FS 7000. GENERAL BIOLOGICAL COMMUNITY SAMPLING

See also the following sections:

- FA 1000 and 2000 Administrative Procedures
- FC 1000 Cleaning/Decontamination Procedures
- FD 1000-9000 Documentation Procedures
- FM 1000 Field Planning and Mobilization
- FQ 1000 Field Quality Control Requirements
- FS 1000 General Sampling Procedures
- FT 1000 – 2000 General Field Testing and Measurement
- LD 7000 Documentation of Biological Laboratory Procedures
- LQ 7000 Quality Control for Biological Community Analysis
- LT 7000 Determination of Biological Indices

1. GENERAL CAUTIONS

1.1. Many of these biological sampling procedures require specific training and a demonstration of competency due to the expert judgment exercised during field sampling. It is recommend that individuals conducting physical/chemical characterization, habitat assessment, qualitative periphyton, Biorecon, Stream Condition Index, Lake Vegetation Index and Lake Condition Index sampling should train with DEP staff (via workshops and/or participating in field sampling) and pass a field performance test to demonstrate competence. (See FA 5710, section 2.)

1.2. When performing impact assessment in a stream, the contaminant plume from a point source or tributary may flow along one bank of the stream for some distance. Make sure the benthic biota are sampled within the influence of the plume. If nutrients are a problem in the discharge, the phytoplankton may be impacted farther downstream where the water slows down. Therefore, two locations may be needed to best characterize the effects of the discharge.

1.3. A freshwater effluent discharged to marine receiving waters may float at the surface until it mixes at some distance from the boil. Benthic biota beneath the discharge boil may not come in contact with the effluent very often. Checking conductivity or salinity at several depths, or performing a dye study, may aid in determining the degree of mixing and fate of an effluent. Sample the benthos at a point where the effluent is sufficiently mixed for it to contact the organisms.

FS 7001 Solution Preparation

1. Buffered Formalin

1.1. <u>Equipment and Supplies</u>

- Sodium bicarbonate
- 37% formaldehyde (formalin)

- pH meter
- Small containers
- Balance

1.2. Methods

1.2.1. Calibrate the pH meter according to FT 1000 and FT 1100.

1.2.2. Place a clean weigh pan on the balance. Tare the balance.

1.2.3. Weigh out approximately 5.0 grams of sodium bicarbonate.

1.2.4. Gradually add the sodium bicarbonate to your container of formalin. In general, one gram of bicarbonate will buffer about one liter of formalin, but it is necessary to check the pH of the buffered solution, as individual batches of formalin may vary in pH. Close the container and shake vigorously after each addition. Check the pH at each interval until reaching a pH between 7.5-8.0 S.U. The sodium bicarbonate may not all dissolve into the formaldehyde, as this is a supersaturated solution.

1.2.5. Transfer solution to smaller containers for field and laboratory use.

2. LUGOL'S SOLUTION

2.1. Equipment and Supplies

- Balance
- Weigh boat
- Spatula
- Magnetic stirrer
- Teflon stirbar
- Spatula
- 500-mL glass amber bottle
- 20 g potassium iodide (KI)
- 10 g iodine crystals
- 20 mL glacial acetic acid
- 200 mL deionized (DI) water in a 250-mL or larger Erlenmeyer flask
- Dropper bottle

2.2. Methods

2.2.1. Add acetic acid to DI water. Place on magnetic stir plate with stir bar.

2.2.2. Weigh out 20 g of KI and 10 g of iodine crystals.

2.2.3. Dissolve KI and iodine crystals into the above solution. This process may take from 30-45 minutes, and a small amount of residue may remain at the bottom of the flask.

2.2.4. Pour mixed solution into a 500-mL glass amber bottle and store in an air-conditioned part of the lab. Transfer to dropper bottle for use in the laboratory or field.

Before you transfer the solution to the dropper bottle, swirl to mix any material that settled out during storage.

3. Phytoplankton Sampling

For more information on sample collection, see also the following section:

- FS 2100 Surface Water Sampling

4. EQUIPMENT AND SUPPLIES

- 1 L Nalgene wide-mouth dark sample bottle
- Van Dorn Bottle
- Lugol's solution
- Buffered formalin
- Cooler with ice

5. METHODS

5.1. When sampling from a boat, rinse the bottle on the side of the boat opposite from where samples are collected in order to avoid disturbance of the surface algal community. When not sampling from a boat, collect samples upstream from where you are standing.

5.2. When the algae are mostly near the surface, collect the grab sample by scooping the bottle through the top 0.3 m of the surface water. Swirl the bottle to evenly mix the contents. Collect samples from the top 0.3 meters to estimate algal populations that would represent the "worst case" scenario. Algal populations are usually most dense near the surface. When the algae are dispersed through the water column, compositing samples from different depths is acceptable. When a fuel-powered boat is used, collect samples from the bow to avoid contamination from the motor or from sediment sampling activities at the rear of the boat.

5.3. Collect samples directly with the sample bottles for surface collection or with the additional equipment for various water depths.

6. SAMPLE PRESERVATION AND HANDLING

6.1. Place sample on ice.

6.2. Preserve the 1 L sample with 3 mL of Lugol's solution (see FS 7001, section 2) within 8 hours of collection time. For long-term storage, add buffered formalin (see FS 7001, section 1) to achieve a minimum of 2.5% final concentration (approximately 25 mL). Depending on the study objectives and the organisms present, samples can be initially preserved with buffered formalin.

FS 7100. Periphyton Sampling

FS 7110. QUANTITATIVE PERIPHYTON SAMPLING

1. EQUIPMENT AND SUPPLIES

- Periphytometer
- Eight 25 x 75 mm glass microscope slides
- Acetone

- Kimwipes
- Nylon twine
- Two 50-mL screw-top centrifuge tubes
- Deionized (DI) water
- 100% buffered formalin (dilute to 5% during sample preservation)
- Cooler with ice
- Permanent marker

2. METHODS

2.1. Briefly soak slides in acetone (5 minutes is sufficient). Remove slides from acetone rinse and clean on both sides with Kimwipes to remove any oily residue. Rinse slides with DI water.

2.2. Place the eight slides in the periphytometer. When you pack for a field trip, place periphyton racks carefully in a cooler to prevent slide breakage.

2.3. In the field, find a suitable location for periphyton incubation; take care to place samplers from stations to be compared in areas with similar light and flow regimes. (Use this method in freshwater systems only.) Because periphytometers float at the surface, they are often subject to vandalism. Degree of isolation is another consideration when determining periphytometer placement. Light penetration through the canopy is also very important.

2.4. Using the nylon twine, attach periphytometer to some stable substrate, such as a tree branch or log. Orient the sampler with the shield directed upstream. Do not tie rack below water or “diving” may result. Take care to not attach the rack too high, to prevent it from drying-out with water level fluctuations.

2.5. After the 28-day incubation period, collect slides and split for determination of periphyton chlorophyll-*a* and taxonomic analysis. If growth is sparse, place four slides (assuming none are lost or broken) in each of the two centrifuge tubes. When periphyton growth is extensive, place fewer slides in centrifuge tubes, as it is difficult to process (filter, etc.) a dense algal slurry. This decision is made in the field, as slides cannot be removed from a sample later (due to sloughing of algal material into the tube). Fill the centrifuge tubes (for chlorophyll-*a* and taxonomic analysis) with DI water.

2.6. Use the permanent marker to label the centrifuge tubes with the station, date collected, and the intended analysis. It is helpful to have this information filled out in advance, if possible. Place the sample tube directly on wet ice.

3. SAMPLE PRESERVATION AND HANDLING

3.1. If slides will not be scraped within 24 hours, add 5 mL of buffered formalin (see FS 7001, section 1) to the sample. As long as samples are kept on ice and in the dark, this step is not necessary most of the time. It is preferable to wait until after slides are scraped to fix the sample with formalin, since scraping slides in formalin solution is unpleasant and potentially dangerous.

3.2. Store samples for chlorophyll-*a*/biomass in DI water.

FS 7120. QUALITATIVE PERIPHYTON SAMPLING

Adapted from *Stevenson, R. J. and L. L. Bahls. 1999. Periphyton protocols. Pages 6-1 through 6-22 in: M. T. Barbour, J. Gerritsen, and B. D. Snyder, editors. Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, Second Edition. EPA 841-B-99-002 U. S. Environmental Protection Agency, Office of Water, Washington.*

This sampling procedure requires specific training and a demonstration of competency due to the expert judgment exercised during field sampling. It is recommended that individuals conducting this procedure should train with DEP staff (via workshops and/or participating in field sampling) and pass a field performance test. See also the following section: FT 3000 Aquatic Habitat Characterization

1. EQUIPMENT AND SUPPLIES

   - Disposable pipettes (2 mL)
   - Preservative (buffered formalin, Lugol's solution, or M3)
   - Permanent marker
   - Plastic container with approximate 8-cm inner diameter lid
   - Scissors or knife for removing pieces of plants, roots, or snags (optional)
   - 50-mL plastic tube to hold final sample
   - Completed Physical/Chemical Characterization Field Sheet (FD 9000-3)
   - Completed Stream/River Habitat Sketch Sheet (FD 9000-4) or site map
   - Completed Habitat Assessment Field Sheet (FD 9000-5 or FD 9000-6)

2. METHODS

   2.1. Complete the Physical/Chemical Characterization Field Sheet (FD 9000-3), Stream/River Habitat Sketch Sheet (FD 9000-4) or site map, and Habitat Assessment Field Sheet (FD 9000-5 or FD 9000-6) forms appropriate to the water body in which you are sampling (see FT 3000-FT 3400). Visually determine the substrates where algae will be collected within 0.5 m of the surface. Targeted substrates include removable portions of vascular plants or mosses, snags, roots, leaf packs or mats, and rock. All sampled substrates are combined into a single, composite sample for a particular site. For sampling, target substrates in flow and sunlight conditions that are representative of the aquatic system being sampled. If algae are visible on a substrate, preferentially include that substrate for sampling, as opposed to sampling an adjacent substrate of the same type where algae are not visible. A thin film of algae (usually diatoms) may be present on some substrates, but not visible to the naked eye, although this algal film may feel somewhat slimy to the touch. If algae is not visible at a site, sample in areas where algae would be expected to grow (stable, "seasoned" substrates) in the top 0.5 m of the water column, where incident light should be available. Avoid depositional areas or substrates with excessive silt or sand. Do not sample the sediments.

   2.2. Collect algae from approximately an 8-cm diameter area from each targeted substrate within a 100 m reach (in a stream) or 100 m diameter (in a wetland). After carefully removing (to avoid loss of algae) a measured amount of substrate (approximately an 8-cm diameter portion) from the water, place it into a jar containing 100 mL of site water. Using your fingers, rub algae from the substrate into the water, rinsing your fingers in the

same jar. Agitate this slurry sufficiently to homogenize the aliquot. Then, using a pipette, transfer 4 mL of the slurry into the 50-mL tube. Collect a total of 10 of these aliquots from points spaced throughout the site, representing a cross-section of the kinds of algae expected to be present. Sample all removable habitats where periphyton growth is expected, such as snags, roots, leaf packs/mats, vascular plants/mosses, and rock. Apportion the 10 aliquots as equally as possible among productive substrates (as defined by the habitat assessment; see FT 3100) according to the following:

1 productive substrate = 10 aliquots

2 productive substrates = 5 aliquots each

3 productive substrates = 4 from most abundant substrate, 3 from each of the remaining substrates

4 productive substrates = 3 from the two most abundant substrates, 2 from each of the remaining substrates

5 productive substrates = 2 aliquots from each substrate

Note: For a targeted substrate consisting of less than 2 m^2, (i.e., not a major productive habitat according to Habitat Assessment SOP FT 3100), collect a single periphyton aliquot from that substrate, and follow the above apportioning rule as closely as possible in the remaining major (greater than 2 m^2) habitats.

When collecting each aliquot, it is important to keep the amount of water placed into the jar (100 ml) and the amount of slurry transferred into the tube (4 ml) consistent for all 10 aliquots.

3. Removable hard substrates (snags, rocks): Remove substrate from water and rub algae from an approximately 8- cm diameter area into jar filled with 100 mL of site water. Pipette 4 mL of resulting slurry into sample container. Discard remaining slurry.

3.1. Removable soft substrates (plants, mosses, leaf packs, macro-algal mats- if representative of the site, roots): Remove approximately 8-cm diameter area of substrate from water, place into jar containing 100 mL of site water, and rub algae (using fingers) from substrate. Pipette 4 mL of resulting slurry into sample container. Discard remaining slurry. When sampling plants, sample algae from the dominant plant species, including both emergent and submerged varieties.

3.2. Sequentially place all 10 of the 4 mL aliquots from the sampled substrates, as described in Section 2.2, into the 50-mL tube sample container, which should be clearly marked with the permanent marker. Include site, date, collector, sample type (qualitative periphyton), and preservative information.

4. SAMPLE PRESERVATION AND HANDLING

4.1. Add 2 mL of buffered formalin (see FS 7001, section 1) or M3 per 40 mL of periphyton slurry for samples for taxonomic identification.

FS 7130. RAPID PERIPHYTON SURVEY

1. INTRODUCTION This sampling procedure requires specific training and a demonstration of competency due to the expert judgment exercised during field sampling. It is recommend that individuals conducting this procedure should train with DEP staff (via workshops and/or

participating in field sampling) and pass a field performance test. See also the following section: FT 3000 Aquatic Habitat Characterization

2. Equipment and Supplies

- Implement to measure a 5 cm distance
- Rapid Periphyton Data Sheet (FD 9000-8)
- Completed Physical/Chemical Characterization Field Sheet (FD 9000-3)
- Completed Stream/River Habitat Sketch Sheet (FD 9000-4) or site map
- Completed Habitat Assessment Field Sheet (FD 9000-5 or FD 9000-6)

3. Method

3.1. Measure a 100m segment of stream, placing flags every 10m.

3.2. Conduct a standard stream habitat assessment. If desired, the following algal observations can be done during the habitat mapping process.

3.3. Beginning at the “0” flag, establish a transect of 9 approximately equidistant points across the stream. Point 1 would be located approximately 0.1 m from the right bank, point 5 is at the middle, and point 9 located 0.1 m from the left bank. The remaining points are sequentially distributed between these points, approximately equidistantly.

3.4. Each observation point, within the above distance parameters, should be chosen haphazardly. That is, choose the actual point without looking (thereby potentially biasing your selection).

3.5. After selecting the observation point:

3.5.1. If the substrate can be reached, grab a handful of material at the observation point, being careful not to lose material when bringing it to the surface. Examine the material, first out of the water, and then with the hand located approximately 2 cm below the surface. Note on the datasheet if algae are visible and measure its average thickness, perpendicular to the substrate, with a ruler. Record these data using the rank thickness classifications on the data sheet as follows; “0” indicates a rough surface with no algae, “1” for algae less than 0.5 mm OR no algae visible but surface is slimy (including muck), “2” for 0.5 mm to 1 mm, “3” indicates an algal cover of greater than 1 mm but less than 6 mm, “4” for 6-20 mm and “5” for algae over 20 mm. NOTE: examine the first substrate you encounter, which may not always be the stream bed (e.g., snag, plants, roots). Make your observations at the point where the hand encounters the substrate (i.e., if a 0.5 m long snag is grabbed, record the algal thickness based on where the hand touches it, not elsewhere). Take the measurement where the algae is representative of the entire amount in your hand (i.e. avoid measuring a single long filament when most of substrate has only a thin coating).

3.5.2. Record the type of algae seen (filamentous, diatoms, or other).

3.5.3. If the substrate can not be seen (e.g., in tannic waters) and can not be reached with the hand, record an “X”, for that point, indicating observations are not possible using this method.

3.5.4. If algae can be seen but not reached, then record as “visible” and visually estimate the average thickness perpendicular to the substrate.

3.5.5. If the substrate cannot be brought to the surface (e.g., a large rock or snag) but it is reachable, then rub the surface of the substrate and visually inspect to determine the presence/absence of algae and approximate thickness.

3.6. Additionally, at point 5 on each of the 11 transects, canopy cover should be measured using a spherical densiometer. The densiometer consists of a concave mirror with gridwork that creates 24 ¼-inch etched boxes. Each box can be subdivided into 4 smaller squares, via an imaginary dot in the center of each box, to create a total of 96 smaller squares that can be counted within the entire densiometer. Hold the instrument far enough away from the body so the operator's head is just outside the grid. Count the number of small squares (out of a total of 96) that DO NOT have tree canopy. Subtract this number from 96 to determine the number of dots WITH tree cover. Record this number (number of dots WITH canopy cover) for each transect under the Canopy column for point 5.

3.7. Repeat of the above procedures every 10m, including the 100m mark, for a total of 99 periphyton observation points.

FS 7200. Macrophyte Sampling

FS 7220. LAKE VEGETATION INDEX (LVI) SAMPLING

1. INTRODUCTION

This method is a rapid screening tool for ecological condition; it determines how closely a lake's flora resembles that of an undisturbed lake. The LVI is applicable for use from April 1 to November 30 of each year, due to impacts of freezing events on the macrophyte community.

This sampling procedure requires specific training and a demonstration of competency, as outlined in FA 4330, due to the expert judgment exercised during field sampling. It is recommend that individuals conducting this procedure should train with DEP staff (via workshops and/or participating in field sampling) and pass a field performance test. Each sampling entity must maintain a plant reference collection. See also the following procedures:

- FT 3001 Physical/Chemical Characterization
- FT 1000 General Field Testing and Measurement
- LQ 7310 Lake Vegetation Index
- FA 4330 Proficiency Criteria for Lake Vegetation Index (LVI) Sampling

2. EQUIPMENT AND SUPPLIES

- Aquatic and wetland plant identification manuals
- GPS unit
- Hand lens
- Binoculars
- Map of lake separated into 12 sections
- Boat
- Implement to measure a 5 m distance

- Frotus
- Plastic bags
- Permanent marker
- Cooler
- Ice
- Lake Vegetation Index Data Sheet (FD 9000-7)
- Completed Physical/Chemical Characterization Field Sheet (FD 9000-3)

3. Methods

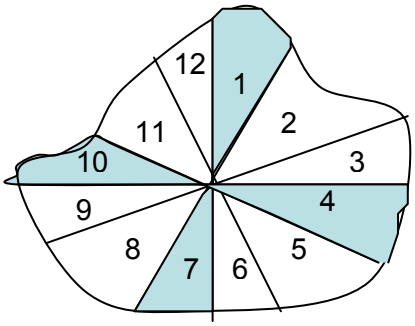
3.1. Prior to visiting the lake:

3.1.1. Generate a GIS map of the lake. (An aerial photo is most helpful for locating features on the lake). Divide the lake into four quadrants by drawing a north-south midline and an east-west midline. Then, logically divide the entire lake into twelve total sections (three in each quadrant), with approximately equal shoreline distances and known latitude/longitude coordinates generated from the GIS mapping effort.

3.1.2. Number the sections 1-12 in a clockwise fashion, starting with 1 as the section immediately clockwise from the north-south line in the northeast quadrant.

3.1.3. Select section 1, 2, or 3 as the starting section by using a random numbers table or some other random number generator (e.g. rolling a die).

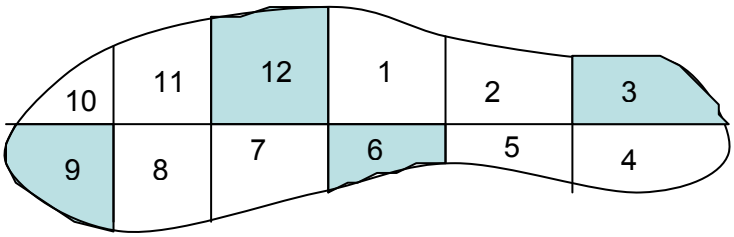
3.1.4. If section 1 is selected as the starting section, sections 1, 4, 7, and 10 will be sampled. If section 2 is selected, sections 2, 5, 8, and 11 are sampled. If section 3 is selected, sections 3, 6, 9, and 12 are sampled. On the Lake Vegetation Index Data Sheet, record the sections of the lake associated with each sampling unit (data column). There are four sampling units per lake. See figure below for examples of different sampling strategies.



Sampling strategy if

1 is randomly selected.

Plants identified in sections 1, 4, 7, and 10 are recorded in individual columns on the data sheet.



Sampling strategy if 3 is randomly selected. Plants identified in sections 3, 6, 9, and 12 are recorded in individual columns on the data sheet.

3.2. In the field:

3.2.1. Note that four data columns will be used on the Lake Vegetation Index Data Sheet for this LVI sampling procedure, resulting in four species lists for the lake. Record the total species from each “drive-by” effort combined with its associated transect portion (to include all five frotus deployments) for each sampling unit as one cumulative species list for the sampling unit. Mark each column on the data sheet to match the sampling unit (see section 3.1.4).

3.2.2. Use the map of the lake and GPS unit to go to the emergent zone of the first sampling unit. Travel at idle or slow speed and record the presence of aquatic and wetland plant species seen from the boat on the Lake Vegetation Index Data Sheet. Identify aquatic and wetland plants to species level. If this not possible, bring the plant back for expert identification. Binoculars may be used to aid in observation. Collect any specimens within easy reach that are not identifiable by observation from the boat. Identify collected specimens, or bag them for return to the lab. Do not leave the boat or spend effort on difficult to access areas. This is a quick “drive-by” survey. Continue this sampling throughout the section.

3.2.3. Identify a location within the sampling unit to set up a transect 5 m in width. Choose a location where you will be able to navigate the boat approximately perpendicular to the shoreline. Where possible, choose a location that is species rich and/or contains species which were not identified in the “drive-by” survey. Within the emergent zone, measure 5 m for the width of the transect. Orient the transect perpendicular to the shoreline, with the boat being the centerline of the transect. Record the presence of all aquatic and wetland plant species within the 5 m belt transect, from the point where aquatic and wetland plants begin, to the estimated ordinary high water mark of the lake. If practical, get out of the boat and wade, to identify plants that are

small, hidden or in areas too shallow to reach by boat. Record the presence of species in the transect (as described in 3.2.1) on the Lake Vegetation Index Data Sheet.

3.2.4. Deploy the frotus five times per section within the 5 m transect, moving from the shoreline to open water. Record the presence of species from the frotus deployments (as described in 3.2.1) on the Lake Vegetation Index Data Sheet.

3.2.5. Repeat steps 3.2.2-3.2.4 for the three other sampling units.

3.2.6. Determine one dominant or two co-dominant species for the sampling unit by estimating the plant with the largest areal extent. (Dominance can be by emergent, floating, or submersed species.) Record dominance or co-dominance on the Lake Vegetation Index Data Sheet. If no one dominant or two co-dominant species are present, do not assign a dominance code for that sampling unit. Return all dominant and co-dominant species to the laboratory for verification by an independent taxonomist.

3.2.7. Place any specimens that can not be readily identified in the field in plastic bags. Label each bag so that the species name can later be added to the correct sampling unit species list(s). Store bags on ice while returning to the laboratory. Plants kept refrigerated can be identified fresh within a few days.

3.3. After visiting the lake:

3.3.1. Prepare unknowns for herbarium identification or verification by a consulting botanist as voucher specimens.

3.3.2. Identify and record the correct names of the unknown specimens on the Lake Vegetation Index Data Sheet.

3.3.3. Calculate the LVI score for the lake as described in LT 7500.

4. Recommended references for plant identification:

4.1. Godfrey, R. and J. Wooten. 1979. Aquatic and Wetland Plants of Southeastern United States: Monocotyledons. Univ. Ga. Press, Athens.

4.2. Godfrey, R. and J. Wooten. 1979. Aquatic and Wetland Plants of Southeastern United States: Dicotyledons. Univ. Ga. Press, Athens.

4.3. Wunderlin, Richard P. 1998. Guide to the Vascular Plants of Florida. University Press of Florida, Gainesville.

4.4. Tobe, John T. et. al. 1998. Florida Wetland Plants: An Identification Manual. Florida Department of Environmental Protection, Tallahassee.

4.5. Langeland, K.A. and K. Burks. 1998. Identification and Biology of Non-Native Plants in Florida's Natural Areas. Florida Department of Environmental Protection, Tallahassee.

FS 7300. Benthic Macroinvertebrate Sampling

FS 7310. RAPID BIOASSESSMENT (BIORECON) METHOD

This sampling procedure requires specific training and a demonstration of competency due to the expert judgment exercised during field sampling. It is recommend that individuals conducting this procedure should train with DEP staff (via workshops and/or participating in field sampling), complete the training requirements outlined in FA 5710 and pass a field performance test. See also the following sections:

- FT 3000 Aquatic Habitat Characterization
- LT 7100 Biorecon Determination

1. INTRODUCTION

1.1. This method was designed to be primarily a screening tool.

2. EQUIPMENT AND SUPPLIES

- Completed Physical/Chemical Characterization Field Sheet (FD 9000-3)
- Completed Stream/River Habitat Sketch Sheet (FD 9000-4)
- Completed Stream/River Habitat Assessment Field Sheet (FD 9000-5)
- Biorecon Field Sheet (FD 9000-1)
- Forceps
- Transfer pipettes
- White picking pans
- 10X hand lens
- Jars filled with alcohol (80% ethanol)
- D-frame dip net with No. 30 mesh and handle marked in 0.1-m increments
- Meters (DO, pH, Conductivity)
- Wide-mouth jug
- Cooler with ice

3. METHODS

3.1. Visually examine the area or reach to be sampled. Either walk or boat throughout the aquatic system, paying close attention to its physical and habitat characteristics. Be very careful when walking through the system not to disturb aquatic habitats. Such disturbances could lead to inaccurate biorecon and/or habitat assessment results. In fairly small (1st to 4th order) streams, the length of a discrete station consists of a 100-m stretch of stream, and the width is from bank to bank. In very large systems it may be necessary to establish more than one station to adequately characterize the biota. Do not sample during flood stage or recently dry conditions.

3.1.1. If the stream is known to have been dry recently, wait a minimum of three months (90 days) after dry conditions have abated and the stream returns to normal flow before performing a Biorecon.

3.1.2. If flood conditions (water level >0.5 meters above normal) prevent access to substrates that are inhabited by invertebrates, delay sampling until those substrates are accessible or wait 28 days for the invertebrates to colonize the newly inundated substrates. If water levels are ≤ 0.5 meter above normal, sample habitats at the normal stream shoreline, not the flooded shoreline.

3.2. Complete the Physical/Chemical Characterization Field Sheet (FD 9000-3), Stream/River Habitat Sketch Sheet (FD 9000-4), and Stream/River Habitat Assessment Field Sheet (FD 9000-5; see FT 3000-FT 3400). The percent coverage of substrate type refers to how much of each habitat type is actually present at the sampling site. Take care

to accurately determine the spatial extent of each substrate type (in a three-dimensional context) for habitat scoring procedures.

3.3. Determine the "best" or "most productive" habitats, which, when sampled, will provide an accurate estimate of the site's maximum biological diversity. Generally, the most productive habitat types are as follows: leaf packs, snags, aquatic vegetation, roots, and rocky outcrops, all can be considered "productive" habitat. Sand and mud/muck are sampled as "minor" habitats. For sampling purposes, habitats receiving good water velocity (>0.2 m/sec) should be selected over those in more sluggish areas. If fewer than four productive habitats are available, select a particularly productive habitat (based on the number of taxa obtained in earlier sweeps) sampled in the earlier sweeps until completing four sweeps.

3.4. Perform four separate 0.5-meter sweeps with the D-frame dip net in the "most productive" habitats as determined by the above procedures. Sort and remove all organisms between each sweep. If conditions pose a hazard (e.g., severe weather), the material from all four sweeps may be stored in jugs, placed on ice, and sorted at the laboratory within a 24-hour period. Several passes (three or more) over the same 0.5 meter area are recommended in an attempt to capture all organisms. This sampling effort in a discrete 0.5 meter spot is considered to be one sweep. Catch organisms by allowing them to flow into the net and also by sweeping the net towards disturbed material. Where a continuous 0.5 m sweep is not possible, take two 0.25 m sweeps in the same area to obtain a full 0.5 m sweep. It is recommended, due to the potential spatial heterogeneity in the distribution of the organisms, that two separate 0.25 m sweeps be taken in two separate areas of a given productive habitat to comprise the 0.5 m "sweep." If you are sampling from a boat, you can get out of the boat and wade in shallow shore areas to obtain the sweeps. You can also approach a habitat with the boat from downstream, agitate and sweep the reachable portion of the habitat (typically by leaning from the bow of the boat), to capture organisms. See FS 7420 section 2.4 for further discussion on proper sweeping techniques.

3.5. There are two picking options for this step. Use the field sorting method unless field conditions are such that that the laboratory sorting method is more appropriate. Read both methods and decide if the laboratory sorting method is allowed. For both options, refer to Table FS 7000-1 to determine the number of individual specimens in each target taxonomic group to retain for laboratory identification. Do not retain more than the specified number of individuals. For both options, perform a QC Check as outlined in 3.5.3.

3.5.1. Field Picking Biorecon Method: This is the preferred sorting method for the Biorecon procedure. Return to a comfortable spot on the bank with the dip net containing the sampled material. Place small aliquots of the detritus plus organism matrix in a picking pan diluted with a small amount of site water. Make sure the density of detritus is low enough so organisms are easily seen and captured. Scan the entire pan for organisms. When an organism is found, remove it with forceps or a pipette, examine it with the hand lens, determine its identity to the lowest possible taxonomic level (usually family or genus), record on the Biorecon Field Sheet (FD 9000-1), and place the organism in an alcohol-filled jar. Repeat these procedures until **all** the material in the net has been examined. Repeat until material from four sweeps has been processed. Record all data on FD 9000-1. In the jar filled with alcohol, **save up to, but do not exceed, the target number of specimens as indicated in Table FS 7000-1** for laboratory verification. Examining habitats separately helps the investigator learn which taxa are found in which habitat type and aids in future Biorecon sampling. Examining habitats separately also helps determine which habitat(s) to target for the fourth sweep if only three productive habitats are available. If field conditions are tolerable (e.g., no

hard rain that would interfere with sorting efficiency) and time spent in the field is not a limiting factor, this option helps the investigator gain a better understanding of aquatic ecology. Organisms from all habitat types may be combined in the jar for laboratory verification. Store samples in ethanol, on ice.

3.5.2. Laboratory Picking Biorecon Method: If conditions are hazardous at the site (e.g., severe weather), this option may be used, but it is not recommended for routine use. To employ this option, sorting must be done within 24 hours on iced samples (do not formalin preserve). Transfer the material in the net to a wide-mouth jug, performing a cursory examination of the diversity of organisms collected in that given habitat. Repeat until material from four sweeps has been collected and placed into a wide-mouth jug. If fewer than four productive habitats are available, select a particularly productive habitat sampled in the earlier sweeps until four sweeps have been completed (see section 3.3). Store on ice and sort within 24 hours. Do not store with formalin. After returning to the laboratory, sort the material with the naked eye, and save up to, but do not exceed, the target number of specimens as indicated in Table FS 7000-1 for identification.

3.5.3. Perform a quality control check on all samples sorted in the field or laboratory.

3.5.3.1. Have a second taxonomist randomly select a minimum of 10% of the sorted aliquots and perform a QC check by examining the aliquot and retrieving and counting any new taxa found. The sorting and QC check can be performed simultaneously by the sampling team. The aliquot can be discarded after the team reaches a consensus that no new taxa are present.

4. A result of fewer than 100 individuals in the four D-frame dip net sweeps suggests that conditions are fairly inhospitable to invertebrates. This could be due to recent desiccation, habitat limitation, toxicity, and/or other factors. If you encounter fewer than 100 individuals, it is recommended that you perform a follow-up Stream Condition Index sampling (see FS 7420) to further evaluate the stream.

5. See LT 7100 for laboratory verification of organism identification, Biorecon score determination, and Biorecon score evaluation.

FS 7320. STREAM CONDITION INDEX (D-FRAME DIP NET) SAMPLING

(Based on *Plafkin, et al., 1989, Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish, EPA/444/4-89-001*. See also *Barbour, et al., Rapid Bioassessment Protocol Manual, EPA/841/B-99-002.*)

This sampling procedure requires specific training and a demonstration of competency due to the expert judgment exercised during field sampling. It is recommended that individuals conducting this procedure should train with DEP staff (via workshops and/or participating in field sampling), complete the training requirements outlined in FA 5710 and pass a field performance test. See also the following sections:

- FT 3000 Aquatic Habitat Characterization
- LT 7200 Stream Condition Index (SCI) Determination

1. EQUIPMENT AND SUPPLIES

- Completed Physical/Chemical Characterization Field Sheet (FD 9000-3)
- Completed Stream/River Habitat Sketch Sheet (FD 9000-4)

- Completed Stream/River Habitat Assessment Field Sheet (FD 9000-5)
- D-frame Dip net with No. 30 mesh and handle marked in 0.1-m increments
- Two 4-liter wide-mouth plastic jugs (Take extra in case more are needed.)
- Buffered formalin (See FS 7001, section1.)
- Brush
- Permanent marker

2. METHODS

2.1. Visually examine the area or reach to be sampled. Walk or boat throughout the aquatic system, paying close attention to its physical and habitat characteristics. Be very careful when walking through the system not to disturb aquatic habitats. Such disturbances could lead to inaccurate SCI and/or habitat assessment results. In fairly small (1st to 4th order) streams, the length of a discrete station consists of a 100 m stretch of stream, and the width is from bank to bank. In very large systems it may be necessary to establish more than one station to adequately characterize the biota. Do not sample during flood stage or recently dry conditions.

2.1.1. If the stream is known to have been dry recently, wait a minimum of three months (90 days) after dry conditions have abated and the stream returns to normal flow before performing an SCI.

2.1.2. If flood conditions (water level >0.5 meters above normal) prevent access to substrates that are inhabited by invertebrates, delay sampling until those substrates are accessible or wait 28 days for the invertebrates to colonize the newly inundated substrates. If water levels are ≤ 0.5 meter above normal, sample habitats at the normal stream shoreline, not the flooded shoreline.

2.2. Complete the Physical/Chemical Characterization Field Sheet (FD 9000-3), Stream/River Habitat Sketch Sheet (FD 9000-4), and Stream/River Habitat Assessment Field Sheet (FD 9000-5; see FT 3000-FT 3400). The percent coverage of substrate type refers to how much of each habitat type is actually present at the sampling site.

2.3. Determine the number of sweeps to perform in each habitat type out of the twenty total sweeps per station. First, use the Stream/River Habitat Sketch Sheet (FD 9000-4) and the Physical/Chemical Characterization Field Sheet (FD 9000-3) to determine the number of productive habitats at the site. Then, use the following conventions to determine the number of sweeps to perform in each habitat type:

2.3.1. If one productive habitat is present, perform 10 sweeps in that habitat and 10 sweeps in a minor habitat (e.g., sand).

2.3.2. If two productive habitats are present, perform 7 sweeps in each of those habitats and 6 sweeps in a minor habitat (e.g., sand).

2.3.3. If three productive habitats are present, perform 5 sweeps in each of those habitats and 5 sweeps in a minor habitat (e.g., sand).

2.3.4. If four productive habitats are present, perform 4 sweeps in each of those habitats and 4 sweeps in a minor habitat (e.g., sand).

2.3.5. If five productive habitats are present, perform 3 sweeps in each of those habitats and 5 sweeps in a minor habitat (e.g., sand).

Generally, the most productive habitat types are as follows: leaf packs, snags, aquatic vegetation, roots, and rocky outcrops. Sand and mud/muck are sampled as “minor” habitats. If sufficient material is not available for performing the specified number of sweeps in a given productive habitat, do as many as possible in that habitat type, and perform extra sweeps in the other habitats to total 20. Proper interpretation of benthic collections requires that samples be collected from multiple habitats that are representative of the site.

2.4. Perform 20 discrete 0.5 m sweeps with the D-frame dip net. Sample the available substrates as determined by the above procedures.

2.4.1. In streams with sufficient water velocity, the most effective way to capture invertebrates is to place the bottom rim of the dip net directly downstream of the area to be sampled. Disturb, agitate or dislodge organisms (with hands and/or feet, or brush, where appropriate) from substrates (snags, etc.) working as closely to the net as possible. When performing an upstream/downstream type of study, sample the downstream station first to prevent upstream invertebrates from drifting into a location they did not originally inhabit. If you are sampling from a boat, you can get out of the boat and wade in shallow shore areas to obtain the sweeps. You can also approach a habitat with the boat from downstream, agitate and sweep the reachable portion of the habitat (typically by leaning from the bow of the boat), to capture organisms.

2.4.2. For areas with little to no flow, disturb an area of substrate that is one dip net width wide and approximately 0.5 m long and create flow into the net to ensure the capture of organisms which were living there. Several passes (three or more) over the same 0.5 m area are required to make sure all organisms are captured. This sampling effort in a discrete 0.5 m area is considered as one sweep.

2.4.3. For heavily vegetated areas place the net at the base of the vegetation and dislodge organisms using your hand or a 0.5 m sweeping motion with the net. Where a continuous 0.5 m sweep is impossible, take two 0.25 m sweeps of the same habitat to attain a full 0.5 m sweep.

2.4.4. Sample leaf packs (if present) by disturbing leaf pack areas with hands or feet before scooping 0.5 m worth of material into the net. When sampling leaf mats, make sure that only the top 2 cm of material is collected, and especially make sure that anaerobic leaf material is not included.

2.4.5. Sand, muck, mud, and silt (non-major habitats) can be “swept” or sampled by agitating the top 2 cm of substrate (with hands, feet, or brush). Take three 0.5 m passes over the same area for one sweep. If the net is pushed into coarse sand or coarse detritus, very little of the sand or detritus will be washed through the net, resulting in a sample that contains few organisms and is hard to process, thus compromising the quality of the entire sample.

2.5. Record the number of sweeps for each habitat on the Physical/Chemical Characterization Field Sheet (FD 9000-3).

2.6. Reduce the sample volume after each discrete sample by dislodging organisms from larger debris (but retaining invertebrates in the net) and discarding the debris. Save finer debris plus organism mixture in large wide-mouth jugs. Make every effort to reduce enough of the sample volume in the field so that no more than four liters of material are collected. If this is not possible, put the material into additional jugs. Additional sample reduction will occur in the laboratory. The relative proportions of the organisms collected must be maintained intact to calculate many community metrics. Indicate on the label in how many jugs the entire sample is contained, e.g., “1 of 2,” “2 of 2.”

3. SAMPLE PRESERVATION AND HANDLING

3.1. Preserve with 10% buffered formalin (do this by adding one part of 100% buffered formalin to the jug with nine parts ambient water or filling up the entire jug with 10% buffered formalin). During laboratory processing, remove the organisms from the detritus and place them into 70% ethanol.

3.2. If samples are immediately iced and can be sorted within 24 hours, the use of formalin is optional. Use 10% formalin, per section 3.1 above, to preserve iced samples that will not be sorted within 24 hours.

3.3. See LT 7200 for laboratory and calculation procedures for Stream Condition Index determination.

FS 7330. HESTER-DENDY SAMPLING FOR THE BIOLOGICAL INTEGRITY CRITERION

1. EQUIPMENT AND SUPPLIES

- Three or four Hester-Dendy (HD) artificial substrates
- Customized HD block, with coupling nuts for attachment of HD samplers and eye bolts for attachment of cable are to be block mounted, or
- Customized HD floating platform with coupling nuts for attachment of HD samplers underneath the platform and eye bolts for attachment of cable, if HD's are to be floated.
- Buoys
- Stainless steel cable or nylon rope of appropriate strength
- Nico-Press tool or two hammers with fasteners
- Whirl-Pak bags or other appropriate containers
- Permanent marker
- Cooler with ice

2. METHODS

2.1. Deploy Hester-Dendys via special Hester-Dendy concrete blocks with coupling nuts, via custom floating platforms, or by tying weighted Hester-Dendys to an overhanging object. For the Hester-Dendy (HD) block method, attach at least three Hester-Dendy artificial substrates (HDs) to the HD block, and place the block at a depth of one meter (or the deepest spot available if shallower than one meter). Take care to place control and test site blocks in areas of similar flow, depth and habitat type. The block has space for four HDs for use in studies requiring additional replication. Knowledge of the system's hydrologic regime is important to make sure samplers will not go dry during the 28 day incubation period. For example, if there is high water and you expect the water to drop one meter in the next few weeks, place the sampler so that it will be one meter deep at the end of incubation. In shifting sand substrates, place the block so that existing snags will deflect sand from being deposited on the samplers. This can be determined by close examination of the bottom topography. If the mean depth of the water body is greater than one meter, and/or there is a chance of the HD samplers that are placed on the bottom being smothered by sand or silt, a floating platform with attachment points for four Hester-Dendys may be used instead. For the HD floating platform method, attach three or four Hester-Dendys to the floating platform. Then attach the platform to a buoy using one meter of stainless steel cable (or less than one meter in shallow streams). Attach the buoy/platform configuration to an anchor (typically a

concrete block). Use a length of stainless steel cable appropriate for the depth of the stream or river. Alternatively, suspend Hester-Dendys one meter (or less in shallow streams) below the water level by attaching them to an overhanging object via string or monofilament, and weighting the bottom of the Hester-Dendy (by tying approximately 250 g of weight to the bottom of the artificial substrate).

2.2. If using the HD block, attach stainless steel cable or nylon rope to a point on the bank sufficiently high to enable recovery even if the water level increases. Wrap the cable or rope around the base of a tree on the bank and use the Nico-Press tool and fasteners to secure the block. If vandalism is a potential problem, attempt to conceal the cable so that no one but you can find it. If the Nico-Press tool is unavailable, the fasteners may be crimped by hammering (two hammers are needed).

2.3. After a 28-day incubation period, recover the HD samplers. Approach the block mounted, floating, or suspended samplers carefully, without disturbance, from the downstream position. Without touching the HDs or any other substrate, place a dip net either downstream or below the samplers to capture escaping organisms. In a deliberate, gentle manner, lift the block straight up from the bottom and immediately place on a flat surface. Wade or use a boat; **do not** pull the block up from the shore. If retrieving suspended or floating HDs, gently lift and place immediately in Whirl-Pak bags.

2.4. Once out of the water, quickly place the Whirl-Pak bags over all the HDs, and detach them from the block or floats. If an organism is observed crawling off a HD, capture it and put it in the appropriate Whirl-Pak. If organisms are found in the dip net, randomly and sequentially place them in one of the Whirl-Paks. Fill the Whirl-Paks with ambient water (so that all the plates are wet), secure them (twirl three times and twist the ends), and place on ice. Whirl-Paks should be pre-labeled with the station, sample date, and replicate number, using the permanent marker.

3. SAMPLE PRESERVATION AND HANDLING

3.1. **Do not** preserve samples until after organisms are scraped from the HD plates unless the HD samplers are not going to be reused. Preservatives will poison the plates, preventing them from being used again.

FS 7340. CORE SAMPLING

1. INTRODUCTION

1.1. Use of coring devices is restricted to sampling fairly soft substrates (silt or muck, with only small amounts of sand or shell), usually in marine systems. Take enough cores so that an area of approximately 675 cm^2 is sampled. Place all replicates in separate sample containers (for statistical analyses). Depending on the study objectives, replicates may also be composited, as long as the number of replicates is equal for each station and clearly recorded so that the number of organisms per square meter can be calculated.

2. EQUIPMENT AND SUPPLIES

- Coring device
- U.S. 30 mesh box sieve (constructed of fiberglass-coated wood and U.S. 30 mesh screen) or dip net with U.S. 30 mesh sieve material
- White enamel pan
- Plastic squeeze bulb

- Small bucket
- Wide-mouth plastic sample containers
- Permanent marker
- Buffered formalin
- Long pole (if needed)
- Rose Bengal dye (optional)

3. METHODS

3.1. When sampling from a boat, use a coring device with a valve near the top attached to a long pole. Collect core samples from the rear and downstream of the vessel to avoid contamination of other types of samples with disturbed sediments. Rinse the box sieve with ambient water and tie it to the side of the boat where samples will be collected. When placed in the water, it will float at the surface. If a box sieve is not available, wash the dredged material in a dip net, provided it is fitted with a U.S. 30 mesh sieve material. The disadvantage of using the dip net is that it requires two people (one to hold the net, one to manipulate the coring device).

3.2. Lower the coring device to the bottom with the valve open. After quickly pushing the device into the sediments, close the valve. The resulting vacuum will keep the material in the tube as it is raised up to the boat. Many clean water organisms are somewhat motile and will elude capture if you are not quick during sampling.

3.3. When collecting samples in wadeable waters, a smaller coring device can be used. This corer utilizes a flapper-valve equipped stopper that is inserted into the top of the pipe. Vacuum inside the pipe holds the material until the stopper is removed. This small corer should be used primarily for non-biological sediment sampling (grain size, metals, etc.), as it is thought to be too small to effectively capture many organisms (e.g., crustaceans or tubiculous worms which are generally large in size) considered useful in impact determination.

3.4. Pull the sampler to the surface, open the valve or remove the stopper, and place it immediately into the box sieve. Disgorge the contents into the sieve, rinsing to assure complete sample purging.

3.5. Swirl the box sieve in the water with a back-and-forth motion to wash the fine sediments through. Concentrate the remaining sample into one corner of the sieve. If a sediment type is especially clayey or mucky, it may be necessary to use a hand to break up clumps and agitate the sample to reduce it. Make sure you rinse any detritus from your hand back into the sieve.

3.6. Fill the small bucket with ambient water and use this water to fill the squeeze bulb. Using the squeeze bulb, rinse the sample from the sieve to the enamel pan. Take care to rinse the entire contents of the sample into the pan. Some organisms may stick to the screen.

3.7. Use the squeeze bulb to transfer the sample from the enamel pan into the pre-marked wide-mouth jug, making sure the location, date, and replicate number are accurate.

4. SAMPLE PRESERVATION AND HANDLING

4.1. Preserve the sample with 10% buffered formalin (see FS 7001, section 1) by adding a 9 to 1 ratio of water to 100% formalin. If laboratory processing is possible within eight

hours, the samples may be stored on ice, without addition of formalin. If desired, add a very small amount of rose bengal dye (approximately 100 mg per liter of material) to the sample as a picking aid.

FS 7350. DREDGE SAMPLING

1. EQUIPMENT AND SUPPLIES

- Ekman or Petite Ponar dredge
- Box sieve (constructed of fiberglass-coated wood and U.S. 30 mesh screen), bucket sieve or dip net with U.S. 30 mesh sieve material
- White enamel or plastic pan
- Plastic squeeze bulb
- Small bucket
- Wide-mouth plastic sample containers
- Permanent marker
- Buffered formalin
- Rose Bengal dye (optional)

2. METHODS

2.1. Use of the Ekman dredge is restricted to sampling soft substrates (silt, muck) in areas with little current. The Ponar dredge may be used for sampling under these conditions and also in areas with a harder substrate (rocks, shells, sand). The number of replicates collected is dependent upon several factors, including the area sampled by the device, the purpose of the study, and the degree of patchiness in the distribution of the organisms at the site. Routinely, take three dredges. Place all replicates in separate sample containers (for statistical analyses). If you are sampling in an exceptionally depauperate area, additional replicates may be required (pilot study needed). In that case, the number of replicates must be equal at all stations to be comparable.

2.2. When you sample from a boat, collect dredge samples from the rear and downstream of the vessel to avoid contamination of other types of samples with disturbed sediments. Rinse the box sieve with ambient water and tie it to the side of the boat where samples will be collected. When placed in the water, it will float at the surface. If a box sieve is unavailable, wash the dredged material in a dip net or bucket sieve, provided it is fitted with a U.S. 30 mesh sieve material.

2.3. Dredges

2.3.1. Ekman: Open the spring-loaded jaws and attach the chains to the pegs at the top of the sampler. Lower the dredge to the bottom, making sure it settles flat. Holding the line taught, send down the messenger to close the jaws of the dredge. Pull the sampler to the surface and place it immediately into the box sieve. Carefully open the jaws and empty the contents into the sieve, rinsing to assure complete sample purging. The spring-loaded Ekman can be dangerous. Hold the dredge firmly above the hinges, and take care to not get pinched by the spring-loaded jaws, which could produce serious injury. Check to make sure the jaws are fully closed and that no sample was lost while lifting the dredge. Discard the grab if the dredge is not fully closed.

2.3.2. Petite Ponar: Open the jaws and place the crossbar into the proper notch. Lower the dredge to the bottom, making sure it settles flat. When tension is removed from the line, the cross-bar will drop, enabling the dredge to close as the line is pulled upward during retrieval of the dredge. Pull the sampler to the surface and place it immediately into the box sieve. Carefully open the jaws and empty the contents into the sieve, rinsing to assure complete sample purging. Check to make sure the jaws are fully closed and that no sample was lost while lifting the dredge. Discard the grab if the dredge is not fully closed.

2.4. Swirl the sieve in the water with a back-and-forth motion to wash the fine sediments through. Concentrate the remaining sample into one corner of the sieve. If a sediment type is especially clayey or mucky, it may be necessary to use a hand to break up clumps and agitate the sample to reduce it. Make sure you rinse any detritus from your hand back into the sieve.

2.5. Fill the small bucket with ambient water and use this water to fill the squeeze bulb. Using the squeeze bulb, rinse the sample from the sieve to the pan. Take care to rinse the entire contents of the sample into the pan. Some organisms may stick to the screen.

2.6. Use the squeeze bulb to transfer the sample from the pan into the pre-marked wide-mouth jug, making sure the location, date, and replicate number are accurate.

3. SAMPLE PRESERVATION AND HANDLING

3.1. Preserve the sample with 10% buffered formalin (see FS 7001, section 1) by adding a 9 to 1 ratio of water to 100% formalin. If laboratory processing is possible within eight hours, the samples may be stored on ice, without addition of formalin. If desired, add a very small amount of rose bengal dye (approximately 100 mg per liter of material) to the sample as a picking aid.

FS 7360. LAKE CONDITION INDEX (LAKE COMPOSITE) SAMPLING

(See also *Development of Lake Condition Indices for Florida, July 2000.*)

See also the following section:

- FT 3000 Aquatic Habitat Characterization

1. EQUIPMENT AND SUPPLIES

- Completed Physical/Chemical Characterization Field Sheet (FD 9000-3)
- Site map
- Completed Lake Habitat Assessment Field Sheet (FD 9000-6)
- Ekman or Petite Ponar dredge (with line marked in 0.1 m graduations)
- Box sieve (constructed of fiberglass-coated wood and U.S. 30 mesh screen), bucket sieve or dip net with U.S. 30 mesh sieve material
- White enamel or plastic pan
- Plastic squeeze bulb
- Small bucket
- Wide-mouth plastic sample containers
- Tape and permanent markers

- Buffered formalin
- Rose Bengal dye (optional)
- Composite Lake Sampling Sheet (FD 9000-2)

2. METHODS

2.1. First, conduct lake habitat assessment according to FT 3200. If the lake has a color level exceeding 20 PCU, LCI invertebrate sampling is inappropriate.

2.2. Prior to visiting the lake, study a map of the system. For lakes less than 1,000 acres, logically divide the lake into 12 sampling units. Collect one bottom grab at a depth between 2 m and 4 m from each of these 12 units. The 12 dredges will be composited into a single sample. For lakes larger than 1,000 acres, divide the lake into two to four major sampling divisions. Collect 12 composited dredge bottom grabs from each major division. Take a copy of the map on the sampling trip so you can mark the location of each benthic grab on the map. Number each major sampling division. Mark the location of each of the 12 benthic grabs within each major sampling division on the map.

2.3. When sampling from a boat, collect dredge samples from the rear and downstream of the vessel to avoid contamination of other types of samples with disturbed sediments. Rinse the box sieve with ambient water and tie it to the side of the boat where samples will be collected. When placed in the water, it will float at the surface. If a box sieve is unavailable, wash the dredged material in a dip net or bucket sieve, provided it is fitted with a U.S. 30 mesh sieve material.

2.4. Dredges

2.4.1. Ekman: Open the spring-loaded jaws and attach the chains to the pegs at the top of the sampler. Lower the dredge to the bottom, making sure it settles flat. Using the graduated line, check to make sure the depth is between two and four meters. Record the depth on the Composite Lake Sampling Sheet (FD 9000-2). Holding the line taught, send down the messenger to close the jaws of the dredge. Pull the sampler to the surface and place it immediately into the box sieve. Carefully open the jaws and empty the contents into the sieve, rinsing to assure complete sample purging. Record the gear type used on FD 9000-2. The spring-loaded Ekman can be dangerous. Hold the dredge firmly above the hinges, and take care to not get pinched by the spring-loaded jaws, which could produce serious injury. Check to make sure the jaws are fully closed and that no sample was lost while lifting the dredge. Discard the grab if the dredge is not fully closed.

2.4.2. Petite Ponar: Open the jaws and place the crossbar into the proper notch. Lower the dredge to the bottom, making sure it settles flat. Using the graduated line, check to make sure the depth is between two and four meters. Record the depth on FD 9000-2. When tension is removed from the line, the cross-bar will drop, enabling the dredge to close as the line is pulled upward during retrieval of the dredge. Pull the sampler to the surface and place it immediately into the box sieve. Carefully open the jaws and empty the contents into the sieve, rinsing the dredge to assure complete sample purging. Record the gear type used on FD 9000-2. Check to make sure the jaws are fully closed and that no sample was lost while lifting the dredge. Discard the grab if the dredge is not fully closed.

2.5. Examine the sediment and document its visual characteristics on FD 9000-2. Swirl the box sieve in the water with a back-and-forth motion to wash the fine sediments through. Concentrate the remaining sample into one corner of the sieve. If a sediment type is

especially clayey or mucky, it may be necessary to use a hand to break up clumps and agitate the sample to reduce it. Make sure you rinse any detritus from your hand back into the sieve.

2.6. Fill the small bucket with ambient water and use this water to fill the squeeze bulb. Using the squeeze bulb, rinse the sample from the sieve to the pan. Take care to rinse the entire contents of the sample into the pan. Some organisms may stick to the screen.

2.7. Use the squeeze bulb to transfer the sample from the pan into the pre-marked wide-mouth jug, making sure the location and date are accurate.

2.8. Repeat steps 2.2-2.6 until 12 benthic grabs have been taken, as determined during step 2.2. If material from all 12 dredges will not fit in one jug, use additional jugs, clearly marked as being part of the same sample (jug 1 of 2, jug 2 of 2).

3. SAMPLE PRESERVATION AND HANDLING

3.1. Preserve the sample with 10% buffered formalin (see FS 7001, section 1) by adding a 10 to 1 ratio of water to 100% formalin. If desired, add a very small amount of rose bengal dye (approximately 100 mg per liter of material) to the samples as a picking aid.

Appendix FS 7000
Figures, Tables and Forms

Form FD 9000-1: Biorecon Field Sheet

Form FD 9000-2: Composite Lake Sampling Sheet (< 1000 ACRES)

Form FD 9000-7: Lake Vegetation Index Data Sheet

Form FD 9000-8: Rapid Periphyton Survey Sheet

Table FS 7000-1: Maximum Number of Specimens Retained for Biorecon Identification

Form FD 9000-1: Biorecon Field Sheet

STORET Station	**Location**
Latitude: N ° ' " Longitude: W ° ' "	**Watershed / Basin**
Date/Time Collected	**Collected, & Sorted By:**
types of habitats sampled	**QC'd By:**

** **R**are (1-3), **C**ommon (4-10), **A**bundant (11-100), **D**ominant (>100).

Taxa	Tally	Abun Code	Taxa	Tally	Abun Code	Taxa	Tally	Abun Code
Diptera			**Trombidiformes**			**Ephemeroptera**		
Ceratopogonidae			Acarina			Acerpenna pygmaea		
Culicidae			**Oligochaeta**			Baetidae		
Empididae			Naiidae			Baetis sp.		
Chironomidae			Lumbriculidae			Baetisca sp.		
Simulium sp.			Tubificidae			Brachycercus sp.		
Stratiomyidae			**Hirudinea**			Caenis sp.		
Tipulidae			**Pelecypoda**			Callibaetis sp.		
			Corbicula fluminea			Centroptilum sp.		
			Elliptio sp.			Heptagenia sp.		
			Pisidiidae			Hexagenia sp.		
						Isonychia sp.		
Gastropoda			**Megaloptera**			Leptophlebia sp.		
Ancylidae			Corydalus cornutus			Neoephemera sp.		
Elimia sp.			Sialis sp.			Stenacron sp		
Physella sp.						Stenonema sp.		
Pomacea sp.			**Hemiptera**			Tricorythodes sp.		
Planorbella sp.			Belostoma sp.					
Hydrobiidae			Corixidae					
Viviparus sp.			Gerridae			**Plecoptera**		
Melanoides tuberculata			Hydrometra sp.			Acroneuria sp.		
			Pelocoris sp.			Amphinemura sp.		
			Pleidae			Hydroperla sp.		
Odonata			Ranatra sp.			Isoperla sp.		
Argia sp.			Velidae			Leuctra sp.		
Aeschnidae						Neoperla sp.		
Boyeria vinosa.			**Other (name groups)**			Paragnetina sp.		
Calopteryx sp.						Perlesta sp.		
Enallagma sp.						Perlinella sp.		
Epitheca sp.						Pteronarcys sp.		
Erythemis simplicicollis						Taeniopteryx sp.		
Gomphus sp.						Talloperla sp.		
Hetaerina sp.								
Ischnura sp.								
Libellulidae						**Trichoptera**		
Macromia sp.						Anisocentropus sp.		
Neurocordulia sp.						Brachycentrus sp.		
Progomphus sp.						Cernotina sp.		
Pachydiplax longipennis						Cheumatopsyche sp.		
			Decapoda			Chimarra sp.		
			Palaemonetes sp.			Diplectrona sp.		
Coleoptera			Procambarus sp.			Hydroptila sp.		
Ancyronyx variagata						Hydropsyche sp.		
Curculionidae						Lype diversa		
Dineutus sp.			**Amphipoda**			Macrostemum sp.		
Dubiraphia vittata			Gammarus sp.			Nectopsyche sp.		
Dytiscidae			Hyalella azteca			Oecetis sp.		
Gyretes sp.			Crangonyx sp.			Oxyethira sp.		
Microcylloepus pusillus						Polycentropus sp.		
Stenelmis sp.			**Isopoda**			Triaenodes sp.		
			Caecidotea (Asellus) sp.					
Column Total: Taxa			**Column Total: Taxa**			**Column Total: Taxa**		

NOTE: The Biorecon field data sheet is to be used as an aid during sampling and organism field sorting. The calculated metrics should be retrieved from the Florida Statewide Biological Database or the same counting and collapsing procedures used, as described in LT 7100.

Form FD 9000-2: COMPOSITE LAKE SAMPLING SHEET (< 1000 ACRES)

Date:		Name of Lake:	Gear used:	PONAR	EKMAN	OTHER
Comments:						
Drop #	**Depth (m)**	**Sediment Type (circle all that apply)**	**Drop #**	**Depth (m)**	**Sediment Type (circle all that apply)**	
1		sand, silt/clay, CPOM, muck, SAV	7		sand, silt/clay, CPOM, muck, SAV	
2		sand, silt/clay, CPOM, muck, SAV	8		sand, silt/clay, CPOM, muck, SAV	
3		sand, silt/clay, CPOM, muck, SAV	9		sand, silt/clay, CPOM, muck, SAV	
4		sand, silt/clay, CPOM, muck, SAV	10		sand, silt/clay, CPOM, muck, SAV	
5		sand, silt/clay, CPOM, muck, SAV	11		sand, silt/clay, CPOM, muck, SAV	
6		sand, silt/clay, CPOM, muck, SAV	12		sand, silt/clay, CPOM, muck, SAV	

CPOM = coarse particulate organic matter; SAV = submerged aquatic vegetation

Paste map here and show location of each dredge drop and where water chemistry parameters were collected.

Table FS 7000-1:
MAXIMUM NUMBER OF SPECIMENS RETAINED FOR BIORECON IDENTIFICATION

Taxon	Target # to Pick
Oligochaeta	5
Acarina	5
Hirudinea	5
Ephemeroptera	
Baetidae	15
Baetiscidae	15
Behningiidae	5
Caenidae	15
Ephemeridae	15
Ephemerellidae	15
Heptageniidae	15
Isonychiidae	5
Leptohyphidae	15
Leptophlebiidae	15
Metretopodidae	5
Neoephemeridae	15
Oligoneuriidae	5
Polymitarcyidae	5
Pseudironiidae	5
Odonata	15
Trichoptera	
Beraeidae	5
Brachycentridae	5
Calamoceratidae	5
Dipseudopsidae	5
Glossomatidae	5
Helicopsychidae	5
Hydropsychidae	15
Hydroptilidae	15
Lepidostomatidae	15
Leptoceridae	15
Limnephilidae	5
Molannidae	5
Odontoceridae	5
Philopotamidae	5
Phryganeidae	5
Polycentropodidae	15
Psychomyiidae	5
Rhyacophilidae	5
Sericostomatidae	5
Uenoidae	5

Taxon	Target # to Pick
Plecoptera	
Capniidae	15
Chloroperlidae	15
Leuctridae	15
Nemouridae	5
Peltoperlidae	5
Perlidae	15
Perlodidae	15
Pteroncyidae	5
Taeniopterygidae	5
Hemiptera (any)	5
Megaloptera (any)	5
Neuroptera	5
Diptera (of any other kind)	5
Ceratopogonidae	5
Chaoboridae	5
Empididae	5
Collembola	5
Amphipoda	15
Decapoda	5
Isopoda	5
Gastropoda	15
Pelecypoda	5
Lepidoptera	5
Coleoptera	
Chrysomelidae	5
Dryopidae	5
Dytiscidae	15
Elmidae	15
Gyrinidae	5
Haliplidae	5
Hydraenidae	5
Hydrophilidae	15
Noteridae	5
Psephenidae	5
Ptilodactylidae	5
Scirtidae	5

Form FD 9000-8: Rapid Periphyton Survey Field Sheet

FDEP Rapid Periphyton Survey 4-19-07											
Site:						Date:		Investigator:			
Transect (m)	Point	Algae Sampled?	Algal Thickness NV (0-1) V (1-5)	Algae Type	Canopy	Transect (m)	Point	Algae Sampled?	Algal Thickness NV (0-1) V (1-5)	Algae Type	Canopy
0	1	Y N		F D O		60	1	Y N		F D O	
	2	Y N		F D O			2	Y N		F D O	
	3	Y N		F D O			3	Y N		F D O	
	4	Y N		F D O			4	Y N		F D O	
	5	Y N		F D O			5	Y N		F D O	
	6	Y N		F D O			6	Y N		F D O	
	7	Y N		F D O			7	Y N		F D O	
	8	Y N		F D O			8	Y N		F D O	
	9	Y N		F D O			9	Y N		F D O	
10	1	Y N		F D O		70	1	Y N		F D O	
	2	Y N		F D O			2	Y N		F D O	
	3	Y N		F D O			3	Y N		F D O	
	4	Y N		F D O			4	Y N		F D O	
	5	Y N		F D O			5	Y N		F D O	
	6	Y N		F D O			6	Y N		F D O	
	7	Y N		F D O			7	Y N		F D O	
	8	Y N		F D O			8	Y N		F D O	
	9	Y N		F D O			9	Y N		F D O	
20	1	Y N		F D O		80	1	Y N		F D O	
	2	Y N		F D O			2	Y N		F D O	
	3	Y N		F D O			3	Y N		F D O	
	4	Y N		F D O			4	Y N		F D O	
	5	Y N		F D O			5	Y N		F D O	
	6	Y N		F D O			6	Y N		F D O	
	7	Y N		F D O			7	Y N		F D O	
	8	Y N		F D O			8	Y N		F D O	
	9	Y N		F D O			9	Y N		F D O	
30	1	Y N		F D O		90	1	Y N		F D O	
	2	Y N		F D O			2	Y N		F D O	
	3	Y N		F D O			3	Y N		F D O	
	4	Y N		F D O			4	Y N		F D O	
	5	Y N		F D O			5	Y N		F D O	
	6	Y N		F D O			6	Y N		F D O	
	7	Y N		F D O			7	Y N		F D O	
	8	Y N		F D O			8	Y N		F D O	
	9	Y N		F D O			9	Y N		F D O	
40	1	Y N		F D O		100	1	Y N		F D O	
	2	Y N		F D O			2	Y N		F D O	
	3	Y N		F D O			3	Y N		F D O	
	4	Y N		F D O			4	Y N		F D O	
	5	Y N		F D O			5	Y N		F D O	
	6	Y N		F D O			6	Y N		F D O	
	7	Y N		F D O			7	Y N		F D O	
	8	Y N		F D O			8	Y N		F D O	
	9	Y N		F D O			9	Y N		F D O	
50	1	Y N		F D O		**Algal Thickness Rank**					
	2	Y N		F D O		0	1	2	3	4	5
	3	Y N		F D O		0 mm rough	<0.5 mm or slimy	0.5-1 mm	>1 to <6 mm	6-20 mm	>20 mm
	4	Y N		F D O							
	5	Y N		F D O		Bottom Visible? If "N", add comment to "Notes" section whether water is dark, turbid, deep, etc.					
	6	Y N		F D O							
	7	Y N		F D O		Algal Thickness:		NV = Not Visible		V = Visible	
	8	Y N		F D O		Algae Type:		F = Filamentous		D = Diatoms	
	9	Y N		F D O				O = Other			
Notes:											

Form FD 9000-7: Lake Vegetation Index Data Field Sheet

Lake Vegetation Index Data Sheet (10-19-06)

Waterbody:	Date:	County:
Analyst:	STORET:	Signature:
Lake water level (circle 1): high, normal, low	Algal mats present (circle 1): yes, no	Lake units sampled (circle one group): 1, 4, 7, 10
Plants in bold=exotic. (1)= category 1 invasive (worst), (2)=category 2 invasive.		2, 5, 8, 11
Except where noted on sheet, species level identification required.		3, 6, 9, 12

It is important to note dominant or co-dominant taxa (by areal extent) for each unit. **Use "D" to denote dominant taxon, or "C" for the 2 co-dominant taxa.**

SPECIES	C of C	1	2	3	4	Specimen collected (check)	Approx. water depth	SPECIES	C of C	1	2	3	4	Specimen collected (check)	Approx. water depth
Acer rubrum	4.65							Habenaria repens	4.58						
Alternanthera philoxeroides(2)	0.00							**Hydrilla verticillata(1)**	0.00						
Azolla caroliniana	1.81							Hydrochloa caroliniensis (Luziola fluit	4.79						
Baccharis glomeruliflora	6.12							Hydrocotyle umbellata	1.92						
Bacopa caroliniana	5.31							**Hygrophila polysperma(1)**	0.00						
Bacopa monnieri	4.49							Hypericum fasciculatum	7.27						
Bidens laevis	7.19							Hypericum hypericoides	5.44						
Bidens mitis	6.31							Hypericum myrtifolium	6.56						
Blechnum serrulatum	7.15							Ilex cassine	7.66						
Boehmeria cylindrica	5.91							**Ipomoea aquatica(1)**	0.00						
Brachiaria mutica	0.00							Ipomoea sagittata	6.42						
Brasenia schreberi	8.79							Itea virginica	7.09						
Cabomba caroliniana	5.07							Juncus effusus	3.25						
Carex albolutescens (C. longii)	3.47							Juncus marginatus	3.65						
Carex verrucosa	7.97							Juncus megacephalus	5.70						
Centella asiatica	1.92							Juncus repens	6.91						
Cephalanthus occidentalis	7.27							Lachnanthes caroliana	3.76						
Ceratophyllum demersum	4.16							Leersia hexandra	5.61						
Ceratopteris thalictroides	2.93							Lemna minor	3.77						
Chara spp.	3.90							Leucothoe racemosa	9.44						
Cladium jamaicense	9.04							Limnobium spongia	4.79						
Clethra alnifolia	5.63							**Limnophila sessiliflora(2)**	0.00						
Cliftonia monophylla	5.63							Liquidamber styraciflua	5.56						
Colocasia esculenta(1)	0.00							Ludwigia arcuata	5.32						
Commelina diffusa	2.02							Ludwigia leptocarpa	3.47						
Crineum americanum	8.67							Ludwigia octovalvis	4.09						
Cyperus alternifolius(2) (C. involucrat	0.00							Ludwigia peruviana	0.62						
Cyperus articulatus	6.64							Ludwigia repens	5.20						
Cyperus haspan	5.68							Lyonia ferruginea	8.39						
Cyperus lanceolatus	2.04							Lyonia ligustrina	8.67						
Cyperus polystachyos	1.56							Lyonia lucida	7.06						
Cyperus prolifer(2)	0.00							Magnolia virginiana	9.44						
Cyperus surinamensis	2.03							Mayaca fluviatilis	8.45						
Cyrilla racemiflora	5.20							**Melaleuca quinquenervia(1)**	0.00						
Decodon verticillatus	7.80							Micranthemum glomeratum	5.85						
Diodia virginiana	4.96							Micranthemum umbrosum	5.66						
Echinochloa crusgalli	0.22							Mikania scandens	1.95						
Eclipta alba	none							**Mimosa pigra(1)**	0.00						
Egeria densa	0.49							Myrica cerifera	3.82						
Eichhornia crassipes(1)	0.00							**Myriophyllum aquaticum**	0.67						
Eleocharis baldwinii	2.82							Myriophyllum heterophyllum	4.77						
Eleocharis cellulosa	7.80							Myriophyllum laxum	5.85						
Eleocharis equisetoides	9.10							Myriophyllum pinnatum	5.85						
Eleocharis interstincta	7.80							**Myriophyllum spicatum(2)**	0.00						
Erechtites hieracifolia	1.37							Najas guadalupensis	5.07						
Eriocaulon compressum	7.50							Najas marina	5.50						
Eriocaulon decangulare	7.50							**Najas minor**	3.64						
Eupatorium capillifolium	0.83							**Nasturtium officinale**	2.93						
Fuirena breviseta	7.60							Nelumbo lutea	6.26						
Fuirena scirpoidea	6.50							Nitella spp.	7.28						
Gordonia lasianthus	9.03														

SPECIES	C of C	1	2	3	4	Specimen collected (check)	Approx. water depth	SPECIES	C of C	1	2	3	4	Specimen collected (check)	Approx. water depth
Nuphar sp.	4.64							**Solanum tampicense(1)**	0.00						
Nymphaea odorata	7.18							Sparganium americanum	6.50						
Nymphoides aquatica	6.09							Spartina alterniflora	7.94						
Nyssa sylvatica var. biflora	9.04							Spartina bakeri	5.98						
Orontium aquaticum	8.39							Spirodela polyrhiza	2.95						
Osmunda cinnamomea	6.44							Spirodela punctata	none						
Osmunda regalis	8.04							Taxodium ascendens	7.21						
Panicum hemitomon	5.82							Taxodium distichum	7.21						
Panicum repens(1)	0.00							Thalia geniculata	7.12						
Paspalidium geminatum	6.36							Thelypteris palustris	none						
Paspalum distichum	5.54							Triadenum virginicum	8.16						
Paspalum repens	6.69							Typha sp.	1.60						
Paspalum urvillei	0.00							Utricularia biflora	6.37						
Peltandra virginica	7.31							Utricularia floridana	6.34						
Pennisetum purpureum(1)	0.00							Utricularia foliosa	6.44						
Phragmites australis	4.39							Utricularia inflata	5.85						
Pinus elliottii	4.21							Utricularia purpurea	6.50						
Pistia stratioides(1)	0.00							Utricularia cornuta	7.46						
Pluchea odorata	4.96							Utricularia radiata	6.01						
Pluchea rosea	5.45							Valliseneria americana	7.28						
Polygonum densiflorum	5.32							Websteria confervoides	none						
Polygonum hydropiperoides	4.02							**Wedelia trilobata(2)**	0.00						
Polygonum punctatum	4.02							Wolffiella spp.	4.37						
Pontederia cordata	5.38							Woodwardia virginica	6.5						
Potamogeton diversifolius	7.15							Zizania aquatica	6.69						
Potamogeton illinoensis	6.64							Zizaniopsis milacea	6.21						
Potamogeton pusillus	7.80														
Potemogeton pectinatus	7.80														
Proserpinaca pectinata	5.50														
Rhynchospora microcarpa	5.29														
Rhynchospora microcephala	6.50														
Rhynochospora tracyi	9.03														
Ricca fluitans	4.64														
Ricciocarpus natans	4.55														
Ruppia maritima	7.24														
Sabal palmetto	4.85														
Sabatia grandiflora	7.09														
Sacciolepis striata	5.35														
Sagittaria kurziana	9.75														
Sagittaria lancifolia	4.96														
Sagittaria latifolia	6.50														
Sagittaria graminea	5.53														
Salix carolina	2.95														
Salvinia minima	2.03														
Sambucus canadensis	1.48														
Sapium sebiferum(1)	0.00														
Saururus cernuus	7.33														
Schinus terebinthifolius(1)	0.00														
Scirpus americanus	6.50														
Scirpus californicus	6.01														
Scirpus cubensis	3.77														
Scirpus validus	5.55														