BULLETIN OF MARINE SCIENCE, 77(3): 453–458, 2005

NOTE

THE EFFECT OF SALINITY AND AMMONIUM ON SEED GERMINATION IN *RUPPIA MARITIMA* FROM FLORIDA BAY

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Ruppia maritima L. (widgeon grass) is one of six species of submerged angiosperms found in Florida Bay. Although *R. maritima* has the widest salinity tolerance of any submerged angiosperm (Kantrud, 1991), within Florida Bay this species is most common in euryhaline environments of the northern basins near freshwater inflows from the Everglades (Fourqurean et al., 2002). However, populations of *R. maritima* in these areas are ephemeral and generally display seasonal die-backs (Montague and Ley, 1993). Recovering from seasonal die-backs is thought to require recruitment via seed germination in this species (Kantrud, 1991).

Germination in *Ruppia* spp. is environmentally regulated (Brock, 1982a; Koch and Seeliger, 1988). Koch and Dawes (1991) found that salinity, rather than temperature, is the prime factor in ecotypic variation in *R. maritima* seed germination between northern and southern populations on the eastern coast of the U.S. and that seeds from Florida had highest germination at lower salinities. Using a statistical model based on field observed water quality and seagrass distribution data, Fourqurean et al. (2003) recently predicted that an increase in seasonal freshwater delivery to Florida Bay, such as that proposed in the Comprehensive Everglades Restoration Program (CERP), would result in an expansion of seagrass beds dominated by euryhaline species such as *R. maritima* and *Halodule wrightii* Ascherson. However, there is some concern that the proposed increases in freshwater inflow may, in addition to lowering salinities, also result in an increase in the total nitrogen pool for central and eastern Florida Bay (Rudnick et al., 1999) and have negative consequences for the submerged angiosperms along the northern euryhaline margins of Florida Bay (Lapointe et al., 2002; Lapointe and Barile, 2004). In this study germination of stratified (i.e., cold treatment, otherwise known as vernalization) and unstratified *R. maritima* seeds from Florida Bay was monitored in response to two salinity manipulations (direct exposure and exposure after incremental increases), two light regimes, and three ammonium treatments. Ammonium levels chosen were based on nutrient concentrations found in northern Florida Bay (Lapointe et al., 2002).

Methods

Seed Collection.—*Ruppia maritima* seeds were collected in Garfield Bight in north-central Florida Bay (25° 09.8′ N, 80° 48.5′ W) in early August 2002 (year one) and 2003 (year two). The location of the seed collections was based on accessibility and the assumption that the population in Garfield Bight was representative of other *R. maritima* populations across the northern bay. In 2002, seeds were collected by sieving (1 and 2 mm sieves) surface sediments and by direct collection from reproductive shoots. In 2003, seeds were only collected from reproductive shoots. Seeds were kept in ambient seawater and transported to the Center for Marine Science, Wilmington, North Carolina. Within 2 d of collection, seeds were rinsed with deionized (DI) water and placed in Magenta GA-7 flasks with 200 ml of autoclaved DI water, 15 seeds were haphazardly selected for each flask. The flasks were stored in a dark growth chamber at 10 °C (year one) or 6 °C (year two) for 2 wks to undergo stratification (Seeliger et al., 1984; Koch and Dawes, 1991).

Direct Salinity Treatment (Year One).—After stratification, 160 individual seeds were randomly placed into screw-cap test tubes containing 100 ml autoclaved Instant Ocean artificial seawater at one of eight treatment salinities (0–70, increments of 10). Twenty replicate tubes were used for each salinity treatment, 10 were placed in a growth chamber with 12:12 light:dark (L:D) cycle [average Photosynthetic Photon Flux Density (PPFD) of 385 μmole $cm^{-2}s^{-1}$], the remaining 10 were placed in a growth chamber with 24 hrs darkness, both growth chambers were kept at 24 °C. Germination was monitored weekly.

An additional 144 *R. maritima* seeds were rinsed with DI water then individually planted (without the 2 wk stratification treatment) into aragonite shell hash from Florida in plastic nursery six-pack containers (5 × 5 × 8 cm). Three replicate 30-l aquarium tanks were set at each salinity treatment (0–70, increments of 10) using Instant Ocean and one six-pack was placed in each tank (n = 18). The aquaria were located in a temperature-controlled (23–29 °C) greenhouse with light-supplemented conditions (eight 500 W metal-halide lamps, 14:10 L:D, yielding an average mid-day PPFD of 1267 μmole $cm^{-2}s^{-1}$). The additional 2 hrs of day-length in the greenhouse, compared with the chambers, coincided with ambient dawn and dusk, at the start of the experiments. The higher light levels in the greenhouse partially compensated for the fact that seeds were buried and in a 30- l tank. Germination was monitored weekly.

Salinity Adjustment and Ammonium (Year Two).—Following stratification, individual seeds were put into screw-cap test tubes containing 100 ml of DI water (salinity 0) in a growth chamber at 24 °C, 12:12 L:D (average PPFD of 385 μmole $cm^{-2}s^{-1}$). After 3 d at salinity 0, the media in all but 10 of the tubes was replaced with water of salinity 2. Ten seeds remained at salinity 0 for the rest of the experimental period. Every 3 d, the salinity in the tubes was raised by two and at every salinity increment of 10, ten seedlings were maintained at that salinity. This increment was chosen to emulate field conditions (Baskin and Baskin, 1998). Upon completion of the salinity-increase adjustments, ten seedlings were at each of eight salinities: 0–70. This salinity-adjustment regime was repeated for each of three ammonium levels: 0, 10, and 20 μM (n = 10 for each salinity at each ammonium treatment). Ammonium was added in the form of ammonium chloride and levels were monitored when each media-change occurred. Salinity media were made with DI water and Instant-Ocean salts and media in each tube was changed every 3 d, whether a change in salinity occurred or not. The pH was monitored before and after media changes and remained between 8.3 and 8.5. Germination was monitored weekly and the salinity at which germination occurred recorded. In both years, low germination rates (see results) precluded statistical analyses.

Results

Direct Salinity Treatment (Year One).—Overall germination of *R. maritima* seeds was low in the test-tube scale experiment. In the light:dark (L:D) chamber, germination occurred in the 0, 10, and 20 salinity treatments (Fig. 1). On each of days 15, 20, 31, and 34, a single seed in the salinity 0 L:D treatment had germinated (40% total germination for that salinity treatment). By day 24, one seed in the salinity 20 L:D treatment had germinated and by day 38, one seed in the salinity 10 L:D treatment had germinated. Only one seed germinated in the salinity 10, 24 hrs dark treatment, after 143 d. No other seeds germinated in the dark treatment over the course of the monitoring period (5 mo), nor in the non- stratified *R. maritima* seeds planted directly into sediments in the aquaria (Fig 1).

Salinity Adjustment and Ammonium (Year Two).—After 121 d post-stratification, 6.25% out of the total 240 seeds had germinated. In the 0 μM ammonium treatment, 11.25% of the 80 seeds germinated. Only 3.75% germination occurred in

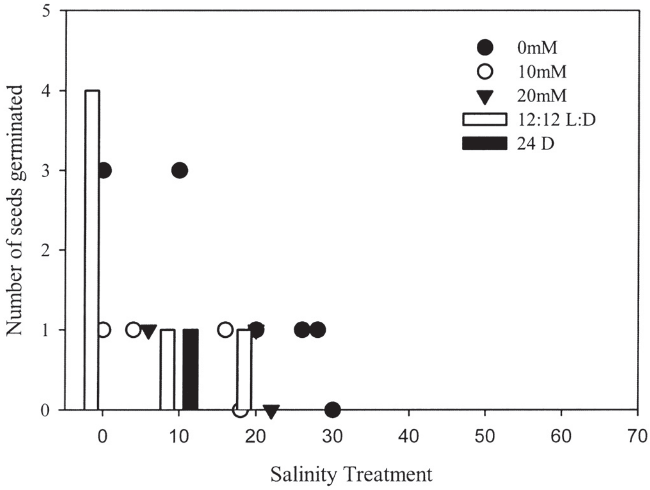

Figure 1. Salinity at which *Ruppia maritima* seeds germinated during the course of the experiment for year one in 12:12 L:D conditions (white bars) and 24 hrs dark (black bars) and year two at the three ammonium and eight salinity treatments: 0 (black circles), 10 (white circles), and 20 μM (black triangles). Note histograms for year one slightly offset so as not to obscure year two data points.

both the 10 and 20 μM ammonium treatments. The number of days post-stratification to germination ranged from 9 to 121 (Table 1). Some germination occurred during the salinity-increase acclimation process at non-treatment salinities. Thus, the salinity of the media at the time at which the seed germinated was recorded. Germination occurred at salinities ranging from 0 to 28, with almost 70% of the observed germination (10 of the 15 seeds) occurring between the salinities of 0 and 10. No seeds germinated above the salinity of 28 (Fig. 1).

Discussion

These results suggest a mechanistic explanation for the prediction that a reduction in salinity in Florida Bay could result in an expansion of *R. maritima* distribution in northern Florida Bay (Fourqurean et al., 2003). Germination of *R. maritima* seeds was greatest at the lowest salinities (0 and 10) in both experiments. However, some germination was observed at mid-range salinities in the second experiment (up to 28) when seeds were exposed to incremental increases in salinity that were more indicative of field conditions (Baskin and Baskin, 1998). An increase in ammonium resulted in decreased germination at all salinities where germination occurred. Although overall germination was low in both sets of experiments, percent germination was comparable to previous shorter-term (< 1 yr) germination results (< 27%) (Seeliger et al., 1984). *Ruppia maritima* beds produce many seeds per reproductive period (Kantrud, 1991); up to thousands of seeds per m^2 (McMillan, 1985; Johnson et al., 2003). Therefore, even with a low annual germination rate the large seed pool and multiyear viability may result in enough production of seedling recruits for population establishment and maintenance.

Table 1. Number of *Ruppia maritima* seeds that germinated, out of a total n = 10, at each salinity and ammonium treatment level (number of days post-stratification when germination occurred).

	Ammonium		
Salinity	0 µM	10 µM	20 µM
0	3 (9, 33, 91)	1 (100)	0
4	0	1 (6)	0
6	0	0	1 (9)
10	3 (18, 24, 84)	0	1 (15)
16	0	1 (27)	0
20	1 (121)	0	1 (30)
26	1 (40)	0	0
28	1 (44)	0	0

Germination did not occur without stratification and occurred almost exclusively in L:D conditions. The former observation suggests an extended-dormancy requirement, beyond a year, for germination, while the latter observation suggests a signal involving transitioning from darkness to light, such as movement to the surface following sediment disturbance, may also be involved in germination.

Previous studies of *R. maritima* germination (Koch and Seeliger, 1988; Koch and Dawes, 1991) reported higher percent germination of up to 60% for seeds collected from sediment cores than found in this study. It is unknown how long seeds from cores have been in the sediment, but their presence in sediment cores suggests the existence of a seed bank as in other submerged angiosperm populations (Brock, 1982b; Inglis, 2000). Seeds from Florida Bay, like other *R. maritima* populations (Seeliger et al., 1984; Koch and Seeliger, 1988), may require a period of dormancy in a seed bank to ensure increased germination in addition to increasing the ability to reestablish a population after disturbance events (Orth et al., 2000). Seeds have been found to be viable for up to 3 yrs in the sediment (Kantrud, 1991). For an ephemeral species like *R. maritima*, in a variable environment such as Florida Bay, the presence of a seed bank helps explain the dramatic temporal population fluctuations observed in this system (Montague and Ley, 1993). The presence of a seed bank and multi-year viability allows for germination and seedling recruitment even after a season of low reproductive success due to environmental variability.

The lower overall germination observed in year one (six seeds versus 15 seeds in year two) may have been due to the relatively high, but more ecologically relevant (Baskin and Baskin, 1998), stratification temperature of 10 °C. This relatively high temperature was chosen because it has previously been suggested that the best germination of *R. maritima* seeds occurs at temperatures corresponding to the lowest winter temperatures at the site of collection (Seeliger et al., 1984). However, using a stratification temperature of 6 °C in year two, similar to that previously used by Seeliger et al. (1984) and Koch and Dawes (1991), but lower than winter minimum temperatures in Florida Bay, resulted in slightly higher germination rates. Other environmental or water-quality factors, such as dissolved-oxygen or nutrient levels (Moore et al., 1993; Brenchley and Probert, 1998), might also affect germination in *R. maritima* from this region. In this regard, we observed that germination decreased with increases in ammonium, but it was not totally inhibited. Were ammonium to increase in conjunction with freshwater increases in Florida Bay, germination would still be expected under the low salinity conditions. Other possible synergistic effects between low salinity and changes in additional water chemistry parameters may be important for resource managers to consider.

Acknowledgments

Authors would like to thank J. Kunzelman from University of North Carolina Wilmington and J. Barr from University of Virginia for their immense help in seed collection. We would also like to thank the reviewers for their insight and valuable comments. Funding was provided by Florida Atlantic University and the South Florida Water Management District (5-50602).

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Date Submitted: 7 January, 2005. Date Accepted: 5 May, 2005.

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