Frozen mussel dissection SOP

SOP derived from protocol developed by Chris Mantegna and Austin Millen and adapted by Ly Vuthy, UW-Tacoma.

Materials:

- Dissecting tools + clean razor blade
- Dissection tray
- Ice for staging container
- Thin, cloth-like sheet material (paper towel, cheesecloth, etc)
    - Cut into ~ 4” x 8” rectangles
- Liquid nitrogen in insulated safety container (**Training Required**)
- Long forceps
- High proof alcohol (70% or greater)
- Soap and water
- Aluminum foil (Cut/Ripped into 2” x 2” square) + Fine Tip Sharpie
- Container with dry ice (interim storage)
- Small stack of paper towels if needed

Preparation for Dissection

- Obtain the dissection tables (one for weights/heights, the other for thickness)
- Label dissection tables with date and initials
- Labeling each dissection table 1-6 + Site name
    - Numbering depends on how many mussels are in the bag
    - Numbering continues from last dissected mussel
- Label pieces of tin foil with the number ID and each tissue type. A total of 8 piece of foil is needed for each mussel dissected
    - L = Left, R = Right
    - L Man, R Man, L Gil, R Gil, L Dig, R Dig, Vis, Add
- Establishing a ‘dry’ and ‘wet’ dissection space

Obtaining Mussels and Measurements

- A sample site bag of frozen mussels is removed from -80 °C freezer storage and placed in an insulated container with ice to slow thawing rate. Recommended doing one bag at a time.
- Multiple bags of mussels can be removed and placed in the -20 °C freezer depending on dissecting skill and speed. Ice bucket can accommodate six mussels at a time, the top row should always be 1-3 and the bottom row should be 4-6. If there are less than six mussels, continue to adhere to this placement convention.
    - Make sure you can recall the order of the mussels and numbering convention

      Note: *All mussels awaiting dissection should be covered in ice. This will prevent the mussel from rapidly thawing.*

IMPORTANT: *Ensure no cross-contamination occurs between dissections. Always clean tools with high-proof alcohol and soapy water and change cloth material between each specimen. THIS INCLUDES THE RAZOR BLADE*

- Measure the height, length, width, and weight of the whole mussel. Keep this information with the respective specimen ID for records

Dissection

- Slide a razor blade along the seam of the two shells, taking care to completely cut the mussel in half (Make sure the mussel is sitting on the table to prevent injuries). Use extreme caution during this step especially around the hinge as mussels can be slippery in this state.

  Note*: all tissues should be collected as quickly as possible before thawing to prevent degradation. Tissues should be flash frozen in liquid nitrogen and immediately stored in a container with dry ice until earliest convenient storage at - 80 °C*

- Take out the foot with forceps or scissors. Then take out the byssal thread by cutting it with scissors

- Quickly tease the first gill from the mantle with a dissecting needle or tool of choice. It often presents as a feathery ice structure with a clear attachment point medially by the solid viscera block. Dissect along this plane, taking care not to include any extraneous tissues

- Once tissue is dissected, placed in correctly labeled tin foil and flash freeze by putting it in the liquid nitrogen. Do this for the remaining tissues.

- The first mantle should be readily apparent and easily detach with light, gentle scraping

- By now, the specimen has thawed slightly enough to separate the frozen viscera block from the other gill and mantle. Perform the previous two steps to collect.

- The mantle and gills typically separate easily from the posterior adductor muscles. The muscle can be scraped off at this point.

- The anterior adductors are not always large or easily distinguishable. If present, collect these now. They are typically found attached laterally near the apical portion of the anterior shell.

- Orient the frozen viscera block in a way that the grey or black digestive gland is visible. The color will be darker the more recently the mussel last ate. Gently tease away the ice, following any natural planes that present during this time. The entire digestive area is usually taken at this time to ensure gland is not left behind.

    Note: *due to the minutely varied nature of individual anatomy, dissection of the frozen viscera block will vary for each specimen. Generally, the digestive gland is readily distinguishable and found in relatively similar states between specimens*

- The gonad is not always large or easily distinguishable. If present, collect separately from the remaining tissue. It is typically found near the posterior end of the body, on either side of the digestive tract.

- The remaining soft tissue mass can be further dissected as desired and flash frozen for future use as-is.

- As you complete your dissection, place the mussel back in an order where you can remember the number ID after all dissections are complete. Once the site group has been completely dissected, take your empty shell weight and shell thickness. A point-to-point caliper works best to measure the irregularly curved shell surface. **Four** thickness measurements should be taken along the length of the shell, and is recorded on the data sheet so the average can be taken.

- Place all tools into an alcohol solution followed by a rinse in DI water after each individual mussel dissection. If there are pieces adhering to the tools, wash with soapy water first and then place in alcohol and rinse. BE SURE TO CLEAN THE RAZOR BLADE

- Step up the following 8 pieces of tin foil and change out the cloth-like material between each mussel

- Repeat steps for all remaining specimens