SALINITY, LIGHT, AND TEMPERATURE EFFECTS ON *RUPPIA MARITIMA* GERMINATION IN FLORIDA BAY

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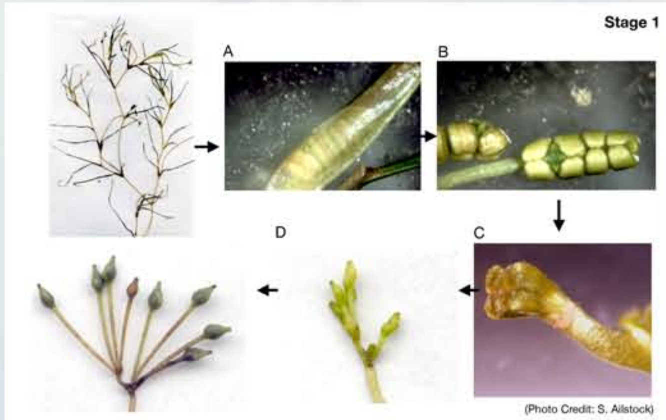
ABSTRACT

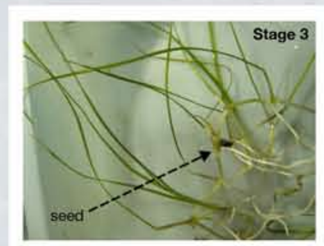
Ruppia maritima is a submerged aquatic vegetation (SAV) species that provides excellent waterfowl and fisheries habitat along the freshwater Everglades-mangrove ecotone in Florida Bay. This species has been identified as a keystone indicator of seagrass community health and a target species for salinity optimization in Florida Bay with Everglades' restoration. Although *Ruppia* is known to dominate in the oligo- to meso-haline region of the bay, it can survive as an adult in hypersaline conditions (>60 psu). To provide data for mechanistic models to forecast seagrass species shifts in the bay under changes in fresh water flows, and in response to climate change scenarios, we investigated salinity (0-45 psu), light (water column [~500 μmol m^{-2} s^{-1}] and sediment [dark]) and temperature (25 and 31°C) effects on *Ruppia* germination and seedling success. We are also monitoring *Ruppia* reproductive potential and germination in the field, and will be implementing seed transplant experiments, in order to further our mechanistic understanding of conditions which promote *Ruppia* dominance in low salinity regions of the bay. In mesocosm experiments, *Ruppia* germination predominantly occurred in the 3 low salinity treatments (0, 5, 15 psu) with few to no germinations at upper salinities (25, 35, 45 psu). However, after 5 months at treatment salinity, salinities were lowered to ≤1 psu, at which time the highest number of germinations occurred. The majority of these subsequent germinations, however, were in tanks previously at marine and hypersaline conditions (35 and 45 psu). These data provide a mechanistic understanding of field observations that *Ruppia* shows greater dominance at sites with highly variable salinities. In addition, although this species is not a true seagrass with strict fidelity to the marine environment, our results provide a better understanding of the role salinity can play in the distribution of this species at marine-freshwater ecotones. These results are now being field validated using long-term data sets, new reproduction monitoring studies, and manipulation experiments in the field.

INTRODUCTION

In this study, we provide information on the germination success of *R. maritima* in Florida Bay to assist in model development and to be able to understand and predict changes in the distribution of this species with Everglades' restoration and climate change. Additional studies are needed on this species' thermal and salinity thresholds initiating ontogenetic development between early life history stages, such as the development of reproductive shoots, seed maturation, seed stratification, seed germination, and finally seedling establishment and growth. Identifying temperature requirements is particularly important in Florida Bay where mean summer temperatures appear to exceed published temperatures that promote *R. maritima* germination and are above optimum temperatures for seed stratification (for review see Kantrud, 1991). Light availability may also affect *R. maritima* success in Florida Bay. *Ruppia maritima* is known to grow poorly in areas with low water clarity (Kantrud, 1991). Brock (1982) found there to be an effect of diurnal cycles on seed germination in *Ruppia* species from Australia. Kahn and Durako (2005) also found seed germination to occur almost exclusively under a 12:12 light:dark cycle, suggesting a light signal may be involved in initiating germination when higher light intensities are seen during the spring and summer. In our first mesocosm experiment, we examined *R. maritima* germination success across a broad range of salinities from 0 to 45 psu. However, in order to assure germination, a stratification pre-treatment of 6°C was employed that had previously been successful in germinating *R. maritima* seeds from Florida Bay. Even though this temperature is lower than what is found in the upper bays of Florida Bay, for this first experiment our goal was to examine the role of salinity in germination. We also evaluated the effects of two temperature levels (25 and 31°C) on germination and seedling development. This temperature range represents the average temperatures in the spring and summer in Florida Bay when germination is highest, despite the fact that the upper temperature has been shown to inhibit seed germination of *R. maritima* in other regions. To reconcile the discrepancy between field temperatures and those considered optimal for successful *Ruppia* seed germination, we will further examine a broader range of pre-treatment temperatures in mesocosms, as well as follow germination and temperature in field monitoring and experimental programs in 2008 to 2010.

RUPPIA MARITIMA DEVELOPMENT STAGES





RUPPIA MARITIMA DEVELOPMENT

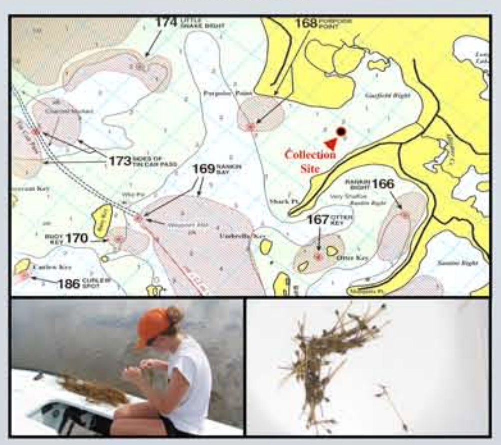
Stage 1 Seed Development: Reproductive shoots of *Ruppia maritima* possess seeds at various stages in their development. During initial seed development, the immature inflorescence is enclosed in a leaf sheath (A), at flower maturation the peduncle elongations and the inflorescence emerges from the sheath (B), each having four carpels (C). After fertilization, the pedicles grow and the small green immature fruit emerge (D) and a maximum of eight individual dark mature fruits are produced (E) which detach from the base of the pedicels (Ailstock and Shafer, 2004).

Stage 2 Seedling Emergence: Upon germination, the leaf and root emerge approximately 180° from each other and approximately 90° from where the seed stalk is attached (this study).

Stage 3 Seedling Shoot Development: The development of the rhizomes and multiple shoots in this colonial plant extend in both directions from the initial seed (this study).

METHODS

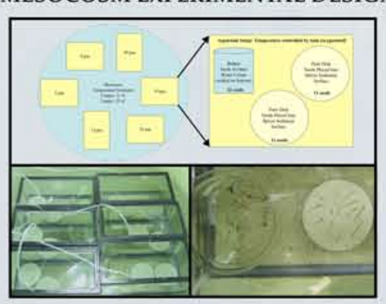
STUDY SITE



Seed Collection, Stratification and Planting

Ruppia maritima reproductive shoots were collected from northern Florida Bay at the mangrove-bay transition zone at Garfield Bight. Mature seeds were taken from reproductive shoots and kept in ambient seawater from the site and transported to the FAU Marine Lab. Approximately 1,000 seeds were recovered and placed in dark Incufridges for two weeks at stratification temperature (6°C). Seeds were kept in Petri dishes filled with artificial seawater (Instant Ocean) at *in situ* salinity from the site of collection (25 psu). Additional Petri dishes were filled with sediment from each collection site and stored in oxygenated coastal seawater in mesocosm aquaria. After two weeks of stratification, seeds were carefully planted in the sediment by lightly pressing the seeds into the sediment and placed in mesocosms.

MESOCOSM EXPERIMENTAL DESIGN



Mesocosm Design and Salinity Treatment - Experiment 1

Half of the seeds (n=22) were planted in sediment and the other half (n=22) placed in 600 mL glass beakers. The mesocosm design consisted of four 500 L tanks (1 m diameter x 1 m height), two at 25±0.8 °C and two at 31±0.4 °C. Within each tank, 5 gallon aquaria confined six salinity treatments (0, 5, 15, 25, 35 and 45 psu). Lights were maintained on a 12:12 hr cycle and 800 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR). Once germination occurred in the beakers, germination was recorded and seedlings were planted in Petri dishes with sediment from the field. At the end of the experiment, total seed germination was calculated and seedlings were dried and weighed to get total biomass and plant normalized biomass.

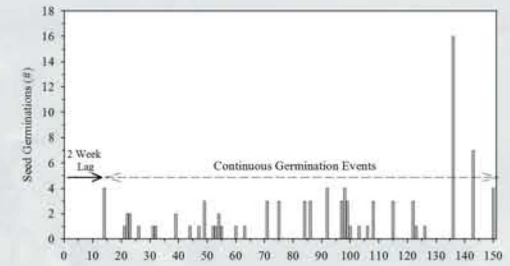
Seed Recovery Post-Salinity Treatments - Experiment 2

At the termination of Experiment 1, the seeds remaining in the sediment and beakers were used to examine the potential recovery of *Ruppia* seeds from hypersaline conditions. After approximately 136 days at treatment salinities, salinity in the aquariums were lowered to 1 psu at a maximum rate of 4 psu d^{-1}. The resulting freshwater conditions (1 psu) were sustained for 24 days in all tanks. The temperature treatments were the same as in Experiment 1 (25 and 31 °C). All new *Ruppia* seed germinations were recorded daily and new emergent seedlings were planted into sediment as in Experiment 1. At the end of the 24 day recovery experiment, all plants were harvested and biomass determined as described above for Experiment 1.

EXPERIMENTAL RESULTS

Total Recovery of Seeds and Percent Germination

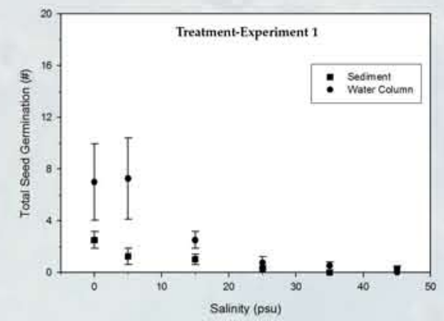
The total percent recovery of seeds for the two experiments, either through successful germination or seed counts in the beakers and sediment was 85%. Of the 1,056 initial experimental seeds 895 were recovered. Of these recovered seeds, 443 or approximately 50% germinated.



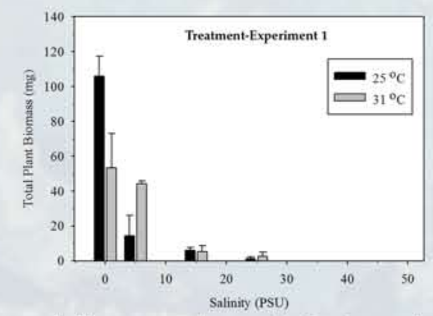
Germination Pattern

No germinations occurred in the sediment or water column post-stratification until 14 days in Experiment 1. After this lag phase, germination was continuous for 136 days without a clear pattern. Thus, seed germination was continuous rather than pulsed post stratification.

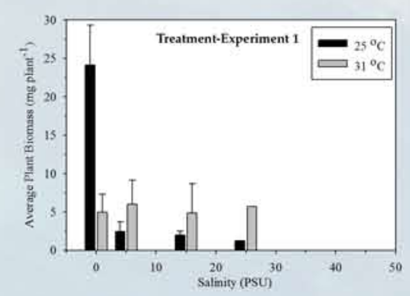
Experiment 1: Treatment Effects



In Experiment 1, salinity was the major factor influencing seed germination ($P<0.01$), with more germinations in freshwater and 5 psu than 25, 35, and 45 psu, and with a majority of the germinations (77%) in salinities ≤ 5 psu.

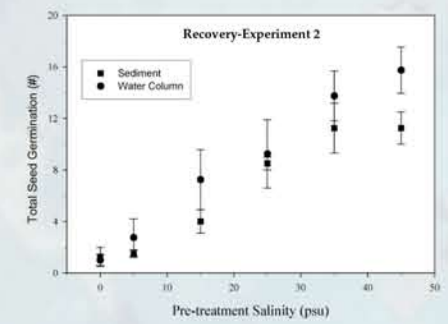


Lower salinities promoted not only *Ruppia* germination, but had a significant affect on seedling biomass development. There was no secondary temperature effect on biomass.

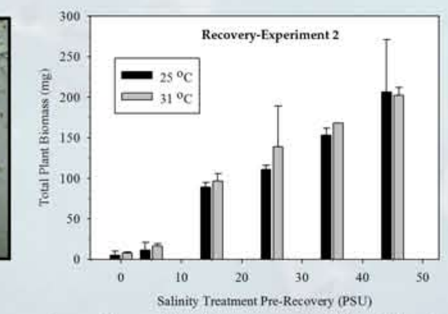


When normalized to plant number, salinity was not a significant factor in biomass development. This normalization is most clearly seen in the 31°C treatment. No significant temperature or salinity treatment was found for final plant-normalized biomass.

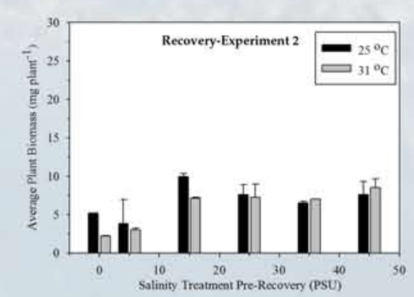
Experiment 2: Recovery



Once salinities were lowered in Experiment 2, the number of germinations increased as a linear function of pre-treatment salinity in the water column ($P<0.01$; $R^2 = 0.99$) and sediment ($P<0.01$; $R^2 = 0.93$).



Biomass showed a positive linear trend with salinity for plants in the 25°C ($P<0.01$; $R^2 = 0.98$) and 31°C ($P<0.01$; $R^2 = 0.96$) temperature treatments and was not influenced by temperature ($P = 0.45$).



Plant normalized biomass had a significant pre-treatment salinity effect; however, there were no significant mean comparisons among salinity levels. No temperature effects were found for plant normalized biomass.

CONCLUSIONS FUTURE RESEARCH

- Based on our experiments on *Ruppia maritima* seeds from Florida Bay, seed germination is induced at salinities ≤ 15 psu, but is optimal at salinities ≤ 5 psu.
- Seed germinations probably control the biomass development potential of this seagrass in Florida Bay.
- Although germination is optimal at very low salinities, seeds exposed to short periods of high salinity promote the subsequent germination of seeds, once salinity levels are lowered to ≤1 psu.
- Although secondary stimulation of germination can occur under high temperatures (31°C), and in the water column compared to the sediment, these factors did not influence seedling development and are secondary to the overriding effects of salinity.
- Field studies are underway to further verify these conclusions.

ACKNOWLEDGEMENTS

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REFERENCES

Ailstock, S., Shafer, D., 2004. Restoration potential of *Ruppia maritima* and *Potamogeton perfoliatus* by seed in the mid-Chesapeake Bay. ERDC/TN EL-04-02. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Brock, M.A., 1982. Biology of the salinity tolerant genus *Ruppia* L. in saline lakes in South Australia. I. Morphology variation within and between species and ecophysiology. Aquat. Bot. 13, 219-248.

Kahn, A.E., Durako, M.J., 2005. The effect of salinity and ammonium on seed germination in *Ruppia maritima* from Florida Bay. Bull. Mar. Sci. 77 (3), 453-458.

Kantrud, H.A., 1991. Widgeongrass (*Ruppia maritima* L.): a literature review. U.S. Fish and Wildlife Service, Fish. Wildl. Res. 10, 58

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