**Turtlegrass**
*Thalassia testudinum*

**Scientific Name**
*Thalassia testudinum* Banks & Sol. ex Koenig

**Common Name**
Turtlegrass

**Group**
Monocotyledon

**Family**
Hydrocharitaceae

**Wetland Indicator Category**
OBL

**Growth Form**
Submerged, marine

**Habitat**
Estuaries, barrier islands, shallow marine environments
**Thalassia testudinum**

**SEED PROPAGATION METHOD**

1. **Seed Collection**
   
   Flowers of *Thalassia testudinum* can occasionally be observed underwater during the summer months. In Florida and along the southern Gulf of Mexico this generally occurs between April and August (Phillips 1960); however, this may vary from year to year depending on seasonal water temperature.

   *Thalassia testudinum* is dioecious (having male and female flowers on different individuals). The flowers are either male (white) or female (light brown), usually solitary, and grow up from the base of the leaf. There tend to be more male flowers than female flowers (Van Tussenbroek et al. 2006).

   Pollen is released from the male flower into the water column, and waves then transfer the sticky mucus pollen string to the female flowers. After fertilization the female flower will produce greenish/yellowish fruits within two to four weeks. The fruits ripen in about eight weeks, are nearly spherical, about 0.8” (2 cm) across with a rough warty surface, and detach to float near the surface of the ocean. When opened, there are typically two to three conical pale green seeds ranging in size from 0.3-0.4” (8-10 mm) (Orth et al. 2006). The fruit can be harvested by collecting them at low tide from the beach and placing them into plastic bags. Not all fruits wash up at the same time and repeated visits may be necessary over one to two weeks to collect additional fruits.

   Seeds of *Thalassia testudinum* do not form a seed bank, with the seeds germinating within 10 days after being released from the fruit (Kaldy and Dunton 1999). Seedlings can become uprooted and float around with the currents before permanently re-attaching (Whitfield et al. 2004).

2. **Fruit and Seed Preparation**
   
   Fruit collection from the plants is not recommended, as the incidence of female flowers is usually less than 10% of the population (Phillips 1960). Flowering on any one individual appears to be sporadic and may only occur every one to three years (Cox and Tomlinson 1988). Fruits washed up on the beach should be collected instead.

   After collection, the fruits should be stored in seawater with aeration (e.g., a small aquarium with air-stone or filter). The fruits will continue to ripen at room temperature until the fruit splits open, allowing the seeds to be released.

   Alternatively, the collected fruits can be manually opened and the seeds removed for immediate planting.

3. **Seed Storage**
   
   There is no seed dormancy in *Thalassia testudinum*. Seeds begin to germinate while still inside the fruit (vivipary). The seeds cannot be stored and must be planted immediately. A tetrazolium red (TZ) dye test can be performed to determine seed viability (see Appendix A).

4. **Seed Germination**
   
   Relatively little is known about the seed germination requirements for *Thalassia testudinum* as they begin developing into seedlings while still inside the fruit. Orth et al. (2006) reported that seedling survival rates vary from year to year, but are typically very low (11%).
5 Seedling Propagation

The seeds collected from the fruits are then rinsed several times with sea water. The rinsed seeds are planted into hydrated peat pellets (Jiffy Products -Jiffy 7) using forceps (tweezers). The peat pellets are buried to a depth of 1-2” (2.5-5 cm) with sand in small plastic or clay pots, maintained submerged in flowing full strength seawater with a salinity of 35 parts per thousand (ppt).

Seedlings must be kept submerged in full strength seawater at all times, and do better if there is some flow of water past the leaves. A small aquarium pump or air-stone should be sufficient. Water movement helps to break down the surface boundary layer on the leaves and enhances gas and nutrient exchange, promoting better growth.

Pots with seedlings are best kept in 15-50 gallon aquaria indoors in a temperature controlled room at 81-86°F (27-30°C) under high output T5 daylight (6,500 K) or blue-spectrum (15,000-20,000 K) fluorescent lights (300-500 W, 150-300 µmol irradiance) on a 18:6 hour (light:dark) photoperiod.

The pots with seedlings can also be placed in 100-500 gallon seawater tanks inside a greenhouse under natural photoperiod. Pots should be submerged with about 6” (15 cm) of water covering them; this is the space the leaves will grow into.

Seedlings are grown for 6-12 months until they reach a size of at least 8” (20 cm). These plants can then be transplanted into larger containers that provide at least 4-6” (10-15 cm) sediment depth (see Transplanting section below).

It may take as much as two to five years for coalescence to 100% cover in this size container, so maintenance costs can quickly escalate when growing *Thalassia testudinum*. For this reason, this species is not recommended for nursery propagation from seeds for restoration projects. A more cost effective method, where appropriate, may be field collections of mature plants, which can be more quickly propagated for future restoration plantings.
**Thalassia testudinum**

**VEGETATIVE PROPAGATION METHOD**

1. **Plant Collection**
   This species, like most submerged aquatic vegetation (SAV), is easiest to propagate by vegetative growth. To do so, mature plants need to be obtained from field locations. In many states this will require the necessary permits from local, state, and federal agencies. In some cases it may take three to six months before the permit is available.

   The best time to collect plants for vegetative growth is in the spring and early summer while they are in the growing phase.

   Plants are easiest to harvest in shallow waters during low tide. Water deeper than waist deep will make efforts much more difficult and should be avoided if possible. The easiest method for harvesting is to collect sods approximately 1 x 1 ft square (30 x 30 cm) using a square-point or flat-head shovel. The shovel should be inserted to a depth of at least 1ft (30 cm) to capture as much of the below-ground root material as possible. It is necessary to cut all four sides of the sod, before trying to lift it out of the ground to minimize damage to the delicate roots. To further minimize damage to the root-zone during transportation, an 15 x 11” (38 x 28 cm) aluminum baking pan or similar container can be used to help support the sod.

   Alternatively, a 6” (15 cm) diameter or larger coring device can be used to rapidly extract a plug of roots and leaves from the seagrass bed while leaving a relatively small disturbance that can be recolonized within a few months by the surrounding plants.

2. **Transportation**
   It is imperative that the plant sod be transported and maintained completely submerged at all times to maximize the survival potential. Even as little as one to two minutes of air exposure will dry out the leaves, killing them. To minimize stress, water from the collection site should be used. We have found that 9 sods in aluminum pans stack well inside a 100 quart (94.6 liter) white cooler. Wood dowels are used as spacers between the pans and reduce the problem of the plants on the bottom getting crushed. Filling the cooler with multiple sods helps to minimize the motion of the water and, therefore, the loss of the delicate sediments around the plant roots.

   Alternatively, plant plugs obtained with a coring device can be placed in large resealable plastic bags and then tightly packed in the cooler in a single layer. The tops of the bags remain open and the entire cooler is filled with water from the collection site.

   The cooler should be kept shaded and cool as much as possible. It may be necessary to bring additional water from the collection site and replace lost volume in the cooler if long transportation times are expected. This will also help to maintain dissolved oxygen concentration and reduce mortality of any associated animals (usually marine worms or clams). Having a portable battery-powered aerator can also help in this regard.

3. **Transplanting**
   On return to the nursery, the plants can be planted immediately or left in the cooler with aeration overnight. It may be beneficial to exchange about half of the water in the cooler with water from the destination tanks to enable the plants to acclimate to the change in salinity and temperature more easily. In no circumstances should the salinity be less than 25 ppt if survival is expected. Temperature changes of greater than 9°F (5°C) are to be avoided. It is better to let the water cool or heat gradually in the cooler until it reaches a temperature similar to the destination tank.

   Planting of the sods into the growing tanks can be done many different ways. Generally an organic soil mixture (1:1 topsoil:sand ratio) works well. More sand in the mix can be used if the collection location was dominated by sandy rather than muddy sediments. The soil mix should be added to at least half-fill the planting container and then fully saturated with water from the destination tank before placing the seagrass sods in them. Do not use tap water for marine seagrass species as the low salinity shock will kill them.
Once the sods are placed, additional soil can be added to completely cover the roots, and additional seawater is added to completely saturate the soil. It is a good idea to add enough water to submerge the leaves also, if the container will not be placed in the destination tank immediately. Minimize the amount of time the plants are out of the water and get them from the cooler into the container, and then the container into the destination tank as quickly as possible.

We have found that larger containers [28 x 20” (71 x 51 cm)] high density polyethylene cement mixing tubs] that are bigger than the sods and provide at least 4-6” (10-15 cm) sediment depth work best, but quickly become very heavy and require two or more people to lift. Two sods can be planted per large tub and allow room for expansion and growth outwards from the sod into the tub.

Smaller plastic tubs [14 x 12” (36 x 30 cm) 12 quart plastic dishpans] are ideal for planting a single sod, and are generally light enough for a single person to handle. Alternatively large pots (8 or 12” plastic or clay) can also be used, however smaller pots are not recommended as too small a container size will reduce the likelihood of vegetative expansion.

This is because *Thalassia testudinum* has a growing region at the end of the horizontal rhizome, called the apical meristem. Without this vital region present, the remainder of the plant will slowly die off and no new growth will occur. Meristem density varies by collection location, but generally only one to two are present in a 1 x 1 ft (30 x 30 cm) area, so larger sods and larger planting containers maximize the potential for success.

4 Growing Methods

Once the newly planted sods in their container have been gently and slowly lowered into the destination tank, it is important to monitor conditions at least weekly. Salinity should be maintained at 30-35 ppt, temperature should be at least 77°F (25°C) and high light levels (at least 300-500 µmol irradiance) are all necessary for success.

It is not recommended to add any fertilizer, as this will just cause the water to go green with phytoplankton and stress the seagrass plants with not enough light. There are more than sufficient nutrients in the soil and the sod to sustain plant growth for at least one to two years.

Because of the high light-levels required for growth, the plants may be better held in outdoor tanks, to minimize electricity costs for lighting, or grown in tanks inside a greenhouse. The local climate will be an important consideration for which option will be best. If cool or cold winters are normal, then it will not be possible to maintain tanks outdoors at a warm enough water temperature for growth. Also, if heavy rainfalls are frequent, then the cost of replacing salt diluted by rain may quickly become prohibitive. For these reasons placing the tanks inside a greenhouse is a better option despite larger initial costs of building a greenhouse.

Shading should be kept to a minimum as the water will filter out a large amount of sunlight even in shallow tanks. It is not recommended to grow the plants in more than 2 ft (60 cm) of water, as low light-levels will be too limiting for success.

Evaporation losses of water from tanks held in the greenhouse will be the main maintenance requirement. Check the salinity to ensure it remains optimal and adjust as needed with additional salt or freshwater.

If indoor growing is necessary (e.g., too cold in winter), artificial lighting is a must. High intensity grow-lights will be needed and the building must be correctly wired to support such a large electricity demand. We have had best success with blue spectrum high output compact fluorescent bulbs (e.g., T5 daylight or deep-blue bulbs in a multiple bulb grow-light fixture). These can output around 300 µmol at a distance of 1-1.5 ft (30 - 45 cm) above the tank and produce very little heat. It is best to use a photoperiod of 20:4 hour (light:dark) to maximize growth at these relatively low light-levels.

Temperature control can be achieved by adding submersible aquarium heaters during the winter, or having an efficient gas burning heater to maintain the air temperature at 9°F (5°C) above the desired water temperature. During hot summer conditions, shade-cloth (50%) can be hung over the tanks, fans can be used to circulate the air, and many greenhouses have some type of active ventilation system to exhaust hot air. Remember these plants are relatively tolerant of warm water temperatures, but care should be taken to ensure water temperatures do not exceed 95°F (35°C) for prolonged periods (many hours per day).

Growth of *Thalassia testudinum* is slow compared to most other SAV species and it will not be unusual for the plants to require three or more months to recover from transplanting shock. It will generally take 12-18 months or longer before the open area of the tub becomes colonized by new shoots under optimal conditions. Sub-optimal growing conditions will prolong this process.

5 Restoration Considerations

Depending on the restoration requirements, the resulting tubs of plants may be transported in their entirety and then the sods placed in sheltered marine conditions. Ideal locations have low wave energy, slow tidal currents, and shallow depths with good water clarity (>50% of surface irradiance reaching the bottom).

Alternatively smaller sods can be extracted from the tub and handled like a transplant sprig. An excellent overview of the different seagrass transplanting methods and the relative success rates can be found in Fonseca et al. (1998). For *Thalassia testudinum* it is best to use larger transplant units, and better to keep the sediments around the roots than to attempt bare-root plantings.

Given the slow transplanting recovery and growth rates of this species, it cannot be recommended as the best option for restoration planting in most locations. More suitable species may include *Halodule wrightii* or *Ruppia maritima*.
**Citations**


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**Thalassia testudinum Propagation Guide**

**Seedling and Plant Propagation Charts**

- **Seed Germination**: 2-3 Weeks
- **Seedlings**: 6-12 Months
- **Container Plants**: 2-5 Years

- **Temperature**
  - 77-95°F (25-35°C) No Light Needed
  - 81-86°F (27-30°C)fluorescent 18:6 Light:Dark
  - <95°F (35°C) Ambient Temperature

- **Light**
  - Full Sun

- **Submerged**
  - Buried Peat Pellets
  - 1-2" Seawater
  - 6" Seawater

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**Further Information**

USDA PLANTS profile:
http://plants.usda.gov/java/profile?symbol=THTE6

Center for Plant Restoration and Coastal Plant Research:
http://sites.google.com/site/coastalplantrestoration/home

Field guide and images of Coastal Mississippi Wetland plant species:
http://jcho.masgc.org/

NRCS Jamie L. Whitten Plant Materials Center:
Plant Guide for Establishing Coastal Vegetation on the Mississippi Gulf Coast

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