# 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

##### General Cautions

##### Many of these biological sampling procedures require specific training and a demonstration of competency due to the expert judgment exercised during field sampling.

##### Study objectives are extremely important in determining the appropriate environmental conditions (e.g., hydrology), site selection, and other factors associated with conducting biological sampling. Because biota respond to natural and human stressors, it is imperative that samplers fully understand the study objectives associated with any biological sampling and take efforts to control for confounding factors that may interfere with the appropriate interpretation of biological data.

**FS 7001 Solution Preparation**

Adapted from section 10200 B, *Plankton* (2011),in Standard Methods for the Examination of Water and Wastewater (see Standard Methods Online, <http://www.standardmethods.org/store/>). This reference is provided for informational purposes only and is not needed for this procedure. Follow manufacturer’s expiration dates if applicable, or follow your laboratory’s quality manual.

##### Buffered Formalin (e.g., for preservation of macroinvertebrate or algal samples)

##### Equipment and Supplies

###### Sodium bicarbonate

###### 37% formaldehyde (formalin)

###### pH meter

###### Small containers

###### Balance

##### Methods

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

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

##### Weigh out approximately 5.0 grams of sodium bicarbonate.

##### 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 formalin, as this is a supersaturated solution.

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

##### Lugol’s Solution (e.g., for phytoplankton samples)

##### Equipment and Supplies

###### Balance

###### Weigh boat

###### Spatula

###### Magnetic stirrer

###### Teflon stir bar

###### 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

##### Methods

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

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

##### 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.

##### Pour mixed solution into a 500-mL glass amber bottle and store in an air-conditioned part of the lab. Once prepared, Lugol’s can be used for one year. 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.

##### M3 Solution (e.g., for algal samples)

##### Equipment and Supplies

###### Balance

###### Weigh boat

###### Spatula

###### Magnetic stirrer

###### Teflon stir bar

###### 500-mL or 1-L glass amber bottles

###### 5 g potassium iodide (KI)

###### 10 g iodine crystals

###### 50 mL glacial acetic acid

###### 250 mL formalin (see section 1 above)

###### 1 L deionized (DI) water in a 1.5-L or larger Erlenmeyer flask

###### Dropper bottle

##### Methods

##### Add acetic acid and formalin to DI water. Place on magnetic stir plate with stir bar.

##### Weigh out 5 g of KI and 10 g of iodine crystals.

##### 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.

##### Store mixed solution in 500-mL or 1-L glass amber bottle 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.

## Phytoplankton Sampling

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

###### FS 2100 Surface Water Sampling

##### Equipment and Supplies

###### 1 L Nalgene dark sample bottle

###### Depth samplers such as an integrated tube, Van Dorn or Alpha bottle

###### Lugol’s solution

###### Buffered formalin

###### Cooler with ice

##### Methods

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

##### Collect water column phytoplankton as a standard surface grab sample (within 0.3 m of surface), following the procedures and cautions described in FS 2000, unless otherwise specified in project objectives.

##### Collect samples directly with the sample bottles for surface collection or with the additional equipment (depth sampler) for various water depths.

##### Sample Preservation and Handling

##### Place sample on ice.

##### Preserve the 1 L sample with 3 mL of Lugol’s solution (see FS 7001, section 2) within 15 minutes of collection, or place samples immediately in wet ice and preserve with 3 mL of Lugol’s solution within 36 hours of collection. For long-term storage, add buffered formalin (see FS 7001, section 1) to achieve a minimum of 2.5% final concentration (approximately 25 mL), or add 3mL of Lugol’s every 6-12 months.

### Phytoplankton Bloom Sampling

Use this procedure to collect water column or surface scum samples of phytoplankton during bloom conditions for toxin (cyanotoxin) analysis or algal enumeration and identification. This SOP includes several sampling techniques for various levels of algal bloom response, ranging from initial determination of taxa present to toxin analysis, and from qualitative to quantitative collection.

Cyanobacteria (blue-green algae) bloom sampling requires knowledge of proper algal sampling techniques, safety protocols and personal protective equipment (PPE). The degree and type of safety measures and PPE required depends on the unique characteristics of the bloom to be sampled. Samplers should use appropriate PPE for conditions sampled to reduce occupational exposure. Typical short-term acute risks include, but are not limited to, contact dermatitis and upper respiratory irritation. Long-term risks are unknown; however certain cyanobacteria are known to produce toxins that are tumor promoters, even at very low doses. Samplers should use appropriate PPE to reduce occupational exposure to these toxins.

See DEP SOP FS 1000, Table 1000-5, for required containers, preservation, and holding times for select cyanotoxins. Contact lab conducting analyses for specifications for analyses not included in Table 1000-5.

See also the following SOPs:

* FA 1000 Regulatory Scope and Administrative Procedures for Use of DEP SOPs
* FC 1000 Cleaning/Decontamination Procedures
* FD 1000 Documentation Procedures
* FM 1000 Field Planning and Mobilization
* FQ 1000 Field Quality Control Requirements
* FS 1000 General Sampling Procedures
* FS 2000 General Aqueous Sampling
* FS 2100 Surface Water Sampling
* FT 1000 General Field Testing and Measurement

##### Equipment and Supplies

###### Cooler with ice

###### Sampling containers

###### Dominant taxa identification to determine type of bloom – any container can be used, and 50-mL screw-top centrifuge tubes work well.

###### Cyanotoxin analysis - 250 mL clean wide mouth amber glass bottles with Teflon® lined caps (plastic bottles should not be used for cyanotoxin analysis due to potential toxin adherence to plastic).

###### Algal enumeration, identification, or biomass - brown plastic or amber glass containers (any type of container can be used for unexpected collections but keep sample on ice and do not expose to light).

###### Do not use containers if defects are present on bottles or caps, or if containers do not appear clean.

###### Depth sampling device (such as integrated tube, Van Dorn or Alpha bottle sampler)

###### Lugol’s solution

###### Buffered formalin

##### Methods

##### Clearly label sample containers with analysis to be conducted and other information as required in FD 1000.

##### If a bloom is suspected while samplers are out for a routine or other monitoring purpose, collect a surface grab sample in any container type for taxonomic identification.

##### Select one or more of the methods described in 2.4-2.7 to collect a bloom sample. Selection will depend on the purpose of the sampling event and intended use of the results.

##### Surface Grab Sample

Collect surface grab sample within the top 0.3 m of the water column directly into the sample container per FS 2000 Section 1.2 with the following additional considerations.

Do not collect the sample by skimming the surface.

##### Do not rinse the interior of the sample container with site water prior to collection.

##### If an algal scum layer is present, avoid submerging the bottle through the algal scum layer as this may introduce algal scum into the sample and may not be representative of the water column. Refer to Surface Scum Sampling procedure in 2.6.

##### Water column samples at depth

##### Collect samples at depth per FS 2000. Document the method of sampling if collecting a sample with an integrated tube, Van Dorn, Alpha bottle, or other secondary sampling device.

##### For cyanotoxin analysis, the intermediate sampling device must be constructed of materials compatible with sampling for cyanotoxins as specified in Table FS 1000-3 (e.g., glass, Teflon®).

##### Record the depth sampled on the sample bottle if multiple depths are sampled. Record the method of collection and sample depth on the field sheet.

##### Surface Scum Sample

##### In order to collect an algal scum layer sample in a repeatable manner that would be representative of a potential recreational exposure, gently mix the scum layer in a 0.5-m diameter area by agitating the surface scum with a sampling pole or other device to homogenize it with the underlying water such that it is dispersed in the upper 10 cm of the water column.

##### Quickly collect a water sample within the top 5-10 cm of the water column directly into the container. *Do not collect the sample by skimming the surface*.

##### Do not rinse the interior of the sample container with site water prior to collection.

##### Sample Preservation and Handling

##### Clean the outside of the containers with water, paper towels or other absorbent materials to remove any spilled sample from the exterior of the container.

##### Protect glass containers from breakage (“bubble wrap” is recommended).

##### For preservation of all cyanotoxin analysis samples, place samples immediately in wet ice, and refer to Table FS 1000-5 for preservation, holding times, and containers. Samples for which a quick turn-around time is needed to identify only the dominant bloom species should be placed on ice and in the dark and submitted without chemical preservation. Consult your laboratory for additional instructions regarding samples in predominantly marine waters.

##### Samples for algal enumeration and identification should be either preserved immediately with a sufficient volume (3 mL/L minimum) of Lugol’s solution in the field or placed immediately in wet ice and then preserved with Lugol’s solution within 36 hours of collection time. Samples preserved with Lugol’s solution in the field do not need to be placed in wet ice but should be kept cool.

##### For long-term storage, add buffered formalin to achieve a minimum of 2.5% final concentration (approximately 25 mL/L [See FS 7001, section 1]). Store sample containers in the dark.

## Periphyton Sampling

### Quantitative Periphyton Sampling

Use this method in freshwater systems only.

##### Equipment and Supplies

###### Periphytometer

###### Eight 25 mm x 75 mm glass microscope slides

###### Acetone

###### Kimwipes

###### Nylon twine

###### Two 50-mL screw-top centrifuge tubes

###### Deionized (DI) water

###### Preservative (100% buffered formalin, Lugol’s solution, or M3)

###### Cooler with ice

###### Permanent marker

##### Methods

##### 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.

##### Pack and transport slides and periphytometer with care. At the sampling site, place the eight slides in the periphytometer.

##### Field samplers must ensure that conditions of flow, depth, salinity (if marine influenced), and other habitat parameters are as similar as possible between deployment sites that are to be compared. 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 should also be considered.

##### 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 the rack below water or the racks may be submersed beyond the zone of light penetration. Because water levels may fluctuate during the incubation period, provide a sufficient length of twine to prevent the periphytometer from either drying out if water levels drop, or from submersing if water levels rise.

##### After the 28-day incubation period, carefully retrieve the rack, ensuring no loss of algae, collect the slides (holding them only on the edges) and evenly divide slides among containers/tubes 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 site water.

##### 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 tubes directly on wet ice.

##### Sample Preservation and Handling

##### If slides will not be scraped within 36 hours, add 2 mL of buffered formalin (see FS 7001, section 1), 1 mL of M3 (see FS 7001, section 3), or 0.5 mL Lugol’s solution (see FS 7001, section 2) to each 50 mL container for identification and enumeration (only thermally preserve periphyton chlorophyll *a* samples). Provided samples are kept on ice and in the dark, and scraped within 36 hours, chemical preservation should be done by the laboratory. It is preferable to wait until the slides are scraped to chemically preserve the sample for identification and enumeration.

##### Store samples for chlorophyll-*a*/biomass in site water.

### 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*. (reference provided for informational purposes only and is not needed for this procedure)

See also the following section:

* FT 3000 Aquatic Habitat Characterization.

##### Equipment and Supplies

###### Disposable pipettes (with at least 2 mL capacity)

###### Preservative (10% buffered formalin, Lugol’s solution, or M3)

###### Permanent marker

###### Plastic container with approximately 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) or other datasheet to capture documentation required in FD 5311

###### Completed Stream/River Habitat Sketch Sheet (FD 9000-4) or other datasheet to capture documentation required in FD 5312, as applicable

##### Completed Habitat Assessment Field Sheet (FD 9000-5) Methods

##### Complete the Physical/Chemical Characterization per FT 3001 and Habitat Assessment per FT 3100 or FT 3200, as appropriate for the water body in which you are sampling. Visually determine the substrate) 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, rock, and if present, the algal mats themselves. All sampled substrates are combined into a single, composite sample for a particular site. For sampling, target substrates in flow and sunlight conditions 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 are 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 is available. Sample all removable habitats where periphyton growth is expected, such as snags, roots, leaf packs/mats, vascular plants/mosses, rock, and algal mats. Avoid depositional areas or substrates with excessive silt or sand. Do not dilute the algal sample with excess sediment or detritus If algal mats are present, be sure to include aliquots from them in your collections. If algal mats dominate the site (i.e. are present as a bloom), see FS 7240.

##### 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 the kinds of algae present. If there are algal mat fragments in the slurry that cannot be transferred with the pipette, collect a 1-cm length portion by pinching with fingers and deposit into sample vial. 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 m2, (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 m2) 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.

##### 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.

##### 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.

##### 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.

##### Sample Preservation and Handling

##### Add 2 mL of buffered formalin (see FS 7001, section 1), 1 mL of M3 (see FS 7001, section 3), or 0.5 mL Lugol’s solution per 40 mL of periphyton slurry for samples for taxonomic identification. Chemical preservation may be delayed for up to 36 hours if samples are kept on ice and in the dark.

### Rapid Periphyton Survey

##### Introduction

##### The Rapid Periphyton Survey (RPS) is a rapid method to quantify the extent and abundance of attached algae (periphyton) in a stream or river and to evaluate the autecological information associated with the dominant algae. The RPS should be done in conjunction with the Physical/Chemical Characterization and Stream and River Habitat Assessment (FT 3000). When this method is used to determine floral health associated with Chapter 62-302.531, F.A.C., perform the method unless the periphyton abundance observation on the physical/chemical characterization is in the categories “not observed” or “rare” and the substrate smothering observation for algae is in the categories “none” or “slight.” In those cases, it is assumed that an algal imbalance is not present, These observations of minimal or no algae must be documented with photographs, and those photographs kept with other documentation for the sampling event. (This rule reference is provided for information only and not needed for use of this SOP.)Individuals who will conduct the RPS shall take and pass the DEP test every five years to demonstrate an understanding of the methods and underlying concepts, per FA 5730.

##### See also the following section:

* FT 3000 Aquatic Habitat Characterization

##### Equipment and Supplies

###### Ruler to measure a 10 cm length

###### Rapid Periphyton Survey Field Sheet (FD 9000-25) or other datasheet to capture documentation required in FD 5323

###### Completed Physical/Chemical Characterization Field Sheet (FD 9000-3) or other datasheet to capture documentation required in FD 5311

###### Completed Stream/River Habitat Sketch Sheet (FD 9000-4) or other datasheet to capture documentation required in FD 5312

###### Completed Stream/River Habitat Assessment Field Sheet (FD 9000-5)

###### Spherical Densiometer (concave or convex)

* 100 m measuring tape
* Flagging tape
* Secchi Disk
* Aquascope underwater viewer (bathyscope)

##### Methods

##### Conduct sampling at a site that is representative of the waterbody of interest, during representative flow conditions, as appropriate based on study objectives. Measure a 100m segment of stream, placing flags every 10m.

##### Complete the Physical/Chemical Characterization per FT 3001 and Habitat Assessment per SOP FT 3100. If the periphyton abundance observation is the categories “not observed” or “rare” and the substrate smothering observation for algae is in the categories “none” or “slight,” it is assumed that an algal imbalance is not present, and the RPS procedure does not need to be conducted for Numeric Nutrient Criteria compliance purposes. These observations must be documented with photographs If the RPS is conducted, the RPS algal observations can be done during the habitat mapping process or following the habitat assessment.

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

##### Each observation point, within the above distance parameters, shall be chosen as haphazardly as possible. While it is recommended to observe the general area prior to sampling as a safety precaution, the actual sampling point shall be selected haphazardly, without biasing your selection for or against algal presence.

##### After selecting the observation point:

##### 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 just below the surface. Determine if algae are present or absent, and if present, measure the average length perpendicular to the substrate, with a ruler. For filamentous algae, measure the average length of filaments rather than thickness of a mat (i.e., a 3 cm thick mat made up of attached filaments that are 20 cm long would be recorded as a “6” as in Figure FS 7230-1). Record these data using the rank classifications described below.

##### “N” = non-problematic; algae are absent, or present at lengths up to 1 mm (includes rough surface with no algae, slimy surface, and algae present with thickness less than or equal to 1 mm),

##### “3” = greater than 1 mm to 6 mm,

##### “4” = greater than 6 mm to 20 mm,

##### “5” = greater than 20 mm to 10 cm, and

##### “6” = algae greater than 10 cm.

##### NOTE: Examine the first substrate you encounter, which may not always be the stream bed (e.g., snag, plants, roots). Make observations at the point where your hand encounters the substrate (i.e., if a 0.5 m long snag is grabbed, record the algal length rank classification based on where your hand touches it, not elsewhere). Take the measurement where the algae are 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). Macroalgae that are structurally more similar to macrophytes (e.g. *Chara*, *Nitella*) are not considered to be algae for RPS purposes. Aquatic mosses (e.g., *Fontinalis*, *Sphagnum*) or liverworts are not algae and are not measured as algae in the RPS.

##### Illustration of how to determine algal thickness ranks by measuring length of algae in the stream flow.  Algal thickness 1.5 cm long given Rank 4.  Algal thickness 12 cm long given Rank 6.

##### **Figure FS 7230-1. Illustration of how to determine algal thickness ranks by measuring length of algae in the stream flow.**

##### If the substrate cannot be seen (e.g., in tannic waters) in depths greater than the Secchi depth, then the length can be presumed to be less than or equal to 1 mm and “N” should be recorded. If the substrate cannot be seen, and the depth is less than the Secchi depth, but cannot be reached with the hand, record an “X” for that point, indicating that observations and measurements are not possible using this method. If there are “X” observations in the survey, include a comment specifying the reasons the point was unable to be assessed (e.g. an obstruction, high flow, bottom stirred up, etc.). Leaving the cell blank indicates the point was not evaluated, so it is important to record the “N” or “X” as appropriate.

##### If the substrate can be seen but not reached, then estimate the length rank. This estimate may be based on visual similarity to other substrates within the stream that were reachable. Note on the datasheet which points were visually estimated. This visual estimation should only be conducted when you cannot reach the substrate. It is recommended to use an aquascope (underwater viewer/bathyscope) when making estimates in nonwadeable systems (e.g. deeper spring runs), for improved clarity and reduction of glare.

##### 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 length.

##### The canopy cover shall be measured using a spherical densiometer, between points 4 and 6 (ideally at point 5) on each transect. Do not take a canopy cover measurement if this section of the transect is unreachable. Only make one canopy cover reading per transect.

##### The densiometer consists of a concave or convex mirror with gridwork delineating 24 etched boxes, each 0.25” squared. Each 0.25“-square box can be subdivided into 4 smaller quadrants, to create a total of 96 quadrants. The densiometer instructions refer to these quadrants as dots.

##### While facing upstream, hold the instrument level at approximately waist height (a bubble on the face of the densiometer indicates when the instrument is level), approximately 12-18” away from the body so your head is just outside the grid.

##### Count the number of quadrants (out of a total of 96) for which at least half of the quadrant is filled by tree canopy cover (branches and/or leaves). Record this number (number of quadrants WITH canopy cover) for each transect in association with point 4, 5, or 6.

##### Repeat the above procedures every 10 m, including the 100 m mark, for a total of 99 periphyton observation points and 11 canopy cover readings.

##### Upon completion of the RPS, determine the percentage of **sampled** points (exclude points assigned an “X”) which have an algal rank of 4, 5, or 6. If this value is 20% or greater, collect a composite sample of periphyton from the 100 m reach per FS 7240 2.2.1, below, targeting any material that is dominant or co-dominant and appears to represent a distinct taxonomic group (e.g. diatoms, filamentous, masses of cyanobacteria, or distinct taxa within these major groups). The purpose of this collection is to identify the dominant algal taxa for additional autecological analyses (i.e., to determine if the type of algae present represents an acceptable vs. adverse condition). Do not add preservative to the sample, as taxonomists will only be identifying dominant or co-dominant taxa, as described above. Place sample on ice.

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### Periphyton Sampling for Taxonomic Identification or Toxin Analysis

Use this procedure to collect samples of algal mats for taxonomic identification, cyanotoxin analysis, or biomass measurements. The resulting sample is not intended to represent an entire site condition, but rather reflect the composition of targeted algal mats.

Cyanobacteria (blue-green algae) bloom sampling requires knowledge of proper algal sampling techniques, safety protocols and personal protective equipment (PPE). The degree and type of safety measures and PPE required depends on the unique characteristics of the bloom to be sampled. Samplers should use appropriate PPE for condition sampled to reduce occupational exposure. Typical short-term acute risks include, but are not limited to, contact dermatitis and upper respiratory irritation. Long-term risks are unknown; however certain cyanobacteria are known to produce toxins that are tumor promoters, even at very low doses. Use appropriate PPE to reduce occupational exposure to these toxins.

See DEP SOP FS 1000, Table 1000-5, for required containers, preservation, and holding times for select cyanotoxins. Contact lab conducting analyses for specifications for analyses not included in Table 1000-5.

See also the following SOPs:

* FA 1000 Regulatory Scope and Administrative Procedures for Use of DEP SOPs
* FC 1000 Cleaning/Decontamination Procedures
* FD 1000 Documentation Procedures
* FM 1000 Field Planning and Mobilization
* FQ 1000 Field Quality Control Requirements
* FS 1000 General Sampling Procedures
* FS 2000 General Aqueous Sampling
* FS 2100 Surface Water Sampling
* FT 1000 General Field Testing and Measurement

##### Equipment and Supplies

###### Sampling containers

###### Dominant taxa identification to determine type of bloom—any container can be used (50 mL screw-top centrifuge tubes work well.

###### Cyanotoxin analysis – 250 mL clean wide mouth amber glass bottle with Teflon® lined caps (plastic bottles should not be used for cyanotoxin analysis due to potential toxin adherence to plastic).

###### Algal enumeration, identification, or biomass – Brown plastic or amber glass containers (any type of container can be used for unexpected collections, but keep sample on ice and do not expose to light).

###### Do not use containers with defects on bottles or caps or if containers do not appear clean.

###### Preservative (buffered formalin, Lugol’s solution, or M3)

###### Permanent marker

##### Methods

* 1. Clearly label sampling bottles with analysis to be conducted and other information required in DEP SOP FD 1000.
	2. Sample for Taxonomic Identification
		1. Collect benthic algal mat samples for taxonomic identification by collecting algal filaments from as many different substrates as are covered by algal mats and/or excessive algal growth to get a representative sample of the algal mats of interest. Sometimes the bases of the filaments are necessary for identification, so be sure to collect the algae where it is attached to the substrate whenever possible.
		2. Place all aliquots into a clean plastic bag or glass container with enough site water to keep the algae submerged.
	3. Sample for Algal Mat Biomass
		1. Use a plastic metric ruler to measure the thickness of the algal mat from the top of the mat to the surface of the substrate to which it is attached.
		2. Record the thickness on the field sheet and the sample container. The thickness measurement can be used along with the diameter of the sample container opening to calculate the volume of water that contained the collected biomass.
		3. Use a wide mouth sampling container as a coring device by first submerging the container to fill it with site water. Once filled, invert the container over the mat and force the container down through the mat until you have reached the bottom substrate.
		4. If the bottom substrate is hard,
			1. Twist the sample container back and forth to shear off any filaments that are trapped between the substrate and the lip of the container. This will result in a core sample within the bottle.
			2. Press down firmly and slide the container the width of the container opening to scrape the entrapped filaments off the substrate.
		5. If the bottom substrate is soft,
			1. Force a wide putty knife blade between the soft substrate and the lip of the container. Remove the sample container from the water with the blade of the putty knife still completely covering the opening of the container. For the best results the front edge of the putty knife should be sharpened (ground on one edge like a flat chisel) to better facilitate cutting off algal filaments.
			2. Carefully lift the lip of the container enough to slide the putty knife blade over the mouth of the container.
			3. With the putty knife covering the mouth of the container, right the container and remove from the water. NOTE: A clean putty knife must be used for each sample, or the device must be decontaminated after each use. If decontamination is to be performed in the field, equipment blanks will need to be collected to document that the decontamination process was successful and subsequent samples were not contaminated by the equipment.
		6. Place the lid on the container and tighten securely.

##### Sample Preservation and Handling

##### Clean the outside of the containers with water, paper towels or other absorbent materials to remove any spilled sample from the exterior of the container.

##### Protect glass containers from breakage (“bubble wrap” is recommended).

##### Place samples for cyanotoxin analysis immediately in wet ice.

##### Samples for which a quick turn-around time is needed to identify only the dominant bloom species should be placed in a cooler on wet ice and submitted without chemical preservation.

##### Samples for algal enumeration and identification should be either preserved immediately with 2 mL of buffered formalin (see FS 7001, section 1), 1 mL M3 (see FS 7001, section 3), or 0.5 mL Lugol’s solution (see FS 7001, section 2) per 40 mL of periphyton slurry, or placed immediately in wet ice and kept in the dark and then preserved within 36 hours of collection.

##### For long-term storage, store sample containers in the dark. Add buffered formalin to achieve a minimum of 2.5% final concentration.

## Macrophyte Sampling

### Lake Vegetation Index (LVI) Sampling (see LVI 1100)

###  Stream and River Linear Vegetation Survey (LVS) Method

### See also the following sections:

###### FT 3001 Physical/Chemical Characterization

###### FT 3100 Stream and River Habitat Assessment

###### LQ 7300 Laboratory Quality Control for Macrophyte Taxonomic Identification

Sampling and Use of the Lake Vegetation Index (LVI) for Assessing Lake Plant Communities in Florida: A Primer (DEP SAS-002/11) (LVI Primer is useful for proper taxonomic level identification).

##### Introduction

This method was designed to be a rapid screening tool for ecological condition, by determining how closely a site’s flora resembles that of an undisturbed condition. It should be done in conjunction with the Physical/Chemical Characterization and Stream and River Habitat Assessment (FT 3000), if macrophytes are determined to be a major habitat. When this method is used to determine floral health associated with Chapter 62-302.531, F.A.C., perform the method only when the total area of macrophytes equals or exceeds two square meters within the wetted area of the 100 m sampling reach. (This rule reference is provided for information only and not needed for use of this SOP.) Individuals who will conduct the LVS shall take and pass the DEP test every five years to demonstrate an understanding of the methods and underlying concepts, per FA 5740.

##### EQUIPMENT AND SUPPLIES

###### Aquatic & wetland plant identification manuals

###### Hand lens

###### Boat (if non-wadeable site)

###### Frotus or ViewScope (if non-wadeable site)

###### Plastic bags

###### Permanent marker

###### Cooler and ice

###### Linear Vegetation Survey Field Sheet (FD 9000-32) or other datasheet to capture documentation required in FD 53212

###### Completed Physical/Chemical Characterization Field Sheet (FD 9000-3) or other datasheet to capture documentation required in FD 5311

###### Completed Stream/River Habitat Sketch Sheet (FD 9000-4) or other datasheet to capture documentation required in FD 5312

###### Completed Stream/River Habitat Assessment Field Sheet (FD 9000-5)

##### METHODS

##### Conduct sampling at a site that is representative of the waterbody of interest, during representative flow conditions, as appropriate based on study objectives. Divide the 100 m reach into 10 sampling units 10 m in length. Complete the Physical/Chemical Characterization per FT 3001 and Habitat Assessment per SOP FT 3100. The LVS observations can be done during the habitat mapping process or following the habitat assessment.

##### At each 10 m sampling unit, visually assess and identify the plants present in the wetted area, either from a boat or by wading. Record the presence of all plant species within this 10 m area (both banks), including submersed, floating, and emergent plants. If you are using the Linear Vegetation Survey Field Sheet (Form FD 9000-32) and you observe species that are not on the datasheet, add them in the empty spaces provided. Identify aquatic and wetland plants to the lowest practical taxonomic level, as described in Section 4.2 of the LVI Primer. Do not include plants on the banks above the current water level. Include macroalgae (e.g. *Chara*, *Nitella*) and aquatic mosses (e.g., *Fontinalis*) that are structurally similar to macrophytes. When this method is used to determine floral health associated with Chapter 62-302.531, F.A.C., do not include tree or shrub taxa unless they can also have a forb/herb growth form. (This rule reference is provided for information only and not needed for use of this SOP.)

##### Determine the codominant or dominant species by estimating the plants with the largest areal extent. Dominance can be by submersed, floating, or emergent species. If two taxa are clearly more abundant than any other taxa, select two codominants If one taxon is at least twice as abundant as any other taxon, select it as a single dominant. If unable to decide on a dominant or two codominants, simply do not assign a dominance code for that sampling unit. If none of the species is abundant (occupies at least 1 m2 of the survey area), then do not assign dominance and record the lack of dominance in the field documentation.

##### Rate the total abundance of macrophytes in each 10 m section into one of the following cover categories: 0-5%, >5 and ≤10%, >10 and ≤25%, >25 and ≤50%, >50%.

##### Any specimens that cannot be readily identified in the field should be labeled and placed in plastic bags. Store bags on ice until returning to the laboratory. Plants kept refrigerated can be identified fresh within a few days. Unknowns for herbarium identification or verification by a consulting botanist should be prepared as voucher specimens. Carefully dispose of exotic plant taxa specimens to prevent the inadvertent and detrimental spread of those taxa.

##### Once specimens are verified, finalize the sampling records with correct taxa names.

##### Repeat procedures 3.2-3.5 for the 10 sampling units.

##### When this method is used to determine floral health associated with Chapter 62-302.531, F.A.C., calculate the mean Coefficient of Conservatism (C of C) and the percent invasive exotics as follows. (This rule reference is provided for information only and not needed for use of this SOP.) Obtain C of C scores and the invasive non-native status from Table LVI 1000-1. Taxa not contained in Table LVI 1000-1 shall not be included in these calculations.

##### The Mean C of C is weighted by taxon occurrence, where the presence of a taxon in a 10 m segment is one occurrence, and the maximum number of occurrences for a taxon within the 100 m reach is ten.  For each taxon, multiply the C of C score by the number of occurrences.  Calculate the Mean C of C as the sum of these products divided by the total number of taxa occurrences in the 100 m reach. For both the numerator and denominator, only include occurrences of taxa for which a C of C score is available in Table LVI 1000-1.

##### Calculate the percent invasive non-native taxa as the number of occurrences of taxa listed as Category I or II by the Florida Invasive Species Council (FISC) in Table LVI 1000-1 in the 100 m reach divided by the total number of taxa occurrences in the 100 m reach. For both the numerator and denominator, only include occurrences of taxa for which FISC status can be determined. Do not include genus-level occurrences if some species of the genus are on the FISC list but some species are not.

### Florida Wetland Condition Index Sampling: Vegetation

##### This method is based on “The Wetland Condition Index (WCI): Biological Indicators of Wetland Condition for Isolated Depressional Herbaceous Wetlands in Florida,” by C. R. Lane, M. T. Brown, M. Murray-Hudson, and M. B. Vivas, 2003, <https://publicfiles.dep.state.fl.us/Labs/lds/reports/15104.pdf> and “The Florida Wetland Condition Index (FWCI): Developing Biological Indicators for Isolated Depressional Forested Wetlands,” by K. C. Reiss and M. T. Brown, June 2005, <https://publicfiles.dep.state.fl.us/Labs/lds/reports/15106.pdf> (references provided for informational purposes only and are not needed for this procedure).

This sampling procedure requires specific training and a demonstration of competency due to the expert judgment exercised during field sampling. Each sampling entity must maintain a plant reference collection.

See also the following SOPs:

###### FD 1000 Field Documentation

###### FT 3001 Physical/Chemical Characterization

###### FT 1000 General Field Testing and Measurement

* LT 7600 Wetland Condition Index Determination

##### introduction

The Wetland Condition Index (WCI) was designed to be a rapid tool for assessing the ecological condition of wetlands by determining how closely a wetland’s biological community compares to biological communities observed in minimally disturbed or control wetlands.  The Department may use the WCI in support of establishing site specific alternative water quality criteria under Rule 62-302.800, F.A.C., conducting a use attainability analysis, or establishing a new or revised designated use(s) under Rule 62-302.400, F.A.C. (These rule references are provided for information only and are not needed for use of this SOP.)  The WCI specifically applies only to isolated herbaceous and isolated forested wetlands, and therefore, this method may only be used in other types of wetland systems on a limited, site-specific basis. No numeric thresholds for interpreting the index have been established.  Therefore, application of the WCI should be limited to carefully designed studies that compare “test” wetland sites with similar, yet minimally disturbed “control” wetland sites to demonstrate that test site water quality is sufficient to support a healthy, well-balanced community of flora and fauna.  In this capacity, the WCI may be used to support alternative water quality criteria development in wetlands.  Due to the current lack of quantitative expectations associated with the WCI, interpretation of results must be made on a site-specific basis.

##### Equipment and supplies

###### Aquatic and wetland plant identification manuals

###### GPS unit

###### Aerial photo of site (on which the sampler will sketch the wetland sampling area)

###### Hand lens

###### Binoculars

###### Compass

###### 2 100-m tape measure

###### 1 m PVC pipe, marked at 0.5 m

###### Plastic bags

###### Permanent marker

###### Cooler

###### Ice

###### Camera

###### Waders

###### Vegetation Wetland Condition Index Field Sheet (FD 9000-33) or other datasheet to capture documentation required in FD 53514– 4 datasheets per site

###### Completed Physical/Chemical Characterization Field Sheet (FD 9000-3) or other datasheet to capture documentation required in FD 5320

##### Methods

* 1. Establishing transects (herbaceous or forested):
		1. Using a compass, locate the 4 cardinal point directions (north, south, east, and west) with respect to the location of the wetland. The 4 transects will begin at each cardinal point on the boundary of the wetland and run into the interior of the wetland. These transects will intersect in the middle and divide the wetland into 4 approximately equal sections (Figure 1). Note the geographic coordinates (latitudes/longitudes) for the beginning and ending points of each transect.
		2. At the beginning of each transect, estimate the boundary of the wetland using principles of the Florida Unified Wetland Delineation Methodology (Chapter 62-340.300, F.A.C; NOTE: full delineation of wetland boundary is not necessary). Use a combination of the predominance of wetland plant species (according to their status assigned in the delineation methodology) and the presence of wetland hydrologic indicators (e.g. lichen lines, moss collars, wrack lines). After determining the approximate wetland boundary, sketch the location of the wetland boundary/sampling area and the transects on the aerial photo. Establish the transect using meter tapes. Start with the 0-meter mark at the wetland edge, and moving toward the wetland’s interior, mark quadrats every 5 m. Quadrats should be back-to-back, and 0.5 m wide on either side of the transect line, so each quadrat is 1 m X 5 m. Transect length may vary based on wetland size but should be 30-50 m long if possible.
		3. If adapting this method to larger wetlands, establish 50 m linear transects through representative plant communities (Figure FS 7330-1). Include multiple transects to incorporate the variability within the full site being characterized.

 Figure FS 7330-1. Schematic of transect locations for isolated wetlands

****

Figure FS 7330-2. Schematic of potential transect configuration when adapting this method to larger connected wetlands

##### Vegetation Sampling

##### Use a separate field sheet (or section) for each transect. On the Vegetation Wetland Condition Index Field Sheet, sampling quadrats are in columns and plant species are in rows.

##### Create continuous quadrats that are 0.5 m wide on either side of the transect line (1 m wide total) and 5 m long.

##### Use “x” to record all living vascular macrophyte species rooted within each quadrat. Use “D” to denote dominance of one species (note: dominance information is not used in metric calculations but is a useful observation).

##### Record plant names using full genus and species names. Give each unknown species a unique ID code using the transect location (ex. N-1) and place it in a bag with the same label. Tag each specimen with masking tape and its label. Collect the entire plant, including its inflorescence and roots, if possible. All plants shall be identified in the field or collected for expert identification. Collect any plants you cannot identify with complete confidence and place them on ice in a cooler for transport to the lab/office. After identification by an internal or external expert, carefully dispose of exotic plant taxa specimens to prevent the inadvertent and detrimental spread of those taxa. Repeat 3.3.2-3.3.4 for each of the four transects.

#####  After Visiting the Wetland:

##### Prepare unknown specimens for verification by an expert as needed.

##### Identify and record the correct names of the unknown specimens.

##### Calculate the Vegetation Wetland Condition Index score using LT 7612 for isolated herbaceous wetlands (e.g. depression marshes) or LT 7622 for isolated forested wetlands (e.g. cypress domes).

##### Recommended References for Plant Identification (references provided for informational purposes only)

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

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

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

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

##### 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.

## Benthic Macroinvertebrate Sampling

### Rapid Bioassessment (Biorecon) Method (See BRN 1100)

### Stream Condition Index (D-Frame Dip net) Sampling (See SCI 1100)

### Hester-Dendy Sampling for the Biological Integrity Criterion (Shannon-Weaver Diversity Index)

##### Introduction

##### Hester-Dendy samplers are artificial substrates which macroinvertebrates colonize over a 28-day period. They provide a means of comparing potential influence of water quality between two sites without the influence of habitat differences. They are typically used to collect data with which to calculate the Shannon-Weaver Diversity Index, for compliance with the biological integrity criterion (62-302.530(10), F.A.C.). (This rule reference is provided for information only and not needed for use of this SOP.) That criterion requires a comparison between a test and control site (e.g., downstream and upstream of a point source discharge) or data at a single site through time. Field samplers must ensure that conditions of flow, depth, salinity (if marine influenced), and other habitat parameters are as similar as possible between deployment sites.

##### 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 if HDs 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 HDs are to be floated.

###### Long cable ties to secure the HD samplers to the platform, if flow is sufficient to uncouple the HD samplers

###### Buoys

###### Stainless steel cable or nylon rope of appropriate strength

###### Nico-Press tool, two hammers with fasteners, or other cable fasteners

###### Large sealable bags or other secure containers to collect condos upon retrieval

###### Permanent marker

###### Cooler with ice

##### Methods

##### 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. If the study system is tidal, be sure that the natural salinity is comparable between the two sites. Knowledge of the system’s hydrologic regime is required to make sure samplers will not go dry during the 28-day incubation period. For example, if there is currently high water and you expect the water to drop one meter in the next few weeks, place the sampler so that it will still be completely inundated 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 Hester-Dendys may be used instead. For the HD floating platform method, attach three or four Hester-Dendys to the floating platform. 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) using 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).

##### 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. If the Nico-Press tool is unavailable, other screw-type fasteners may be used.

##### 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. Wade or use a boat; do not pull the block up from the shore. Without touching the HDs or any other substrate, place a dip net, bucket, or pan 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. If retrieving suspended or floating HDs, gently lift the rack and place each HD immediately in a bag or container.   Alternatively, for floating HDs or when HDs are anchored in shallow water, enclose one HD with a bag or container while still submerged, being careful not to jostle the others.  Gently lift the HD so the top of the bag or container breaks the water’s surface, carefully detach the HD, and then seal the bag.  Try not to lift the other HDs out of the water.  Repeat for each HD.  For either retrieval method, use a dipnet or other means to ensure that organisms are not lost.

##### Once out of the water, quickly place the 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 bag. If organisms are found in the dip net, randomly and sequentially place them in one of the bags. Fill the bag with ambient water (so that all the plates are wet), secure them (twirl three times and twist the ends), and place on ice. Bags should be pre-labeled with the station, sample date, and replicate number, using the permanent marker.

##### Sample Preservation and Handling

##### Bagged HDs shall immediately be placed in wet ice. Do not preserve samples with fixative until after organisms are scraped from the HD plates unless the HD samplers are not going to be reused. Chemical preservatives will poison the plates, preventing them from being used again.

##### Follow laboratory processing and quality control procedures outlined in LT 7710 and LQ 7400.

#####

### Core Sampling for Macroinvertebrates

##### Introduction

##### Use of coring devices is restricted to sampling 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 cm2 is sampled. Sample only the top few inches, where invertebrates would be captured. 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.

##### Equipment and Supplies

###### Coring device

###### U.S. No. 30 (approximately 600 µm) mesh box sieve (constructed of fiberglass-coated wood and U.S. No. 30 mesh screen) or dip net with U.S. No. 30 mesh sieve material

###### White pan

###### Plastic squeeze bulb

###### Small bucket

###### Wide-mouth plastic sample containers

###### Permanent marker

###### Buffered formalin or non-formalin-based fixative that penetrates and stabilizes tissue without compromising analytical capability (e.g. NOTOXhisto®)

###### Long pole (if needed)

###### Rose bengal dye (optional)

##### Methods

##### 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. No. 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).

##### Lower the coring device to the bottom with the valve open. After quickly pushing the device into the sediments, close the valve. If the core is equipped with a flapper valve, the value will automatically close. The resulting vacuum will keep the material in the tube as it is raised up to the boat.

##### 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 that the organism plus sediment mixture is completely emptied into the sieve.

##### Swirl the box sieve in the water with a back-and-forth motion to wash the fine sediments through the standard U.S. No.30 mesh screen. 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 your 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.

##### 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.

##### 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.

##### Sample Preservation and Handling

##### Preserve the sample with prepared 10% buffered formalin (see FS 7001, section 1) or by adding a 9 to 1 ratio of water to 100% formalin or use non-formalin-based fixative that penetrates and stabilizes tissue without compromising analytical capability (e.g. NOTOXhisto®). If laboratory processing is possible within twenty-four hours, the samples may be stored on ice, without addition of fixative. 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.

##### Follow laboratory processing outlined in LT 7720. Follow laboratory quality control procedures outlined in LQ 7400.

### Dredge Sampling

##### Introduction

##### Dredges are used to sample sediment in freshwater or marine environments. They may be used to collect macroinvertebrates for the purpose of calculating the Shannon-Weaver Diversity Index, for compliance with the biological integrity criterion (62-302.530(10), F.A.C.). (This rule reference is provided for information only and not needed for use of this SOP.) The biological integrity criterion requires a comparison between a test and control site or data at a single site through time. Field samplers must ensure that conditions of depth, salinity (if marine), and other habitat parameters are as similar between dredge sampling sites as possible.

##### Equipment and Supplies

###### Dredge sampler such as Ekman or Petite Ponar

###### Box sieve (constructed of fiberglass-coated wood and U.S. No.30 [approximately 600 µm] mesh screen), bucket sieve or dip net with U.S. No. 30 mesh sieve material

###### White pan

###### Plastic squeeze bulb

###### Small bucket

###### Wide-mouth plastic sample containers

###### Permanent marker

###### Buffered formalin or non-formalin-based fixative that penetrates and stabilizes tissue without compromising analytical capability (e.g. NOTOXhisto®)

###### Rose bengal dye (optional)

##### Methods

##### Use of the Ekman dredge is restricted to sampling soft substrates (silt, muck) in areas with little current. The Petite Ponar dredge may be used for sampling under these conditions and in areas with a harder substrate (small rocks, shell hash, 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 replicates. Place all replicates in separate sample containers (so that the results may be statistically analyzed separately). Depending on the study objectives, replicates may also be composited, as long as the number of replicates is equal for each site and clearly recorded so that the number of organisms per square meter can be calculated. If you are sampling in an exceptionally depauperate area, additional replicates may be required to assure that a minimum of 300 individuals are obtained in the total sample. In that case, the number of replicates must be equal at all sites to be comparable.

##### 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. No. 30 mesh sieve material.

##### Dredges

##### 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. A 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 cause 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.

##### Petite Ponar: Open the jaws and insert the spring-loaded peg through the hole in the cross bars. Lower the dredge to the bottom, making sure it settles flat. When tension is removed from the line, the spring-loaded peg will pop out, 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.

##### 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.

##### 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.

##### 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.

##### Sample Preservation and Handling

##### Preserve the sample with a 10% buffered formalin (see FS 7001, section 1) or by adding a 9 to 1 ratio of water to 100% formalin or use non-formalin-based fixative that penetrates and stabilizes tissue without compromising analytical capability (e.g. NOTOXhisto®). If laboratory processing is possible within twenty-four hours, the samples may be stored on ice, without addition of fixative. 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.

##### Follow laboratory processing outlined in LT 7720. Follow laboratory quality control procedures outlined in LQ 7400.

### Lake Condition Index (Lake Composite) Sampling

See also the following section:

###### FT 3000 Aquatic Habitat Characterization

##### Equipment and Supplies

###### Completed Physical/Chemical Characterization Field Sheet (FD 9000-3) or other datasheet to capture documentation required in FD 5311

###### Site map

###### Completed Lake Habitat Assessment Field Sheet (FD 9000-6)

###### Dredge sampler such as Ekman or Petite Ponar (with line marked in 0.1 m graduations)

###### Box sieve (constructed of fiberglass-coated wood and U.S. No. 30 mesh [approximately 600 µm] screen), bucket sieve or dip net with U.S. No. 30 mesh sieve material

###### White pan

###### Plastic squeeze bulb

###### Small bucket

###### Wide-mouth plastic sample containers

###### Tape and permanent markers

###### Buffered formalin or non-formalin-based fixative that penetrates and stabilizes tissue without compromising analytical capability (e.g. NOTOXhisto®)

###### Rose Bengal dye (optional)

###### Datasheet to capture documentation required in FD 5320

##### Methods

##### First, conduct lake habitat assessment according to FT 3200. If the lake has a color level exceeding 20 PCU, LCI invertebrate sampling is inappropriate, unless you are performing a time-series study in a lake for trend analysis.

##### Prior to visiting the lake, study a map of the system. For lakes less than 1,000 acres, logically divide the lake into 12 roughly equal sampling units. Collect one bottom grab at a depth between 2 m and 4 m from each of these 12 units. Composite the 12 dredges 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, at depths between 2 m and 4 m. 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.

##### Collect dredge samples from the rear and downstream of the boat 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. No. 30 mesh sieve material.

##### Dredges

##### 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 2 m and 4 m. Record the depth. 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. A 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 cause 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.

##### Petite Ponar: Open the jaws and insert the spring-loaded peg through the hole in the cross bars. Lower the dredge to the bottom, making sure it settles flat. Using the graduated line, check to make sure the depth is between 2 m and 4 m. Record the depth. When tension is removed from the line, the peg will pop out, 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. 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.

##### Examine the sediment and document its visual characteristics following FT 3002. 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.

##### 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.

##### 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.

##### 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).

##### Sample Preservation and Handling

##### Preserve the sample with a 10% buffered formalin (see FS 7001, section 1), or by adding a 10 to 1 ratio of water to 100% formalin or use non-formalin-based fixative that penetrates and stabilizes tissue without compromising analytical capability (e.g. NOTOXhisto®). If laboratory processing is possible within twenty-four hours, the samples may be stored on ice, without addition of fixative. 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.

##### Follow LQ 7400 and LT 7300 for laboratory and calculation protocols for the Lake Condition Index.

### Florida Wetland Condition Index Sampling: Macroinvertebrates

This method is based on “The Wetland Condition Index (WCI): Biological Indicators of Wetland Condition for Isolated Depressional Herbaceous Wetlands in Florida,” by C. R. Lane, M. T. Brown, M. Murray-Hudson, and M. B. Vivas, 2003, <https://publicfiles.dep.state.fl.us/Labs/lds/reports/15104.pdf> and “The Florida Wetland Condition Index (FWCI): Developing Biological Indicators for Isolated Depressional Forested Wetlands,” by K. C. Reiss and M. T. Brown, June 2005, [\\floridadep\data\DEAR\Labs\Lab5\EAS\Common\AEQA\1 QA\62-160\2022 Rule and SOP edits\SOPs\FS 7000\DEVELOPING BIOLOGICAL INDICATORS\_ISOLATED FORESTED (state.fl.us)](%5C%5C%5C%5Cfloridadep%5C%5Cdata%5C%5CDEAR%5C%5CLabs%5C%5CLab5%5C%5CEAS%5C%5CCommon%5C%5CAEQA%5C%5C1%20QA%5C%5C62-160%5C%5C2022%20Rule%20and%20SOP%20edits%5C%5CSOPs%5C%5CFS%207000%5C%5CDEVELOPING%20BIOLOGICAL%20INDICATORS_ISOLATED%20FORESTED%20%28state.fl.us%29)See also Plafkin, et al., 1989 Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish, EPA/444/4-89-001 and Barbour, et al., Rapid Bioassessment Protocol Manual, EPA/841/B-99-002 (references provided for informational purposes only and are not needed for this procedure).

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. See also the following SOPs:

###### FD 1000 Field Documentation

###### FT 3001 Physical/Chemical Characterization

###### FT 1000 General Field Testing and Measurement

* LT 7600 Wetland Condition Index Determination

##### Introduction

The Wetland Condition Index (WCI) was designed to be a rapid tool for assessing the ecological condition of wetlands by determining how closely a wetland’s biological community compares to biological communities observed in minimally disturbed or control wetlands. The Department may use the WCI in support of establishing site specific alternative water quality criteria under Rule 62-302.800, F.A.C., conducting a use attainability analysis, or establishing a new or revised designated use(s) under Rule 62-302.400, F.A.C.  (These rule references are provided for information only and are not needed for use of this SOP.) The WCI specifically applies only to isolated herbaceous and isolated forested wetlands, and therefore, this method may only be used in other types of wetland systems on a limited, experimental basis.  No numeric thresholds for interpreting the index have been established.  Therefore, application of the WCI should be limited to carefully designed studies that compare “test” wetland sites with similar, yet minimally disturbed “control” wetland sites to demonstrate that test site water quality is sufficient to support a healthy, well balanced community of flora and fauna.  In this capacity, the WCI may be used to support alternative water quality criteria development in wetlands.  Due to the current lack of quantitative expectations associated with the WCI, interpretation of results must be made on a site-specific basis.

##### Equipment and Supplies

###### D-frame dip net with U.S. No.30 (approximately 600 µm) 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, Section 1) or non-formalin based fixative that penetrates and stabilizes tissue without compromising analytical capability (e.g. NOTOXhisto®)

###### Brush

###### Permanent marker

###### Completed Physical/Chemical Characterization Field Sheet (FD 9000-3) or other datasheet to capture documentation required in FD 5311

###### Waders

##### Methods

##### Examine the wetland area to be sampled. Walk through the wetland, as is practical, paying close attention to its physical and habitat characteristics. Disturb the substrate and vegetation as little as possible when walking through the system, so as not to disturb aquatic habitats. Such disturbances could lead to inaccurate macroinvertebrate index results. If sampling is done in conjunction with the vegetation index sampling, do not sample in areas along transects or other areas potentially trampled by samplers. Do not sample the wetland during flood conditions, or if the wetland has recently been dry, or soil has been saturated but not inundated. If flooding has occurred, it is important that the sampler be confident that the “reachable” habitat has been inundated for a minimum of 28 days. In other words, if the water has risen quickly and stays up, the water may be too deep for the sampler to reach habitat that is colonized by organisms. The 28 days gives the organisms time to colonize the newly inundated habitat. It is common for wetlands in Florida to have fluctuating hydroperiods and to go dry seasonally. If a wetland has been dry or soil has been saturated but not inundated, wait a minimum of 3 months after inundation has occurred to attempt sampling.

Determine major vegetation zones based on a quick assessment of the dominant wetland plant species (e.g. for an isolated herbaceous wetland: a fringing *Panicum hemitomon* zone, a deeper *Pontederia cordata* zone). For some isolated forested wetlands, there may be only one zone (e.g. *Taxodium ascendens*), although some of these systems will have multiple zones.

##### Complete portions of the Physical/Chemical Characterization relevant to wetlands per FT 3001.

Perform 20 discrete sweeps with the D-frame dip net. Each sweep shall be 0.3 m (the width of the dip net) X 0.5 m. Evenly apportion the number of dip net sweeps among the vegetation zones. If there are two zones of approximately equal area, perform 10 sweeps in each. If there are two zones and one covers approximately 75% of the wetland and the other covers approximately 25%, perform 15 sweeps in the larger zone and 5 sweeps in the smaller zone.

Because these wetland systems typically have little to no flow, disturb an area of substrate 0.3 m wide X 0.5 m long, and create flow into the net to ensure the capture of organisms living there. Three or more passes over the same 0.5 m area are required to make sure all organisms are captured. This sampling effort is considered one sweep.

For areas with herbaceous vegetation, place the net on the substrate at the base of the vegetation and dislodge organisms using your hand or a 0.5 m sweeping motion with the net, moving up the stalks of the plants. For areas with woody vegetation, place the net on the substrate at the base of the trunk and move up the trunk, using a brush to dislodge organisms from the trunk and from any woody debris (snags or roots) into the net. Where a continuous 0.5 m sweep is impossible, take two 0.3m sweeps of the same habitat, or the number necessary to attain a full 0.5 m sweep. Do not sample muck.

Record the number of sweeps for each vegetation zone.

##### Sample Handling and Preservation

##### If necessary, 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 the finer debris plus organism mixture in large, wide-mouth jugs. Make every effort to reduce the sample volume in the field so that no more than four liters of material are collected. If this is impossible, 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 community metrics. Indicate on the label in how many jugs the entire sample is contained, e.g. “1 of 2, “2 of 2.”

##### Preserve the sample jugs with 10% buffered formalin. Do this by adding one part of 100% buffered formalin to each jug with nine parts ambient water or filling each jug with a 10% buffered formalin. Or use non-formalin based fixative that penetrates and stabilizes tissue without compromising analytical capability (e.g. NOTOXhisto®).

##### If samples are immediately iced and can be sorted within 24 hours, the use of fixative is optional. Preserve iced samples that will not be sorted within 24 hours per section 4.2 above.

##### Calculate the Macroinvertebrate Florida Wetland Condition Index per LT 7613 for isolated herbaceous wetlands and LT 7623 for isolated forested wetlands.