Fish Library Protocols

Species List References

Integrated Taxonomic Information System. 2006. http//www.itis.usda.gov/index.html

Pennsylvania Natural Heritage Program. 2006.
http://www.naturalheritage.state.pa.us/vertebrates.aspx

Collection Protocols

Stream seining:

1. Locate a "typical riffle". Such a riffle would have a stream bed uniformly composed of rocks, ranging in size from ten-inch cobbles down to one-quarter-inch gravel. The water will range in depth from approximately two inches to a foot, with a moderate-swift flow. Avoid riffles located in an area of a stream that has been recently disturbed, such as construction from a pipeline crossing or roadway.

2. Once the riffle has been located, select an area measuring 3 feet by 3 feet which is typical of the riffle as a whole. Avoid disturbing the stream bed above this area, so as not to alter the sample.

3. Prior to entering the stream, examine the net closely. Remove any organisms that might remain from the last time the net was used.

4. **DO NOT STAND IN THE SAMPLING AREA**

5. Have one person place the net at the downstream edge of the sampling area. The net should be held perpendicular to the flow, but at a slight downstream angle. Stretch the net to approximately three feet, but be certain that the bottom edge is lying firmly against the bed. If water washes beneath or over the net you will lose organisms.

6. **STAND BESIDE, NOT WITHIN THE SAMPLING AREA!** Place one foot at the upstream edge of the area as a marker. Remove all stones and other objects two Inches or more in diameter from the sampling area. Hold each one in front of the net and below the water surface as you brush all organisms from the rock surface. Before placing each rock outside the sampling area, examine the surface to be certain you have not missed any organisms.

7. When all materials two inches or larger have been brushed, step into the upstream edge of the sampling area and kick the stream bed vigorously for exactly sixty seconds. Kick from the upstream edge towards the net so that when sixty seconds are up you will have just reached the seine. Try to disturb the bed to a depth of at least two inches.

8. Once Step 7 is completed, carefully remove the net with a forward scooping motion. **DO NOT** allow water to flow over the top of the net or you may lose organisms.

9. Carry the seine to a flat and clean area on the stream bank. Remove leaves, rocks, and other debris. Examine for any attached organisms. Using fingers or forceps, remove the larger organisms from the net and place in the plastic container with water for later identification. Examine the smaller organisms that remain on the net.

10. Record the presence of each type of organism collected and give an estimate of the number of each type using the appropriate letter code on the stream quality assessment form.

11. Go to the edge of the stream; turn over rocks to see if you observe any additional macroinvertebrates that you have not already collected in the seine.

12. If time permits, take another collection in a different area of the same riffle.

13. Consolidate all the collections made on the same day at each individual station to obtain a cumulative index value that will best represent all the taxa present.

14. Determine the stream quality assessment using the procedures listed before.

Commonwealth of Kentucky. 2006.
http://www.state.ky.us/nrepc/water/kicksein.htm

Beach Seining:

Beach seines (sometimes called haul seines because they are hauled or pulled to catch fish and shellfish) were used by the ancient Phoenicians to catch fish in the Mediterranean, and these nets remain basically unchanged today. There are four parts to a beach seine:

- Float line -- supports the top of the webbing and has attached floats.
- Webbing -- usually 1/4-inch or larger, generally 4 feet deep, and of varied ength.
- Lead line -- supports the bottom of the webbing and has lead weights attached.
- Poles -- attached to the ends of the net and are used to drag the seine.

Seines are usually made of cotton or nylon and are available in various mesh sizes. Nylon nets may cost more, but they will last for years. Minnow seines have a 1/4-inch mesh. Larger meshes are used commercially to selectively catch larger fish.

Seines can be ordered in any length or depth. As a general rule, a 4-foot depth is sufficient for minnow seines. Nets over 20 feet long are of questionable value, as they don't catch different species, just more animals.

Below are a few rules for successful seining:

- Keep the net in a half-moon configuration.
- Never tow hard enough to pull the lead line off the bottom or to pull the floats under.
- Keep the poles touching the bottom.
- When pulling the seine onto the beach, keep the lead lineon the bottom or your catch will escape.
- Watch for "hang-ups" that might catch or rip the seine.
- Wear shoes or sneakers when seining. It only takes one broken bottle or sharp shell to ruin your outing.
- Clean and rinse the seine with fresh water when you are finished, and let the net air-dry.

Procedure adapted from:

Hall, William Dr. University of Delaware Sea Grant.
http://www.ocean.udel.edu/seagrant/publications/beachseining.html. Accessed Aug 2006.

Backpack Electrofishing:

ELECTROFISHING CONFIGURATION AND FIELD TEAM ORGANIZATION

All field team members must be trained in electrofishing safety precautions and unit operation procedures identified by the electrofishing unit manufacturer. Each team member must be insulated from the water and the electrodes; therefore, chest waders and rubber gloves are required. Electrode and dip net handles must be constructed of insulating materials (e.g., woods, fiberglass). Electrofishers/electrodes must be equipped with functional safety switches (as installed by virtually all electrofisher manufacturers). Field team members must not reach into the water unless the electrodes have been removed from the water or the electrofisher has been disengaged.

It is recommended that at least 2 fish collection team members be certified in CPR (cardiopulmonary resuscitation). *Many* options exist for electrofisher configuration and field team organization; however, procedures will always involve pulsed DC electrofishing and a minimum 2-person team for sampling streams and wadeable rivers. Examples include:

- Backpack electrofisher with 2 hand-held electrodes mounted on fiberglass

poles, one positive (anode) and one negative (cathode). One crew member, identified as the electrofisher unit operator, carries the backpack unit and manipulates both the anode and cathode poles. The anode may be fitted with a net ring (and shallow net) to allow the unit operator to net specimens. The remaining 1 or 2 team members net fish with dip nets and are responsible for specimen transport and care in buckets or livewells.

- Backpack electrofisher with 1 hand-held anode pole and a trailing or floating cathode. The electrofisher unit operator manipulates the anode with one hand, and has a second hand free for use of a dip net. The remaining 1 or 2 team members also aid in the netting of specimens, and in addition are responsible for specimen transport in buckets or livewells.
- Tote barge (pramunit) electrofisher with 2 hand-held anode poles and a trailing/floating cathode (recommended for large streams and wadeable rivers). Two team members are each equipped with an anode pole and a dip net. Each is responsible for electrofishing and the netting of specimens. The remaining team member will follow, pushing or pulling the barge through the sample reach. A livewell is maintained within the barge and/or within the sampling reach but outside the area of electric current.

FIELD EQUIPMENT/SUPPLIES NEEDED FOR FISH SAMPLING--ELECTROFISHING

- appropriate scientific collection permit(s)
- backpack or tote barge-mounted electrofisher
- dip nets
- block nets (i.e., seines)
- elbow-length insulated waterproof gloves
- chest waders (equipped with wading cleats, when necessary)
- polarized sunglasses
- buckets/livewells
- jars for voucher/reference specimens
- waterproof jar labels
- 10% buffered formalin (formaldehyde solution)
- measuring board (500 mm minimum, with 1 mm increments)[a]
- balance (gram scale)[b]
- tape measure (100 m minimum)
- fish Sampling Field Data Sheet[c]
- applicable topographic maps
- copies of field protocols
- pencils, clipboard
- first aid kit
- Global Positioning System (GPS) Unit

[a] Needed only if program/study requires length frequency information
[b] Needed only if total biomass and/or the Index of Well-Being are included in the assessment process (see Section 8.3.3, Metric 13).
[c] It is helpful to copy fieldsheets onto water-resistant paper for use in wet weather conditions.

The safety of all personnel and the quality of the data is assured through the adequate education, training, and experience of all members of the fish collection team. At least 1 biologist with training and experience in electrofishing techniques and fish taxonomy *must* be involved in each sampling event. Laboratory analyses are conducted and/or supervised by a fisheries professional trained in fish taxonomy. Quality assurance and quality control must be a continuous process in fisheries monitoring and assessment, and must include all program aspects (i.e., field sampling, habitat measurement, laboratory processing, and data recording).

8 Field Sampling Procedures

1. A representative stream reach (see Alternatives for Stream Reach Designation, next page) is selected and measured such that primary physical habitat characteristics of the stream are included within the reach (e.g., riffle, run and

pool habitats, when available). The sample reach should be located away from the influences of major tributaries and bridge/road crossings (e.g., sufficiently upstream to decrease influences on overall habitat quality). The exact location (i.e., latitude and longitude) of the downstream limit of the reach must be recorded on each field data sheet. (If a Global Positioning System unit is used to provide location information, the accuracy or design confidence of the unit should be noted.) A habitat assessment and physical/ chemical characterization of water quality should be performed within the same sampling reach (see Chapter 5: Habitat Assessment and Physicochemical Characterization).

2. Collection via electrofishing begins at a shallow riffle, or other physical barrier at the downstream limit of the sample reach, and terminates at a similar barrier at the upstream end of the reach. In the absence of physical barriers, block nets should be set at the upstream and downstream ends of the reach prior to the initiation of any sampling activities.
3. Fish collection procedures commence at the downstream barrier. A minimum 2-person fisheries crew proceeds to electrofish in an upstream direction using a side-to-side or bank-to-bank sweeping technique to maximize area coverage. All wadeable habitats within the reach are sampled via a single pass, which terminates at the upstream barrier. Fish are held in livewells (or buckets) for subsequent identification and enumeration.

Sampling efficiency is dependent, at least in part, on water clarity and the field team's ability to see and net the stunned fish. Therefore, each team member should wear polarized sunglasses, and sampling is conducted only during periods of optimal water clarity and flow.

U.S. Environmental Protection Agency. Monitoring and assessing water quality. http://www.epa.gov/owow/monitoring/rbp/ch08main.html. Accessed Aug 2006.

Boat Electrofishing:

Boat electrofishing is the use of electricity to stun and capture fish. The electrofishing unit used by the WDFW Warmwater Teams, is an electrofishing boat manufactured by Smith-Root, Vancouver, WA. The 16-18 foot flat bottomed boats are manufactured of welded aluminum. They have an electrical generator onboard. Anode and cathode droppers attached to the bow of the boat, touch the surface of the water. Anodes droppers hang from the front of two booms extended out in front of the boat. Cathode droppers are attached to the bow. Once the boat and generator are running, and the activation pedal is depressed, an electrical field is created in front of the boat.

A control panel allows the operator to adjust the strength of the electrical field. This allows Biologists to efficiently capture fish, while reducing the chance of injury to fish.

During a typical electrofishing survey, the boat is maneuvered through the shallows, following the shoreline of the lake. Electrofishing is usually conducted for 600 seconds of "pedal down" shocking within each randomly chosen section. The surveys are conducted

after sunset, as research has shown that nighttime electrofishing is more effective. In a dc field (the safest type of current for fish and the type that the WDFW uses), a typical reaction of the fish will be to turn towards the anode exhibiting galvanotaxis, (forced swimming with orientation) followed by galvanonarcosis (muscle relaxation). The reactions bring the fish in towards the anode in a stunned state, where they are easily netted off the front of the boat. During a typcial electrofishing survey, stunned fish are netted and placed in a live well on the boat, to later be released or preserved.

Procedure adapted from:

Washington Department of Fish and Wildlife. Warmwater fish enhancement program. http://wdfw.wa.gov/fish/warmwater/surveys.htm. Accessed Aug, 2006.

Fish Preservation Protocol

The following methodology outlines the procedure for the preservation and vouchering of whole fish specimens. The method is not a precise guide for the preparation of specimens for long-term storage. It is a generalized guide instructive in the preservation process and the objectives of each step of the method. Variation in the timing, treatment method, and solution concentrations may be necessary to achieve the proper preservation. Specimens that will be used for taxonomic description should be processed immediately upon collection in a manner that will prepare them to future meristic, morphometric, and possibly genetic procedures. This type of specimen preparation is not discussed in this guide.

Preparation of fresh specimens

Euthansia

Specimens should be euthanized with methods compliant with the American Veterinary Medical Association (AVMA). **See the ARP website for AVMA methods of euthanasia (http://www.research.psu.edu/arp/euthanasia.shtml).**

Cleaning of specimens

Every effort should be made to remove any and all material that is not fish or fish tissue. Plant material has the potential to stain the specimens, thus, should be removed. Rocks and other hard material can damage soft tissues, dislodge scales, and otherwise lessen the quality of the specimens. Such objects should be separated from the collection.

Formalin Fixation

To ensure proper and safe handling of formalin, read the Material Safety Data Sheets (MSDS) before attempting to use formalin (formaldehyde) for any purpose.

Tissue penetration, thus histological fixation, occurs at varying rates that are dependent on temperature, tissue thickness, and tissue lipid content (lipids tend to slow formaldehyde penetration rates). Other factors can increase or decrease penetration rates, but typically are not considerations when fixing fish voucher specimens. Initial migration of formaldehyde can occur at a rate of 2mm per hour, at room temperature. The process of fixation acts against formaldehyde migration, slowing the rate of fixation as time progresses. Small fishes (8 cm or less) can be completely fixed within a 24 hour period. When fixing a fish collection of fishes of mixed sizes, it is our practice to allow 2 weeks for the fixation process. Complete fixation has likely occurred within the first week. The additional time allows for a complete even distribution of formaldehyde and more uniform fixation.

Ideally, specimens should be subjected to a concentration of 10% formalin during the tissue fixation process. To achieve the desired concentration of 10% formalin, consideration must be given to a number of factors; the size of the specimens, the volume of the fixation container, the concentration of the formalin solution, the volume of tissue to be fixed. For this reason, you may need to use full-strength formalin solution (37%-40%) when preparing formalin solutions for fixation of specimens in the field. For example, if you are using a container that has a 3.8 L volume and you intend to fill it to capacity with specimens and solution, you would need to first add 950 mL of 40% stock solution to the 3.8L container containing the fish and then top to 3.8L. This would result in a solution concentration of 10% that takes into account both the amount of fish tissue and the container volume. If formalin solution concentration is less than 10% there is a high likelihood that the specimens will decompose. Fish of large size (>20 cm) must have formalin directly injected into the abdominal cavity and, possibly the thick dorsal muscle tissue, particularly for larger specimens (>30 cm). When injecting formalin into muscle tissue, use full-strength solution (37%-40%). This will allow for a faster migration of the formaldehyde gas through the tissue, thus increasing the quality of the voucher specimens by minimizing tissue degradation through a more rapid fixation. Injection sites should be no further apart than 4 cm. Greater distances will result in greater potential for poor tissue fixation. The needle can be tracked and formalin injected into a larger area through the same injection site by altering the track of the needle to closely parallel the axis of the fish. As the needle is being backed out, formalin should be continuously injected. Long needles can be used on larger fish to inject formalin more evenly through thick muscle tissue. By using a large gauge needle ( ) and syringe ( ) you will expedite the injection process by minimizing the chance of needle fouling and need for syringe refills. When working with larger fish, it may be desirable to cut a slice into the abdominal cavity to permit the flow of formalin into the abdominal cavity. This cut should be made on the right side of the fish close to the ventral midline. The length of the incision should be about 1/3 of the abdominal cavity length. Take care to make sure the cut is completely through the abdominal wall. It is considered a standard conventional when vouchering specimens to make every effort to leave the left side of the specimen unaltered for taxonomic, photograpic and/or analytical purposes.

<u>Clearing of Formaldehyde</u>

Fish specimens must be cleared of formaldehyde before being placed in alcohol for long term storage. Either a 50/50 isopropyl alcohol/water solution or a 70/30 ethyl alcohol/water solution is used for long term storage. Most accredited museums (i.e., Smithsonian, British Museum of Natural History) use 70/30 ethyl alcohol.

The formaldehyde clearing process involves soaking the specimens in multiple changes of water until the presence of formaldehyde in no longer detectible. Detection involves gently smelling the specimens, but is only done after the 3rd or fourth water changes. Water changes can follow these general guidelines. Generally, the greater the water to specimen ratio, the faster the clearing process occurs. Separate the specimens from the formaldehyde solution. Rinse the specimens with fresh water. Place the specimens into a container and top with fresh water. The first water change can be done the following day. If all the specimens are small in size (<6 cm), you may only need 3 to 4 water changes over a 1 week period. Collections with fishes of mixed sizes or large specimens generally require 6-8 water changes over a 2 week period. Once clearing has been accomplished, place specimens in suitably sized glass containers, insert labels containing all the necessary information, add the alcohol solution, and catalogue for reference purposes. The specimens are now ready for long term storage and vouchering.

Procedure compiled by Tim Stecko