

Field Protocol for *Thalassia testudinum* Productivity Measurements

The *Thalassia* leaf punching method was first published by Jay Zieman in 1974. Our revised method is based on the facts that 1) *Thalassia* shoots usually have 3 to 5 blades, and 2) the oldest blade in each shoot grows very slowly, if at all. To measure leaf growth rates, a hypodermic needle is pushed through the bases of all the leaves in a *Thalassia* shoot in situ. The punched shoots along with attached roots and rhizomes are gently harvested after 9-10 days. In the lab, the shoots are cut near the lateral meristem near the base of the shoot, and the distance between the cut line and the punch mark on each blade is measured. As noted, the oldest blade will not grow more than a couple of millimeters in the period after punching. Younger blades can grow several millimeters per day. Linear leaf growth rates are summed for all the leaves in a shoot, and the productivity of the shoots is calculated as total leaf elongation rate multiplied by the leaf width.

- I. Setting out productivity quads and punching shoots- Four 20 cm x 20 cm PVC quadrats are deployed at each productivity site. Each quadrat is placed on the sediment surface in a *Thalassia* bed (other seagrass species can be present), taking care not to squash leaves or shoots under the PVC quadrat frame. The four corners of each quadrat are held in place with landscape staples. Each *Thalassia* shoot inside the quadrat is punched. All the leaves of each shoot are trained together, so a 20-gauge hypodermic needle that punches through one leaf will punch all the way through all the other leaves in the shoot. The first needle punch passes through all the leaves in each shoot near the top of the dead leaf sheaths, and the second punch mark is made about 5 mm above the first punch. All the *Thalassia* shoots in each 20 cm x 20 cm quadrat are punched, but other seagrass species are left alone. After punching all the *Thalassia* shoots in the 4 quadrats at a site, fill out the productivity data sheet completely, including the approximate time of punching and the time zone used. Two important caveats: 1) Feel for the place where the shoot changes profile from cylindrical to flat and punch the flat part of the shoot base, and 2) **BE SURE to record the GPS coordinates of the plot** and make a map of the quad locations—you will need this at harvest time! At each site, separate quads by at least 1 meter. It's not a great idea to put a float or a stake at the quad locations because curious boaters will

pull up stakes and quads. If you DO place a stake or a float, put it a fixed distance and heading from the quads and record this information on the productivity data sheet.

- II. Nine to ten days later, the shoots are harvested for growth measurements in the laboratory. The shoots must be harvested carefully to avoid separating blades- especially old and brittle blades- from their shoots. Shoots are harvested along with roots and rhizomes and all other seagrass and algal biomass (live and dead) to estimate total *Thalassia* biomass in each quadrat. A keyhole saw or drywall saw is used to sever the rhizomes by cutting vertically into the sediments around the entire inside perimeter of each quadrat. If the sediments are soft enough, it is now possible to lift the entire “sod” of *Thalassia* shoots, roots, and rhizomes out of the quadrat (including all other seagrasses and macroalgae present as well). The material is gently placed in a mesh laundry bag, and the sediment is very gently rinsed from the seagrass material (don’t slosh it up and down off the side of the boat; better to have someone in the water to carefully and slowly move the bag and contents in the water to remove sediment). The bag contents are then gently transferred to a sorting tray in the field, and all the intact *Thalassia* shoots are placed together in a Ziploc bag, with a completed label. Remaining loose blades, rhizomes, and roots, dead seagrass material, shoots of other seagrass species and macroalgae are placed in another bag along with another completed label. Both bags are labeled with the site name, quadrat number, and date. Bags are held in a cool location until transfer to a refrigerator. Prior to analysis in the lab, the shoot samples should be held in a cool refrigerator but not frozen.