Productivity Quad Processing

In order to quantify the biomass and productivity of *Thalassia testudinum* and the biomass of other seagrass species and macroalgae, biomass cores or quads of a defined area are collected. Square quads, 20 cm by 20 cm in area, are used to measure *Thalassia* productivity using the blade punching technique. During the collection of each quad, divers are careful to collect all above- and below-sediment biomass, including macroalgae, other seagrasses, and dead or detrital material. The collected material is gently washed in the field to remove as much sediment as possible as well as any large organisms and shell or rock material. Because these samples are collected to quantitatively estimate the amount of seagrass and macroalgal biomass on an areal basis, it is essential to carefully handle and process these samples so no biomass material is lost. For productivity quads, if time permits, at the time of collection, the punched shoots will be separated from the remainder of the biomass collected and carefully placed in a separate bag. When segregating the punched shoots, be very careful to keep all blades attached to the shoot. The oldest blade of the shoot is a critically important part of the productivity measurement. The quads are refrigerated until lab processing.

Initial Lab Processing:

Lab processing is a time-intensive effort, so it is a good idea to start with the punched shoots, and hold the remaining material in a refrigerator. The seagrass tissues begin to degrade after 3 to 4 days in the refrigerator, and sometimes the presence of animals and sulfidic sediments will cause a big odor buildup. If sediments really smell strongly of sulfide, the processing can take place in a hood for safety reasons. If there are many quads to process, then it is acceptable to freeze the sample portion that does not contain the punched shoots. It is important, though, to measure morphometrics within 3–4 days of collection on 20 shoots, even if unpunched shoots have to be used to get the shoot count to 20 for each quad.

1. Carefully rinse the quad material with tap water and drain the muddy water through a sieve to prevent excessive sediment and large rocks from going down the sink drain. Retrieve any biomass material that escapes.
2. Find the identifying label and record the site id and collection date on the Quad Morf Data Sheet.
3. On the Sample Log, identify the sample you are working on. This log will allow us to determine if any samples are missing, etc.
4. If punched shoots have already been segregated, then count the number of punched shoots. If the number is <20, then find unpunched *Thalassia* shoots in the remaining biomass to reach a total of 20 shoots for morphometrics. Record the number of punched shoots on the Quad Morf Data Sheet.
5. If punched shoots have not been segregated from the whole sample, then carefully go through the sample and:
    A) Identify and segregate the punched shoots, being very careful to keep all blades attached to the shoot. The oldest blade of the shoot is a critically important part of the productivity measurement.

B) If there are <20 punched shoots, then find enough unpunched shoots to make 20 shoots and place these shoots with the punched shoots. If the total number of *Thalassia* shoots is <20, then measure morphometrics on every shoot in the sample.
C) Identify any macroalgae present, clean, and place into drying dishes by species. Label the drying dishes with the site id, collection date, quad number and algal genera or species.
D) Place the remaining biomass material (remaining live *Thalassia* shoots, roots, rhizomes, dead shoots, loose blades, other seagrasses, etc.) back into the sample bag with an id tag and refrigerate or freeze, if it will be a week or more before the sample will be processed. These will be processed at a later time.
E) If you observe flowers or seeds on the *Thalassia* shoots, please note this on the Quad Morf Data Sheet, and save these with the rest of the shoot sample.

***Thalassia* morphology measurements:**

Use a separate Quad Morf Data Sheet for each quad. Before starting measurements, be sure to fill in the quad identifying information completely at the top of the data sheet. Be sure to enter quad id information on all subsequent data sheets for a particular sample, and paginate as well. The page-1of the Quad Morph Data Sheet is different from the subsequent data sheet pages and has several other count-boxes that will be filled in when you process the unpunched portion of the quad.

1. Place the *Thalassia* shoots you have chosen to process in a tray with a small amount of water (tap water is fine). The blades need to stay hydrated so it might be helpful to place a wet paper towel over the shoots as well.
2. Begin with the punched shoots.
3. Remove any live roots and rhizomes from the punched shoot, rinse these materials, and place them in a drying dish labeled LVRORH-1. Cut off the short shoot where it connects with the rhizome. If roots are attached to the short shoot, clip these off and place in the LVRORH-1 drying dish. If the short shoot is missing but the shoot blades are still attached to each other, this shoot can be processed, but make a note that the short shoot was missing on the Quad Morf Data Sheet. All live root and rhizome materials from measured shoots can be placed in the same drying dish.
4. Select a shoot and lay it out on the measuring board. Carefully cut off the short shoot where the blades begin to emerge but definitely below the punch marks. Measure the length of the short shoot in cm (00.0 cm). Carefully keep all the blade and sheath material of the shoot together and in their original orientation if at all possible. This will make blade numbering and measurements more consistent.
5. Once the short shoot has been measured, place it in a plastic zip bag, with an internal label, listing site id, date collection, and quad number. All measured shoots go into one bag and are frozen for future analysis.

Note 1: Each biomass fraction is dried together in a single drying dish. For example, all the live roots and rhizomes from a quad sample will go into one dish and will be dried together, as will all the blades from all the punched shoots in a quad sample. See Appendix 1 for a list of biomass fractions and their codes.

Note 2: Often on the outside of a shoot, sheaths are present. These are what are remaining of old blades that have senesced and fallen away; sheaths are white or translucent. Sheaths are placed with the short shoots. In some cases, punched sheaths can be used as the "reference blade" if that blade is missing: see below.

6. Assign each shoot a number, starting at 1 and increasing consecutively. Blades must also be numbered, with the oldest blade (or sheath, see below) assigned #1.
7. Beginning with the oldest, outermost (and usually least healthy looking) blade, measure and record the following:
   A) Total length from the point where it was cut at the base to the tip or end, in cm (00.0 cm);
   B) Green length—the length of the blade in cm (00.0 cm) that is green. If some browning is present, count the blade as green up to where the blade area is at least 50% green. If the transition between green and not green is not sharp, choose a central position in the color transition and measure from there.
   C) Punch length—the distance from where it was cut at the base to the punch mark, in cm (00.0 cm).

Note 3: The punch marks in the oldest blade will be used as the reference mark for calculating productivity; therefore it is essential to have this measurement. Some shoots, however, may only have one or two live blades. If that is the case, try to find a punch mark in a sheath (remainder of an old, dead blade near where the blades emerge from the short shoot) and use this sheath as the "oldest blade" and therefore the punch reference distance. Note on the data sheet that you are measuring the punch length on a sheath and only measure the distance from the punch mark in the sheath to where the shoot was cut from the short shoot.

Note 4: There are likely 2 punch marks on each shoot. Decide which punch you will use for measuring purposes and be sure to use the same punch mark for each blade in the shoot.

   D) Blade width—take 3 measurements along the width of the blade and write down the average of the 3 in cm (0.00 cm). If the blade is narrow, use an ocular micrometer on a dissecting scope to measure an accurate width.
   E) Estimate how much (by percent) of the blade is green and how much is covered by epiphytes. You can use the markings on the board to assist with these estimates.
   F) Note the tip condition (see Appendix 2 for codes), whether complete (P=point) or broken (B), and the type of damage evident on the blade. See Figure 1, below, for description of the types of damage. Note the location of the damage, the extent and pattern. You may note >1 location and pattern, but write down just one entry for extent.
   G) Finally note the type of epiphytes (there may be >1), using the codes in Appendix 2.

H) After all measurements and notations are complete, gently scrape the blade with a single-edge razor blade or sponge to remove epiphytes and sediment. Once the blade is clean, rinse it off and place it into a tissue drying dish labeled with the site ID, quad id, collection date, and LVPBLD-1 if the shoot is punched or LVBLD-1 if the shoot is not punched.

8. After measuring the oldest blade, proceed to the next oldest blade, usually on the opposite side of the shoot. Assign this blade as #2, and measure as above.
9. Continue in this fashion, measuring from oldest to youngest blade, being sure to search for very short emerging new blades near the base of the shoot.
10. If any of the younger blades do not have a punch mark (they may have emerged after punching), enter “0.0” in the punch length column of the data sheet.
11. For shoots that were not punched, enter “0.0” in the punch length column for the first, oldest blade and all other blades. This indicates to data analysts that the shoot was not punched.
12. When you are finished measuring the blades of one shoot, be sure you have filled in the data sheet completely for that shoot before proceeding to the next shoot.
13. Continue measuring shoots until all punched *Thalassia* shoots in the quad sample have been measured or until you have measured 20 shoots, whichever comes first. If there are not 20 punched shoots in the sample, measure unpunched shoots, if available, to meet the 20 shoot requirement. If there are not 20 *Thalassia* shoots in the whole sample, then measure all the shoots present. Keep the blades from unpunched shoots separate from blades from punched shoots. The drying tray holding punched blades is called LVPBLD-1 and the drying tray holding unpunched blades is called LVBLD-1.
14. When 20 (or all) shoots have been measured in a sample, carefully rinse the material in the drying dishes with tap water, without losing any sample. Be sure each dish is properly labeled and place the dishes in a drying oven to dry at about 50^{o}C.
15. After about a week, the biomass fractions are weighed on a 3-place balance, and each fraction is stored in a quart Ziploc bag (or equivalent) with an identifying label placed inside the bag with the tissues.

Processing of Remaining, Frozen Biomass Quads:

After removal of macroalgae and live *Thalassia* shoots for morphometric measurements, the remainder of the biomass quads are refrigerated or frozen. The night before planned processing of frozen quads, remove several from the freezer and allow to thaw in the refrigerator overnight. If the quad samples are still frozen on the day of analysis, the bags can be placed in a tub of warm water to speed thawing.

The purpose of this process is to remove, sort, and clean all seagrass and macroalgal biomass, both dead and alive, prior to drying.

1. Select a sample and log it in on the Sample Login Sheet by indicating the date of second processing.

2. Find the Quad Morf Data Sheets from the first processing effort and log in the second processing date and your name. Keep these sheets with you because you will have to write in additional data.
3. Rinse the sample in the bag with tap water, and drain through a sieve, being sure not to lose any biomass material. Put the sample in a large tray.
4. Sort the sample into biomass fractions.
    a. Count the number of live and dead *Thalassia* shoots and/or short shoots and record the number on the Quad Morf Data Sheet. Place all dead short shoots in a drying dish labeled with the quad id, collection date and the fraction DDSS-2.
5. If any live *Thalassia* shoots remain, separate them, and cut the blades from the short shoots, and remove the short shoots from roots and rhizomes if necessary. Place the live short shoots in a drying dish labeled with the quad id, collection date and the biomass fraction LVSS-2. Place the live roots and rhizomes in a dish labeled LVRORH-2 along with the site id, collection date and quad number.

Note 4: if any punched shoots remain in the sample, treat them the same as the other live *Thalassia* shoots.

6. Carefully scrape and clean all blades from the live blades to remove epiphytes. Place all the cleaned blades in a drying dish labeled with the site id, quad id, collection date and the biomass fraction LVBLD-2.
7. Identify and separate out all loose live *Thalassia* blades and count them. Enter the count on the Quad Morf Data Sheet. Scrape and clean them and place all of them in a drying dish labeled with the site id, quad id, collection and the biomass fraction LVLBLD-2.
8. Identify and separate out all dead loose *Thalassia* blades and count them. If there are >25 dead loose blades, estimate the number in units of five. Scrape and clean them and place all of them in a drying dish labeled with the site id, quad id, collection date, and the biomass fraction DDLBLD-2.
9. Separate the dead roots and rhizomes from the live roots and rhizomes. In general, dead roots and rhizomes are darker in color, are squishy and often hollow inside. Clean sediments from these fractions. Note and count the total number of rhizome apices (where the end of a rhizome is a point). Enter the count on the Quad Morf Data Sheet. Place live roots and rhizomes in a drying dish labeled with the site id, quad id, collection date, and the biomass fraction LVRORH-2, and place dead roots and rhizomes in a drying dish labeled with the site id, quad id, collection date, and the biomass fraction DDRORH-2.
10. If there is any remaining biomass or detrital material that is hard to identify or is very decomposed, rinse it well and place it in a drying dish labeled with the site id, quad id, collection date, and the biomass fraction ODB (old dead biomass). A small 500 mm sieve is very helpful in rinsing ODB.
11. Repeat this process for any other species of seagrass that might be present. Be sure to count the number of shoots, both live and dead, for each species of seagrass present. No measurements are needed, but blades need to be cleaned. Record these number on the Quad Morf Data Sheet. Dry biomass fractions of each species of seagrass separately using the same biomass coding but be sure to write the seagrass species on the drying dish.

12. If any macroalgae were overlooked during initial processing, list the species on the data sheet, clean the macroalgae, and place each type in a separate drying dish labeled with the site id, quad id, collection date and the appropriate algal name with "-2" after the name.
13. At this point there should be no plant or algal biomass remaining in the sample. Discard the rocks, shells, sediment, etc., and rinse all the biomass fractions in their respective drying dishes with tap water. Place the dishes in a drying oven to dry for at least a week at about 50^{o}C.
14. Return the Quad Morf Data Sheets to the appropriate notebook.
15. After about a week, the biomass fractions are weighed on the 3-place balance, and each fraction is stored in a quart Ziploc bag (or equivalent) with an identifying label placed inside the bag with the tissues.

Appendix 1: Biomass Fraction Identification Key

Quad Biomass Fraction Identification			
Biomass Fraction	Code	1st Processing Date	2nd Processing Date
Live Punched short shoots	LVPSS	-1	.
Live non-Punched short shoots	LVSS	-1	-2
Live Punched blades	LVPBLD	-1	.
Live non-Punched blades	LVBLD	-1	-2
Live Loose blades	LVLBLD	.	-2
Dead Loose blades	DDLBLD	.	-2
Dead short shoots	DDSS	.	-2
Live roots & rhizomes	LVRORH	-1	-2
Dead roots & rhizomes	DDRORH	.	-2
Old, Dead, detrital material	ODB	.	-2

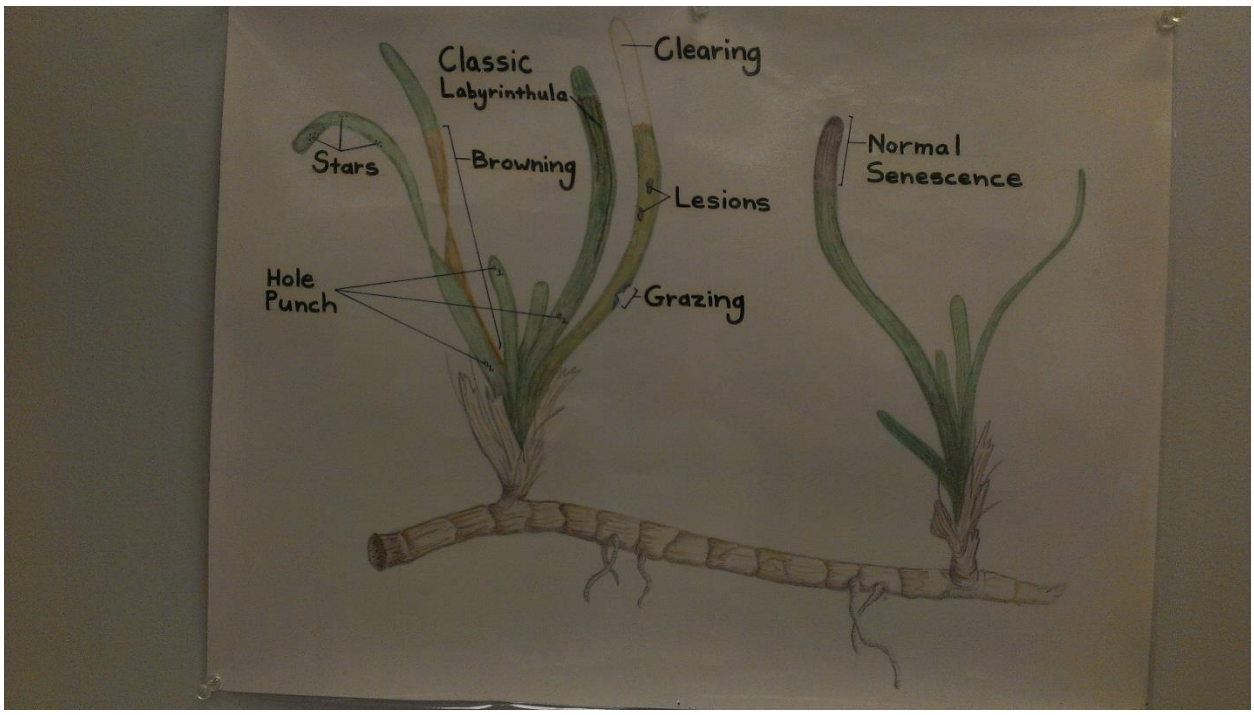

Figure 1. Types of damage to Thalassia blades.

Appendix 2: Codes for Seagrass Blade Characterization

Tip Condition:

P - normal point
B – broken

DAMAGE: (enter n/a if none)

LOC (location):
A – apex
M – middle
B – base
S – sides
(may use >1 entry)

EXT (extent):
N – none
S – slight
M – moderate
E – extensive
T - total
(use only one code)

PATT (pattern):
G – grazing
L – *Labyrinthula*

Epiphyte Codes:
1- sediment
2- calcareous algae
3- epiphytic algae
4- *Spirorbus*
5- mussels, sponges, misc.
6- *Corophium*
7- Foraminifera
(may use >1 entry)

B – browning
C – clearing
N – normal senescence
S – stars
(may use >1 entry)