

Cultivation Guide for *Ruppia maritima* from Seeds (Indian River Lagoon, Florida)

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Bethune-Cookman University

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Ruppia maritima L.

Ruppia maritima L. (Wigeongrass) is a euryhaline submerged aquatic vegetation (SAV) and one of the seven seagrass species found in the Indian River Lagoon (IRL), Florida. Unlike other seagrass species, *R. maritima* tolerates a wide range of salinities (ranging from near freshwater to hypersaline conditions; Kantrud 1991; Cho et al. 2009). *R. maritima* is known to grow seasonally and even sporadically in some of the brackish backwater bodies within the IRL. *R. maritima* is reported and observed in many of mosquito control impoundments within the IRL (Fig. 1).

Fast growth and production of abundant seeds are *Ruppia*'s strategies to thrive in ephemeral salt marsh ponds and ditches. Seeds of *R. maritima* are protected by sturdy coats (Cho and Poirrier 2005; Cho and Biber 2010) allowing resistance to desiccation (Cho and Sanders 2009) or even harsh gut-passages of waterfowl and fish, which also aids in long-distance dispersal of the propagules (Figuerola and Green 2004). The unique biology and ecology (growth, reproductive and dispersal strategies, etc.) of *R. maritima* should be integrated into the long-term restoration of other resident seagrasses in IRL.

This guide provides protocol for cultivating *R. maritima* in a laboratory or in a seagrass nursery from field collected seeds.



Figure 1. *Ruppia maritima* growing in the T-10-F impoundment in April 2022 at the Merritt Island National Wildlife Refuge. Lower left insert: reproductive shoots.

Ruppia maritima Seed Collection, Viability Test, and Germination

Seed collection through reproductive shoots:

Harvest reproductive shoots with seeds from flowering beds, leaving the base and the roots/rhizomes of the plants intact (Fig. 2). The flowering season and duration vary with location and habitat type (Fig. 3). The best times to look for harvestable seeds are March through early May in spring; and October to November in the fall.

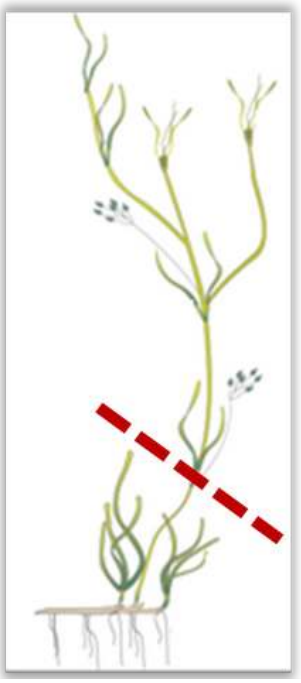


Figure 2. Reproductive shoots of *Ruppia maritima* (Image source: www.teachoceanscience.net). The suggested cutline is indicated with a red dashed line.

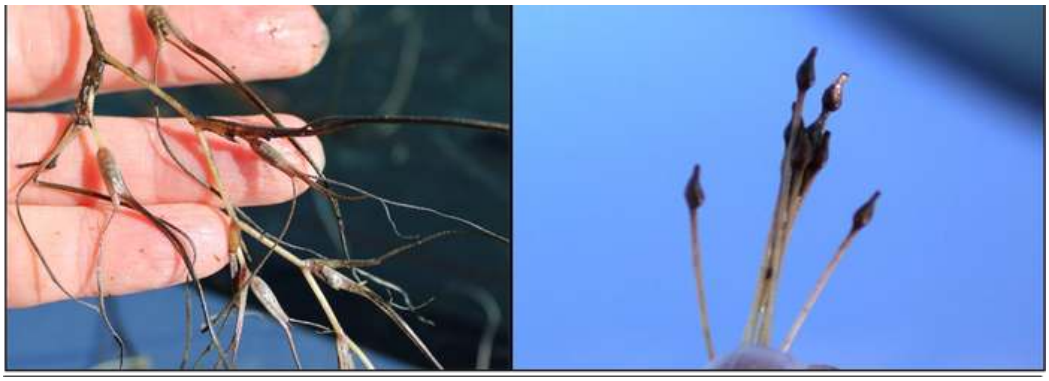


Figure 3. *Ruppia maritima* inflorescences in leaf sheaths that are not ready for seed harvesting (left). Stalks of immature seeds (right). The immature seeds should be harvested with the reproductive shoots as they will not mature without being attached to the reproductive shoots.

Transport the collected reproductive shoots in a cooler with habitat water of the harvest site to a wet laboratory or a seagrass nursery. In the laboratory, gently separate and place the shoots into aquarium tanks filled with salt water (salinity adjusted close to the habitat condition) prepared using aquarium sea salt (e.g. Instant OceanTM) to allow for seed maturation (Fig. 4). Immature seeds should not be detached from the shoots.



Figure 4. Immature green, elongated seeds of *Ruppia maritima* (left). Mature seeds are rounder with dark and hard seed coats (middle and right). Note: immature seeds should not be detached from the shoots.

When using indoor tanks, air-stones should be added for air and water circulation and to also prevent the overgrowth of epiphytes. Aquarium lighting emulating daylight should be set at the 12L:12D cycle. When using outdoor tanks, check and adjust salinity two - three times a week to compensate for the water volume change from evaporation or rainfall.

The seeds reach maturity after a couple of weeks, but may also take up to two months. Seed maturation is evidenced by rounder and darker seeds, seed coats hardening, and seed stalks becoming thinner and about to break (Fig. 4). Manually harvest mature seeds by detaching them from the shoots at the stalk; the contents at the bottom of the containers need to be sieved with a 1 mm mesh sieve to collect any seeds that naturally detached after maturation and handling.

Seed collection from sediment:

In an area known to have persistent *R. maritima*, scoop the top layer (top 5 cm) of sediment using a gardener trawl or a small shovel and place the sediment into resealable plastic bags. Transport the collected sediment samples in a cooler with ice to a wet laboratory or a seagrass nursery. Sieve the sediment samples through a 1 mm mesh sieve to separate and collect seeds from other materials. Seed bank collection from sediment is more flexible than seed collection from reproductive shoots as sediment can be collected from known areas of *R. maritima* at any time throughout the year. However, this collection results in lower seed numbers and viability rates than those harvested from reproductive shoots. Another downside of seed collection from sediment is the process of sorting the tiny seeds from the wetland mud that is mixed in with large amounts of other dark and coarse fragments.

For example, an average of 29 seeds (N = 5) were harvested from ½ gallon of sediment sample as opposed to 553 seeds (N = 5) from the same volume of reproductive shoots from the same location (T-10-F impoundment in April 2022 at the Merritt Island National Wildlife Refuge). The viability of the seeds collected from the sediment was 50-55%, as opposed to 80-95% from the freshly collected seeds.

Seed viability test:

Small batches (e.g. 5 replicates; 10-20 seeds per replicate) of the collected seeds can be used for viability testing. Prepare a solution of 5%- 2,3,5 Triphenyl Tetrazolium chloride or bromide dye (DifcoTM, Becton Dickinson & Company). Puncture or crack the seed coat by gently squeezing each seed using the tips of forceps. Place the seeds in glass Petri dishes and submerge them in a thin layer of the prepared dye solution and leave for 24 hours to allow ample staining time (Fig. 5). Count the number of dyed embryos (pink to red coloration indicates viability; Fig. 5) and undyed (white embryo) under a dissecting microscope to determine viability rate (# dyed seeds/total number of seeds tested).



Figure 5. Harvesting seeds from reproductive shoots (top left); Setup for seed viability test (top right; from White 2014); Prepared dye solution and seed soaking in the dye solution (bottom left; from White 2014). Embryo dyed in red, indicating the seed was viable (bottom right; from Cho and Sanders 2009).

Seed Germination:

Induce germination by placing seeds in 1 cm deep of harvest site water or in artificially prepared salt water using glass vials or glass Petri dishes (Fig. 6). Seeds tend to germinate faster in freshwater (tap water) while the final germination rates are similar at varying salinity ranges of the habitat conditions (Cho and Sanders 2009). Signs of seed germination occur within one - three days (Fig. 7); and the number of cumulated germination plateaus after two - three weeks. Embryos mature before the seed coat fully matures (Cho and Sanders 2009); therefore, some seeds with slightly pre-mature seed coats can germinate.



Figure 6. Seed germination is induced in glass Petri dishes with 1 cm deep of tap water (0 ppt) or prepared saltwater (left). Seeds being germinated in glass vials (right)



Figure 7. Germinating seed (left). Seedling 3 days after germination (right).

Once germinated (Fig. 7), remove the germinated seeds from the Petri dish and place them in separate 500 mL glass beakers with harvest site water or artificial saltwater to facilitate seedling growth (Fig. 8). If the seeds were germinated in freshwater, expose the seedlings to gradual salinity increases at a rate of 1-2 ppt increase per week. When seedlings become longer than one inch, they need to be planted with substrate (sand or sand mixed with potting soil) in a tank (Figs. 8 and 9). Seedling growth, and development of reproductive shoots, flowers, and fruits (seeds) are documented in Figs. 8, 9, 10, and 11.

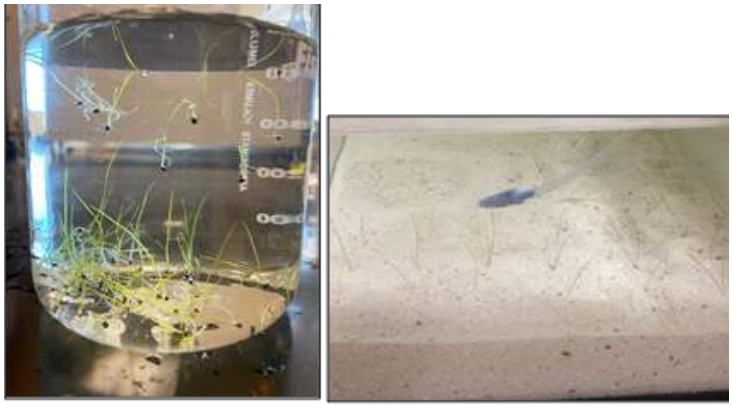


Figure 8. Seedlings are transferred to a 500 mL glass beaker (left). Seedlings planted in sand in a tank (right).



Figure 9. Seedlings one week (left) and two weeks (right) after germination. The seedlings are planted in sand or sand mixed with potting soil.



Figure 10. Reproductive shoot with immature flowers enclosed in a sheath are seen after 3 months. Growth rate and development depend on water temperature.

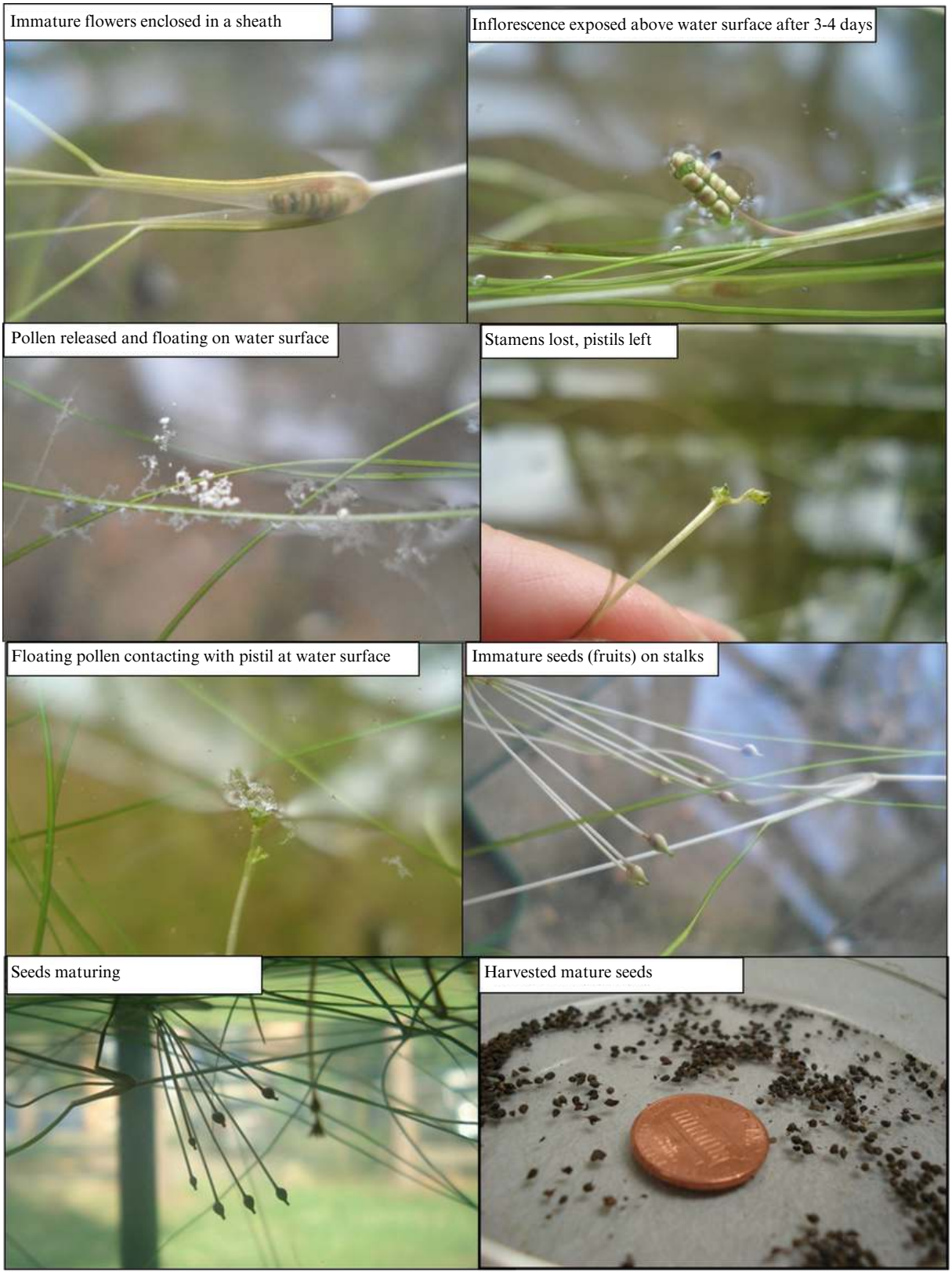


Figure 11. Flowers and fruits of *Ruppia maritima* grown from seeds collected from the field.

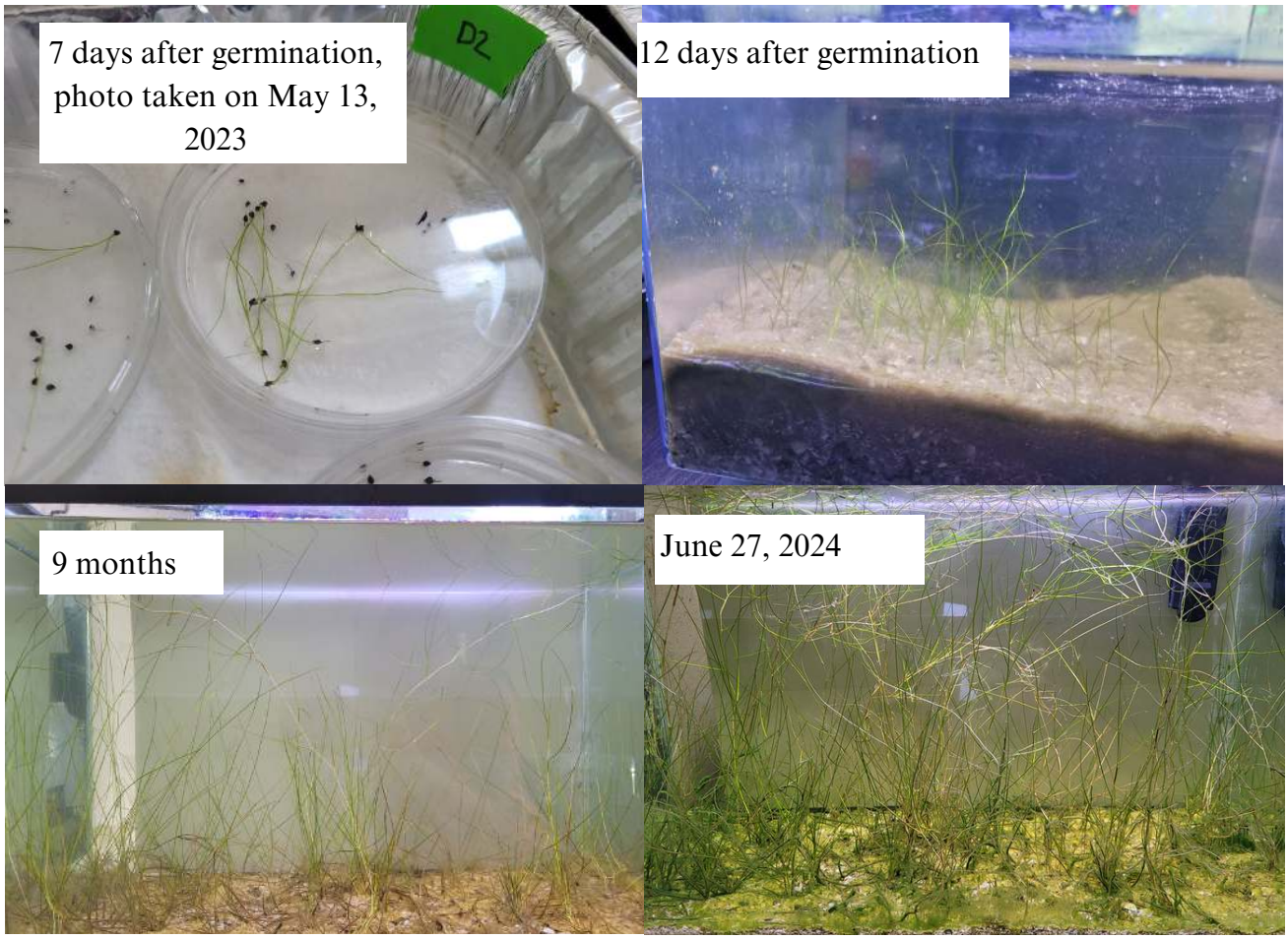


Figure 12. Growth progress of *Ruppia maritima* in an indoor tank from seeds collected from the sediment of a Merritt Island impoundment.

Treatment for Seed Germination & Long Term Storage

Treatment for Seed Germination

The thick and hard seed coats enable the *R. maritima* seeds to endure desiccation and other perturbation (e.g. grazer's gut passages). However, the seed coats can delay germination time and termination rate. Scarification of seed coats induces early germination by facilitating water intrusion. Scarification can be made by puncturing the coat with a needle or a tip of fine forceps. Scarred seeds need closer monitoring as they are prone to fungal growth in low salinities (< 2 ppt). If scarification is used for faster germination, induce germination in saltwater (> 5 ppt).

Two alternatives can be used to induce early germination: (1) induce germination when the seed coats are not fully hard; and (2) acid treatment of seeds for acid scarification (Baskin and Baskin 2003).

Long-term Storage of Seed

Dry storage:

Mature seeds can be air-dried for long-term storage. Dry-storage, however, reduces viability and final germination rates. One study showed that 28 days of dry-stratification significantly reduced the seed viability (mean of 54%) when compared to the viability of non-stratified seeds (85%; White 2014). Almost half (45%) of the dried seeds germinated when placed in freshwater at room temperature (White 2014). Cho and Sanders (2019) found that up to 30% of dry-stratified seeds were still viable and able to germinate after one year of storage. Although drying reduces viability over time, it is still a good method for the long-term storage of *R. maritima* seeds.

Cold storage:

Storing seeds in cold fresh tap water at 1.5-3˚C also suppresses germination. White (2014) found that 28 days of cold storage also significantly reduces seed viability (61 %). Germination rates of cold-stratified seeds were 16% in laboratory conditions and 23% in field testing (White 2014).

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