**Octocoral Physiology Prep Protocols**

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*Each of the steps in bold can be done on a different day, or back to back on the same day!*

**Measuring branch width**

*We may chose to compare branch width across the depth gradient, so we need to do these measurements before we freeze-dry!*

1. For each sample, measure the branch width at three points along the branch using calipers.
2. Record the three widths.

**Freeze Drying**

1. Prepare the cooler with the following materials:
   1. Gloves
   2. Tube rack
   3. Pieces of parafilm
   4. Very fine/pointy tweezers
   5. Ice packs
2. Put the frozen samples in the cooler and drive them to bay campus.
3. Get the samples in the McMahon oven room freezer as quickly as possible.
4. Follow the McMahon procedure for freeze drying.
5. Freeze dry samples for ~12hrs.
6. Once samples are done in the freeze-dryer, put the caps on and keep them at room temperature.

**Grinding**

1. Clean out mortar and pestles using 70% ethanol and a kim wipe.
2. Once ethanol has evaporated, place dry coral tissue in the mortar.
3. Grind the sample into a powder. Remove core with tweezers as early as possible. Put core back in the tube for safe keeping.
4. Tare a piece of weigh paper on the scale.
5. Pour the ground sample onto the of weigh paper.
6. Weigh sample and record sample weight.
7. Carefully pour sample into the 1.5ml tube.
8. Store at room temp until ready to move on.

**Aliquoting**

*This step measures out two aliquots of the freeze dried material for future phys work. One will be for chlorophyl and the other will be for symbiont counting and protein analysis.*

1. Prepare two 1.5 ml tubes per sample
   1. Project + Sample name + “CHL”
   2. Project + Sample name + “Sym”
2. Tare a piece of weigh paper on the scale
3. Weigh 15 mg of sample.
   1. Weight must be +/- 0.1mg so between 14.9 and 15.1mg
4. Record the weight in the correct column (chl\_weight or sym\_weight)
5. Dump into tube C
6. Repeat for tube S
7. Store at room temp and transfer to main campus

**Aliquot re-saturation**

1. Prepare two more 1.5 ml tubes per sample
   1. Project + Sample name + “CHL”
   2. Project + Sample name + “Sym”
2. Re-saturate sample and leave sclerites behind by doing 3 rinses:
   1. Add 0.5ml of DI II to each of the tubes containing the measured sample
   2. Vortex for 3+ minutes (extra time doesn’t hurt)
   3. Use pipette to gently take up the liquid and transfer it to the new corresponding tube. Leave liquid behind rather than taking up any sclerites
3. Repeat step 2 two more times, for a total of three rinses, resulting in 1.5ml of liquid in the final tubes.
4. The sym tube is done.
5. Spin the CHL tubes in the centrifuge for 3mins at 13,000g.
6. Use a pipette to discard the supernatant liquid from the CHL tube.
   1. Careful not to disturb the pellet
7. Store all re-hydrated samples and CHL pellets at -80.

**Phys Assays**

1. Follow the coral CHL protocol using the CHL pellet.
2. Use the Sym tube for sym density and protein concentration assays.