**Tissue Sampling for Summer Flounder Sea Grant project (2013-2016)**

***Sampling locations:***

1. North Carolina: far south
2. North Carolina: just south of Cape Hatteras
3. Virginia: southern
4. Virginia: northern
5. New Jersey: southern
6. New Jersey: northern
7. Massachusetts/Rhode Island: south of Cape Cod
8. Massachusetts: Georges Bank

***Sampling materials***

1. Surgical scissors
2. Forceps (tweezers)
3. Squeeze bottle of deionized (DI) water for cleaning between samples
4. Kimwipes or other clean tissues
5. Squeeze bottle of 95% (or higher) ethanol for sample preservation
6. 125 sample tubes (e.g., twist top 1.5mL tubes)
7. Two sample boxes
8. Pencil
9. Heavy cardstock or RiteInTheRain paper
10. Fine-tip sharpie marker
11. Field notebook
12. 5 gallon bucket for rinse water (if working inside)

***Sampling procedure:***

1. Samples for DNA should be taken at the dock from fish recently caught by commercial fishermen. We need at least 50 fish from each sampling location (preferably 60 fish). Be sure the fisherman can identify the location of catch to within 10’ latitude and 10’ longitude (the more accurate the better).

2. DNA cross-contamination is always an issue that can compromise results from molecular studies (this is the contamination of one sample of DNA with DNA from another sample), and it is a particular concern with the next-generation sequencing that we will do. After every fin clip is taken from a specimen, the scissors and forceps must be cleaned thoroughly of any tissue, slime, blood, etc. Rinse them with DI water and wipe carefully with clean Kimwipes. THIS IS TO BE DONE BETWEEN EVERY INDIVIDUAL.

3. Before beginning to sample (this can be done ahead of time in the office), prepare 125 sample tubes. Label the exterior of the tube with a fine-tip Sharpie. Also add a small interior tube label written in pencil on card stock or other heavy paper (RiteInTheRain is good). The labels should be of the form “SS ### YYYY-MM-DD III” where SS is the state (NC, VA, NJ, or MA), ### is the sample ID (1 to 125), YYYY is the year of collection, MM is the month, DD is the day, and III are your initials. Please also write “P. dentatus” on the label.

4. Close each tube and return them to the sample boxes. This will help keep the tubes organized and avoid adding a tissue sample to the wrong tube.

5. Prepare your field notes in spreadsheet form. Data columns should include the state abbreviation (NC, VA, NJ, or MA); the sample ID (1 to 125); total length (cm); sex; the latitude and longitude of catch; the latitude, longitude and place name of the location where the specimen was fin-clipped; the date of collection; collectors’ name; and a notes column for any additional information that you wish to provide.

6. At the dock, pick your first fish, assign the next ID number, measure total length, determine sex, and enter the data in your field notebook.

7. Rinse off a section of the tail fin (or other fin if the tail is unavailable) with DI water. With sharp (clean) scissors, clip a 1 cm x 0.5 cm section of the fin. Once the clip is successfully removed, place it into the properly labeled tube (outside and inside labels already added).

8. Fill the tube with 95-100% EtOH from the squeeze bottle.

9. Clean the scissors and forceps thoroughly with DI water and tissues. **THIS IS TO BE DONE IN BETWEEN EVERY SINGLE INDIVIDUAL.**

10. Move on to the next fish specimen (Step 6).

**Storing Tissue Samples**

Keep the samples cool in the field (e.g., not on a car dashboard), and place them in a freezer when you return to the lab or office. Maintaining samples at a cooler temperature prolongs the life of the usable DNA.

**Shipping Tissue Samples**

EtOH is a flammable liquid, and the simplest thing is to hand-carry them to Rutgers. However, these samples are also small enough that they fall under the FedEx “Dangerous Goods In Excepted Quantities” exception and can be shipped normally if provided with enough documentation. Contact Malin (malin.pinsky@rutgers.edu) for instructions if you need to ship samples.

*Instructions adapted from http://www.cypriniformes.org/tissue\_fish\_%20sample\_col\_protocols.html. Thanks to Richard Mayden and Casey Dillman.*