elevated ammonium also had a negative effect on the hypo-salinity tolerance of *T. testudinum* seedlings. Ammonium may be toxic to aquatic plants, even at relatively low levels (Smolders et al., 1996; Katwijk et al., 1997). Seagrasses take up their ammonium and other nutrients from sediment pore water and the water column. Since *T. testudinum* has a greater affinity for  $NH_4^+$  uptake through the leaves than the roots (Touchette and Burkholder, 2000), this may play a role in the relative magnitude of impact that elevated ammonium levels would have on seedlings that are floating in the water column and have no established root systems.

#### 4.2. Fluorescence and respiration

Rapid light curve analyses indicated that, initially, rETR<sub>max</sub> and  $\alpha$  exhibited bell-shaped curves across salinities from 0 to 60 PSU, with the greatest values at 30 PSU and almost total mortality at 70 PSU. This indicates that although plants may initially survive and grow in salinities outside of the optimum range, establishment or expansion of the population may not occur because of decreased photosynthetic performance and increased mortality.

It is known for other mature seagrasses that hypoand hyper-salinity negatively impact photosynthetic performance. The euryhaline species, Ruppia maritima, exhibits decreased photosynthetic performance under both extreme hypo- and hyper-saline conditions (Murphy et al., 2003). Halophila johnsonii Eiseman (Fernández Torquemada et al., 2005) and Halophila ovalis (R.Br.) Hook. f. (Ralph, 1998) showed similar photosynthetic responses to salinity stress. For H. ovalis the decrease in quantum yield in hyposaline conditions was attributed to damage of photosystem II via an ionic imbalance and seepage due to the difference in water potential between the less saline media and more saline leaf tissue. A study by Xia et al. (2004) also suggested that reaction centers of photosystem II are the target of salinity stress. Ralph (1998) found that under hypersaline conditions, H. ovalis decreased maximum fluorescence, showing a greater initial tolerance to hypersaline conditions until photosynthetic inhibition occurred gradually at the point where the plant could no longer tolerate the extreme osmotic stress. T. testudinum seedlings in this study could be exhibiting similar reactions to the salinity stress, whereby the capacity to maintain internal osmolality negatively affects the photosynthetic performance both by damage of photosystem II as well as the energy cost of maintaining hyperosmolality to the surrounding environment.

Our respiration measurements suggest that an increased rate of respiration was due to an increase in metabolism, rather than an increase in stress on the plant. Previous studies on mature *T. testudinum*, under salinity stress, also found no effect on respiration under hypo- and hyper-saline conditions (Berns, 2003). Respiration of *Zostera* sp. exhibits no consistent correlation between salinity and respiration (Biebl and McRoy, 1971; Kerr and Strother, 1985). Although the patterns among ammonium treatments and salinities varied, the highest rates of respiration were found in plants, which according to growth data were the most productive. However, the lack of pattern in respiration

rates at extreme salinities may reflect low survival and thus, small sample size.

## 4.3. Osmolality

Leaf tissue remained consistently hyperosmotic to the media regardless of salinity or ammonium levels. For the plants that remained at 30PSU, average osmolality at ambient ammonium levels was ca 1400 mmolkg<sup>-1</sup>. These patterns are similar to those reported for mature T. testudinum short shoots (Koch et al., submitted for publication). Another study of mature T. testudinum reported a decrease in osmolality with decreased salinity at both the intra- and intercellular level (Berns, 2003). These results provide evidence that regardless of environmental parameters (i.e. salinity or other stressors) or age, the internal osmolality of T. testudinum tissue is maintained at a near-constant elevated level relative to the external environment. However, this osmotic adjustment to maintain the water potential may be taxing on the photosynthetic capacity of the plant (Xia et al., 2004; Ralph, 1998). To deal with physiological changes, the productivity of the seedlings may be reduced by both a decrease in the energy allocated for photosynthesis as well as from possible damage occurring to photosystem II.

# 4.4. Conclusions

In conclusion, T. testudinum seedlings tolerate salinity changes in their environment approximately 10PSU outside their optimum 30-40PSU range. However, our results suggest they are less adaptable than mature short shoots at surviving hypersaline conditions (Koch et al., submitted for publication). In this study of seedlings, growth and survival were the best indicators of tolerance and stress for both salinity and the interaction with ammonium. The influence of increasing ammonium concentrations varied among the salinity treatments. At optimal salinity conditions (30-40PSU) ammonium was not an influential factor in growth responses. However, at lower salinities, increasing the level of ammonium negatively impacted growth. This suggests that were the input of fresh water into Florida Bay, containing higher levels of nitrogen, to increase, this would more negatively impact T. testudinum seedling growth than would reduced salinity alone. This is important to consider when modeling the ecological impacts of a water-flow change on Florida Bay. Previous models have shown that the proposed change in water flow into Florida Bay would likely alter

the seagrass distribution (Fourqurean et al., 2003; Rudnick et al., 2005). This seedling study supports that the impact of fresh water in combination with increased ammonium and possibly other nutrients may influence the distribution of the dominant *T. testudinum* and could decrease the ability of new generations of genetically variant seedlings to successfully establish. Our results also suggest that it is important to investigate effects of environmental factors in combination and at different life stages in order to examine possible synergistic effects on stress tolerance of impacted organisms.

That *T. testudinum* leaf tissue remains hyperosmotic to the media even at salinities greater than seawater should be further examined. The accumulation of ions may damage the photosystems as well as influence cellular water potential, therefore the extent to which the inner cellular structures can tolerate these changes needs to be determined. Other biochemical processes may be affected by salinity-induced ionic imbalances affecting how plants adjust physiologically, particularly in the presence of other chemical stressors. Understanding these responses, especially at the young, less-developed seedling stage, may lead to better predictions of salinity tolerance in these plants.

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