Illinois Environmental Protection Agency

Bureau of Water

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Standard Operating Procedure for Method to Collect Aquatic Macroinvertebrates from Wadeable Streams for Biotic Integrity Assessments

Surface Water Section

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1.0 Scope and Application

The instructions outlined in this Standard Operating Procedure (SOP) describe how to collect and preserve aquatic macroinvertebrate samples from perennial streams and rivers using a quantitative multiple-habitat, 20-jab method (see below). Use these samples to calculate an index of biotic integrity (IBI) and assess the physical, chemical and biological condition of the resource. Collect samples between June 1st and October 15th. These protocols apply to perennial streams and wadeable rivers that typically range in size from 2nd through 6th order. In some situations, these protocols may apply to intermittent streams that meet specific base flow criterion (see below in Section 6.1). Use the macroinvertebrate Index of Biotic Integrity (mIBI) to evaluate 20-jab samples.

Aquatic macroinvertebrates are invertebrates that are visible to the unaided eye, retained in a U.S. Standard No. 30 sieve (595-micron mesh size) and live at least a portion of their lives in the water. Refer to Appendix A for the list of IBI-applicable aquatic macroinvertebrates.

The framework outlined in this SOP is a modification of the "*Multihabitat Approach: D-frame dip-net*" collection methodology that was outlined in EPA, (1999). The modifications consist of additional dip allocation criteria, which improve dip allocation consistency when collecting macroinvertebrates. Hereafter, refer to this modified macroinvertebrate collection method as, the "20-jab" approach.

2.0 Summary of Method(s).

- Collect aquatic macroinvertebrate samples from perennial, 2rd through 6th order wadeable streams.
- Collect aquatic macroinvertebrate samples between June 1st and October 15th.
- Collect the sample with 20- dip-net jabs.
- Use a 600-micron mesh, 18x9-inch rectangular-frame, dip-net.
- Clean equipment between each sample.
- Sample an 18x18-inch area of habitat with each dip-net jab.
- Allocate 20 jabs in the sample reach.
- Use the mean water width of the sample reach to determine how to allocate the 20 jabs among the edge-zone and bottom-zone (*e.g., $\bar{x}$ stream width = bank-zone jabs: <10ft=10, 10-29ft=8, 30-59ft=6 and ≥60ft=4*). See Table 1, in Appendix B.
- Establish the relative amounts of each of the pertinent habitat types within the bottom- zone and bank-zone.
- Bottom zone habitats are: *coarse particle substrate, fine particle substrate, vegetation and plant detritus.* See Table 1, in Appendix B.
- Edge zone habitats are: *submerged terrestrial vegetation, submerged tree roots and brush-debris-jams.* See Table 1, in Appendix B.
- Distribute the bank-zone and bottom-zone jab allotments proportionally among the pertinent habitat types within each of the two respective zones (see Equation 1 and Section 6.2.4).
- Summarize the number of jabs allocated to each habitat in the sample reach.
- Perform 20 jabs.
- Use the jab allocation summary and perform the allocated number of jabs in each of the pertinent habitat types.
- Take jabs in the more productive areas of each habitat type. More productive areas typically occur where current velocity is relatively high and the substrate is relatively stable.
- Distribute multiple jabs within more productive areas, of a particular habitat type, equally along the entire length of the sample reach when possible.

- Composite the collected materials into a 600-micron sieve bucket or a suitably sized watertight plastic tub after each jab is completed.
- Sieve the collected materials to remove excess water (as needed). As needed, discard excess debris after removing all clinging macroinvertebrates- retain these macroinvertebrates in the sample.
- Preserve the sample with 95% ethanol and return it to the lab for subsequent sub-sampling and identification.

3.0 Interferences and Corrective Action.

- Macroinvertebrate communities vary seasonally. Collect aquatic macroinvertebrate samples between June 1st and October 15th.
- Specimens left on the equipment between samples can give false results. Remove all specimens from the equipment before subsequent samples are collected.
- Improper sample preservation makes specimen identification more difficult. Take care to use large enough containers and adequate amounts of 95% ethanol during the initial preservation of the sample. Safeguard preserved samples that contain a relatively large amount of organic matter by decanting the original, spent, preservative and refilling the container using fresh 95% ethanol - perform this maintenance within 1-week of sample collection. Repeat as needed to maintain the sample preservative.

4.0 Safety.

Follow the general field-safety guidelines in the Illinois EPA, Bureau of Water's Surface Water Section, Field Safety Manual (Document Control Number 151) (Illinois EPA, 1994).

5.0 Equipment and Supplies.

- Nitex bottom kick net with an 18x9-inch rectangular opening and a 600-micron mesh size.
- Sieve bucket with a 600-micron stainless steel mesh bottom.
- Plastic rinse pan
- Forceps
- Sample bottles
- 95% ethanol

6.0 General Guidelines
Collect Macroinvertebrate Samples with the 20-jab Method.

6.1 Select a sampling reach that:

- has instream and riparian habitat conditions typical of the entire assessment reach,
- has flow conditions that approximate typical summer base flow,
- has perennial flow, or if intermittent, has continuous width and depth that extends throughout the sampling reach, which should be at least 300 feet. The length of the sample reach should not exceed 800 feet.
- has no highly influential tributary streams,
- has at least one riffle/pool sequence or analog (i.e., run/bend meander or alternate point-bar sequence), if present.,

6.1.1 The 20-jab approach is applicable if:

- Conditions allow the sampler to collect macroinvertebrates (i.e., to take jabs with a dip-net) in all pertinent bottom-zone and bank-zone habitat types that occur in the sampling reach.

AND

- Conditions allow the sampler to apply the 11-transect habitat-characterization, as described in Section E of this manual or to estimate the amount of each of several bottom-zone and bank-zone habitat types using the non-transect method (Appendix B).
- In general, the 20-jab approach applies if more than 50% of the stream is accessible and adequately characterized.

- Between each sample, pick equipment clean of macroinvertebrates and debris. Rinse all sampling equipment in ambient water before collecting the sample.

6.2 Jab allotment (sample reach)

6.2.1 Determine jab allotment for the bottom-zone and bank-zone.

- Follow the instructions provided in *Appendix B* to identify bottom-zone and bank-zone jab allotments. Specifically, use Table 1 (Appendix B) and mean water width of the sample reach to determine the number of jabs allocated to the bottom-zone and bank-zone. Essentially, allocate fewer jabs along the edge as stream size increases. Depending on stream size, jab allotment will range from 4-10 jabs in the bank-zone and from 10-16 jabs in the bottom-zone.

6.2.2 Identify the type and amount of bank-zone and bottom-zone habitat.

- Identify the relative amount of pertinent habitat type in each of the respective bank-zone and bottom-zone. Follow the instructions provided in *Appendix B* to identify the presence and relative proportion of pertinent habitat in the sample reach. Specifically, use the appropriate habitat characterization method, 11-transect versus non-transect method, in Appendix B., to determine the relative proportion of pertinent habitat in each collection-zone. Pertinent bottom-zone habitat types are *fine substrate*, *coarse substrate, detritus and vegetation (Table 1, in Appendix B)*. Pertinent bank-zone habitat types are *submerged terrestrial vegetation, submerged tree roots and brush-debris jams (Table 1, in Appendix B)*.

6.2.3 Summarize and record the relative amount of each of the pertinent habitat types in the collection-zones.

6.2.4 Determine jab allotments for each of the various habitat types within the bottom-zone and bank-zone.

- Take the jab allocation assigned to a particular collection-zone (Section 6.2.3) and in turn, redistribute this allotment of jabs proportionally among the various habitat types within the respective zone (Section 6.2.4). Essentially, allocate more jabs to the more frequently encountered habitat types.
- Based on the definition of each bottom-zone habitat type (Table 1 in Appendix B) and Equation 1 (see below), translate the relative percentage of the given habitat type (Section 6.2.3) to the number of jabs allocated to *fine substrate, coarse substrate, detritus and vegetation*. Similarly, translate the relative percentage of the given bank-zone habitat type to the number of jabs allocated to *submerged terrestrial vegetation, submerged tree roots* and *brush-debris jams*.

- If rounding results in more than 20 jabs for the total allocation across all habitat types, *decrease* the number of jabs allocated to the most-abundant habitat type to limit the total to 20.

- If rounding results in less than 20 jabs for the total allocation across all habitat types, *increase* the number of jabs allocated to the most-abundant habitat type to expand the total to 20.

Number of jabs to perform in a particular habitat type within a particular bank-zone or bottom-zone =				
Percentage or length of pertinent habitat type (Table 1 in Appendix B)	÷	Sum of percentages or lengths of all pertinent habitat types	x	Number of collection zone jabs allocated (Table 1 in Appendix B)

6.2.5 Summarize jab allocation

- Within each of the two collection-zones (bank-zone and bottom-zone), summarize the jabs allocated among each pertinent habitat type. For each zone, if the relative percentage of a habitat type is less than 5%, do not allocate jabs to that type. When transforming relative amounts of habitat types into numbers of jabs, round to the nearest whole number (Equation 1).
- In certain circumstances, rounding to the 20 jab target is allowable (see section 6.2.4).
- Occasionally a stream reach will lack bank-zone habitat. In this instance, allocate all 20 jabs to the bottom-zone habitats.

6.2.6 Record the number of jabs allocated to each bottom-zone and bank-zone habitat type.

6.3 Perform 20 jabs.

6.3.1 General guidelines for jabs:

- Collect macroinvertebrates with 20, dip-net jabs, according to the computed jab allotment summary obtained in Section 6.2.6.
- Use an 18x9-inch rectangular dip-net with a Standard #30 (600-micron) mesh size.
- One person performs all jabs taken within both collection zones (bank-zone or bottom-zone).
- For each of the given habitat types, take jabs in the more productive, areas. More productive areas generally occur where current velocity is relatively high. To minimize the potential for sampling bias attributable to uneven spatial distribution of macroinvertebrates throughout an entire sampling reach, distribute multiple jabs in more productive, areas of a particular habitat type as evenly as possible throughout the sampling reach-- providing the particular habitat type and multiple productive areas within the particular habitat type occur throughout the sample reach. In each of the two collection-zones, bank versus bottom, if there is not enough sampling area to allow performing all of the jabs allocated to a particular habitat type in the given zone, then, perform the remaining jabs among the remaining habitat

types in the given zone. Allocate these remaining jabs in proportions as close as possible to the original allotments. If the bank-zone lacks bank-zone habitat, re-allocate all 20 jabs proportionally among bottom-zone habitats.

- Between jabs (as needed), composite the dip-net contents into a 600-micron sieve bucket or large plastic tub. These containers facilitate debris removal and provide convenient storage when compositing the materials collected with the 20 jabs prior to sample preservation.

- Debris removal (e.g., coarse-particle substrates) greatly facilitates laboratory sub-sampling and sorting of the preserved sample. To remove large objects from the sample, place the material in a dip net, sieve bucked or watertight tub and vigorously agitate, rinse, brush, or pick the objects (as needed) to remove attached organisms. Discard the debris after all macroinvertebrates are removed. Retain all detached macroinvertebrates and return them to the sieve bucket. To remove ultra-fine particles (e.g., sitl), rinse the sample in a sieve bucked until the unwanted particles are washed away. Be careful to not splash out any organisms. Remove sand by transferring the collected materials from the dip-net into a shallow plastic pan. Add a small amount of water to the pan (e.g., ~1-inch deep) and swirl the contents being careful to not splash out any organisms. Pour the water and dislodged organisms into the sieve bucket while retaining as much of the sand as possible in the rinse pan.
- After rinsing, drain, bottle and preserve the sample.

6.3.2 Specific instructions:

- To perform a jab, place the net immediately downstream from the targeted bottom-zone or bank-zone habitat type and dislodge macroinvertebrates by disturbing an 18x18-inch area of substrate. At higher water velocities, these activities will flush dislodged substrate and macroinvertebrates directly into the stationary dip-net. At lower velocities, the same activity becomes ineffective and an additional step is required. Immediately after the upper layer of an 18x18-inch patch of substrate is collected, direct several net sweeps through the plume of materials that forms and is momentarily-suspended directly above the just-sampled patch of streambed. If possible, sweep in an upstream direction.

- When large, coarse-particle substrates (e.g., cobble and boulders) are sampled, wash, brush, or pick surface-clinging organisms from each object. Retain all of the dislodged organisms and discard the cleaned object. As needed, use a dip-net, sieve bucket or watertight tub to composite the materials collected with each jab(s). Use the sieve bucket or dip-net to remove excess water or fine particle materials from the sample.

- When fine-particle streambed substrates (e.g., silt/mud, sand) are sampled, disturb and collect bottom materials from the upper 1-inch of streambed in an 18x18-inch patch by repeatedly bumping the leading edge of the dip-net along the streambed surface until the entire sample area is disturbed. Immediately after the upper layer of material from the 18x18-inch patch is collected, complete the jab by repeatedly sweeping the net upstream through the plume of materials that becomes dislodged and momentarily-suspended over the disturbed area of streambed. Before preserving the sample, remove excess fines and sediment (see above).

- Large, pieces of wood are sampled if they occupy an 18x18-inch sample area AND if their dimensions allow fitting these objects into the dip-net AND they have microbial conditioning. When sampling large woody-debris, wash, brush, or pick surface-clinging macroinvertebrates from the collected materials and retain these organisms in the dip net. After all macroinvertebrates are retained, discard the woody debris.

7.0 Sample Preservation.

After completing 20-jabs, sieve and transfer the collected materials to an appropriate leak-proof jar(s). Label the container and preserve the sample with 95% ethanol by immersing the sample material. If a sample contains large amounts of organic debris, check for sufficient preservation within five days (or sooner) of initial "fixing". As needed, decant the spent preservative and add more 95% ethanol to ensure continued sample preservation. Thereafter, periodically check and re-preserve the sample as needed.

8.0 Literature Cited

EPA. 1999. Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers Periphyton, Benthic Macroinvertebrates, and Fish Second Edition. EPA 841-B-99-002. United States Environmental Protection Agency. Office of Water 4503F Washington, DC. 20460.

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