# Appendix E:

# Methods of Collecting Macroinvertebrates in Streams

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## A. Methods of collecting stream macroinvertebrates for determining biological integrity

### A-1. General instructions

#### A-1.1 Collect macroinvertebrates during June 1 through October 15.

#### A-1.2 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 no highly influential tributary streams,
- contains one riffle/pool sequence or analog (i.e., run/bend meander or alternate point-bar sequence), if present, **AND**,

where the multi-habitat method is applicable (see below),

- is at least 300 feet long

#### A-1.3 Determine applicability of the multi-habitat method.

**The multi-habitat method is applicable if :**

- Conditions allow the sampler to collect macroinvertebrates (i.e., to take dips with a dipnet) in **all** bottom-zone and bank-zone habitat types that occur in the sampling reach. These habitat types are defined explicitly later in this document.

  **AND**

- Conditions allow the sampler to apply the 11-transect habitat-sampling method, as described in *"Wadable Streams Transect Approach"* in *Appendix 1, Section E: Stream Habitat and Discharge Monitoring*, in *Quality Assurance Project Plan* (Illinois EPA 1994) or to estimate with reasonable accuracy--via visual or tactile cues--the amount of each of several bottom-zone and bank-zone habitat types. If conditions (e.g., inaccessibility, water turbidity, or excessive water depths) prohibit the sampler from estimating with reasonable accuracy the composition of the bottom zone or bank zone throughout the entire sampling reach, then the multi-habitat method is not applicable. In most cases, if more than half of the wetted stream channel cannot be seen, touched, or otherwise reliably characterized by the sampler, it is unlikely that reasonably accurate estimates of the bottom-zone and bank-zone habitat types are attainable; thus, the multi-habitat method is not applicable.

### A-2. The multi-habitat method of collecting stream macroinvertebrates

The multi-habitat method for sampling stream macroinvertebrates (hereafter called the "multi-habitat method") provides information useful for determining the biological integrity of a stream, as reflected in selected attributes of the macroinvertebrate assemblage living in the stream. These biological attributes represent how macroinvertebrates respond to and integrate the chemical, physical, and biological effects of human-induced impacts (both negative and positive) on streams and their watersheds, e.g., point- or nonpoint-source impacts, stream-restoration efforts. The multi-habitat approach allocates sampling effort based on the relative amounts of several predefined macroinvertebrate habitat types that occur in the sampling reach.

## A-2.1 Identify several predefined macroinvertebrate-habitat types (listed below) based on conditions at the time of macroinvertebrate sampling. Determine the amount of each habitat type in the sampling reach:

**Bottom-zone habitat types (four types):**

- Fine substrate: streambed surface predominantly comprising silt/mud to fine gravel (i.e., particles <0.3 inches in diameter of intermediate dimension)

- Coarse substrate: streambed surface predominantly comprising medium gravel to boulder (i.e., particles >0.3 inches in diameter of intermediate dimension)

- Plant detritus: streambed surface predominantly comprising nonliving plant material (e.g., leaves, twigs)

- Vegetation: streambed surface predominantly comprising living plant material (e.g., aquatic macrophytes, filamentous algae, submerged terrestrial plants)

**Bank-zone habitat types (three types):**

- Submerged terrestrial vegetation: living, terrestrial plants (along stream banks) of which submerged portions provide cover or attachment sites for macroinvertebrates

- Submerged tree roots: living tree roots (along stream banks) of which submerged portions provide cover or attachment sites for macroinvertebrates.

- Brush-debris jams: non-living, submerged, woody material (e.g., branches, twigs, or smaller logs) that occurs above the streambed surface and that appear to have microbial conditioning. Excludes recent deadfall that lacks microbial conditioning.

### A-2.1.1 For qualified, trained personnel having fewer than 2 years of experience in measuring and characterizing instream physical habitat (including stream-bottom composition) for purposes of natural-resource management, use the 11-transect habitat method to determine the amount of each habitat type:

- When applicable, measure and estimate habitat conditions by applying the appropriate parts of the 11-transect habitat method as described in *"Wadable Streams Transect Approach"* in *Appendix 1, Section E: Stream Habitat and Discharge Monitoring,* in *Quality Assurance Project Plan* (Illinois EPA, 1994). Specifically, use the 11-transect method to identify the "substrate" (see below) or "bottom type" (see below) at each of many points distributed regularly on the wetted stream bottom throughout the entire sampling reach. Also, per each of ten segments in the sampling reach, visually estimate the length of space occupied by each of the "instream cover type"s.

**Substrates:**

| Name | Particle-Size Range |
|---|---|
| Silt/mud | < 0.062 mm |
| Sand | 0.062 – 2 mm |
| Fine gravel | 2 – 8 mm |
| Medium grave l | 8 – 16 mm |
| Coarse gravel | 16 – 64 mm |
| Small cobble | 64 – 128 mm |
| Large cobble | 128 – 256 mm |
| Boulder | 256 – 4000 mm |
| Bedrock | > 4000 mm |

**Instream Cover Types**:

Submerged terrestrial vegetation
Submerged tree roots
Brush-debris jam
Boulder (not embedded)
Undercut bank
Rock/clay ledge
Log
Aquatic vegetation
Other (please specify)

**Bottom Types:**

Claypan/Compacted soil
Plant detritus
Vegetation
Submerged log
Other (please specify)

- Based on the definition of each bottom-zone habitat type (see section A-2.1), translate each of the observations of "substrate" and "bottom type" into the appropriate bottom-zone habitat type and calculate and record the relative percentage of each bottom-zone habitat type in the sampling reach as:

*Relative percentage of each bottom-zone habitat type =*

| *Sum of the points (from all transects) at which the bottom-zone habitat type occurred* | ÷ | *Sum of the points (from all transects) at which any of the four bottom-zone habitat types occurred* | **x 100** |
|---|---|---|---|

- When using the 11-transect habitat method, spatial coverage of each bank-zone habitat type is visually estimated within each of the ten stream segments delineated by the eleven transects. Estimate and record *Submerged terrestrial vegetation* and *Submerged tree roots* as the length of bank covered by each habitat type in the sampling reach. Estimate and record the amount of *Brush-debris jams* in the sampling reach. Estimate the single longest dimension covered by each brush-debris jam and then sum these lengths to yield the total length of *Brush-debris jams*. Consider all brush-debris jams as bank-zone habitat, regardless of occurrence within the assumed bank zone (see Table 1)—provided that the brush-debris jam occurs at a depth and water velocity that allow safe and sufficient sampling of macroinvertebrates with a dipnet.

- If water turbidity or excessive depth prevents seeing the entire wetted stream channel throughout the sampling reach, the sampler may use tactile cues or knowledge of the channel morphology and streambed to obtain a reasonably accurate estimate of the amount of each bottom-zone and each bank-zone habitat type. However, in most cases, if more than half of the wetted stream channel cannot be seen, touched, or otherwise reliably characterized by the sampler, it is unlikely that reasonably accurate estimates of these amounts are attainable; thus, the multi-habitat method is not applicable.

### A-2.1.2 For qualified, trained personnel having 2 or more years of experience in measuring and characterizing instream physical habitat (including stream-bottom composition) for purposes of natural-resource management, visually estimate the amount of each habitat type:

- Wade, walk the stream banks, or float (via boat or canoe) the sampling reach and visually estimate and record the percent surface area of the wetted stream bottom that consists of each of the four bottom-zone habitat types. Similarly, visually estimate and record the length of space occupied by each of the three bank-zone habitat types. For estimating *Submerged terrestrial vegetation* and *Submerged tree roots*, use the length of bank covered by each. For estimating *Brush-debris jams*, estimate the single longest dimension of each brush-debris jam and then sum these lengths to yield the total length of *Brush-debris jams* in the sampling reach. Consider all brush-debris jams as bank-zone habitat, regardless of occurrence within the assumed bank zone (see Table 1)—provided that the brush-debris jam occurs at a depth and water velocity that allow safe and sufficient sampling of macroinvertebrates with a dipnet.

- If water turbidity or excessive depth prevents seeing the entire wetted stream channel throughout the sampling reach, the sampler may use tactile cues or knowledge of the channel morphology and streambed to obtain a reasonably accurate estimate of the amount of each bottom-zone and each bank-zone habitat type. However, in most cases, if more than half of the wetted stream channel cannot be seen, touched, or otherwise reliably characterized by the sampler, it is unlikely that reasonably accurate estimates of these amounts are attainable; thus, the multi-habitat method is not applicable.

## A-2.2 Allocate effort for the multi-habitat method:

- Allocate 20 dips of effort to the bank zone and bottom zone. Based on mean wetted width of the sampling reach, determine the number of dips to perform in the each zone by consulting Table 1. If the 11-transect habitat method was not used, calculate mean wetted width based on measurement of the wetted width of at least three transects judged to reflect best the wetted width of the entire sampling reach.

- For sampling within the bank zone or within the bottom zone, further allocate dips based on the relative amounts of each corresponding habitat type (from sections A-2.1.1 and A-2.1.2). For each habitat type in each zone (bottom vs. bank), transform the relative amount into the number of dips to perform as follows:

  *Number of dips to perform in a particular bottom-zone or bank-zone habitat type =*

$$\boxed{\frac{\textit{Percentage or length of habitat type}}{\textit{Sum of percentages or lengths of all habitat types}}} \times \textit{Number of dips allocated (from Table 1)}$$

  For each zone, if the relative proportion of a habitat type is less than 0.05, do not allocate dips to that type. When transforming relative amounts of habitat types into numbers of dips, round to the nearest whole number. If rounding results in more than 20 dips for the total allocation across all habitat types, decrease the number of dips allocated to the most-abundant habitat type to limit the total to 20. Record the number of dips allocated to each bottom-zone habitat type and each bank-zone habitat type.

For example, for a stream having a mean wetted width of 37 feet, 14 dips are required from bottom-zone habitats and 6 dips are required from bank-zone habitats. Suppose the percent surface areas of the four bottom-zone habitat types are 48% *Fine substrate*, 22% *Coarse substrate*, 7% *Plant detritus*, and 12% *Vegetation* (please note that these percentages do not necessarily have to sum to 100% because the stream bottom may consist of other materials). Based on these amounts, the 14 bottom-zone dips should be allocated as:
8 dips in *Fine substrate* ( $[48 \div [48 + 22 + 7 + 12]] \times 14 = 7.55 \cong 8$ ), 3 dips in *Coarse substrate*, 1 dip in *Plant detritus*, and 2 dips in *Vegetation*.
Suppose the lengths of the three bank-zone habitat types are 5 ft. of *Submerged terrestrial vegetation*, 100 ft. of *Submerged tree roots*, and 50 ft. of *Brush-debris jams*. Based on these amounts, the 6 bank-zone dips should be allocated as: 4 dips in S*ubmerged tree roots* and 2 dips in *Brush-debris jams*. An insufficient relative amount (i.e., $(5)/(5+100+50) = 0.032$, which is $\leq 0.05$) of *Submerged terrestrial vegetation* occurs to allocate even a single dip.

**Table 1. Bank-zone and bottom-zone sampling-effort allocation.**

| Mean wetted width (to nearest foot) | Assumed width of bank-zone | Bank-zone dips | Bottom-zone dips |
|---|---|---|---|
| < 10 ft | 25% of wetted width per bank | 10 | 10 |
| 10-29 ft | 20% of wetted width per bank | 8 | 12 |
| 30-59 ft | 15% of wetted width per bank | 6 | 14 |
| 60-99 ft | 10% of wetted width per bank | 4 | 16 |
| ≥100 ft | 5% of wetted width per bank | 2 | 18 |

## A-2.3 Perform the 20 dips.

### A-2.3.1 General guidelines:

- One person performs all 20 dips.

- Take dips in the most-productive, stable areas represented by each habitat type. Most-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 stream reach, distribute multiple dips in (most-productive, stable areas of) each habitat type as evenly as possible throughout the sampling reach. [[ <u>Should we address situations in which not enough sampling space is available in the reach to accommodate the complete number of allocated dips ? How often does this occur? ]]</u>

### A-2.3.2 Specific instructions:

- Use an 18x8-inch rectangular net with a Standard #30 (600-micron) mesh net.

- To perform a dip, place the net immediately downstream from the target area of the appropriate bottom-zone or bank-zone habitat type and dislodge macroinvertebrates by disturbing an 18x18-inch area. At higher water velocities, dislodged macroinvertebrates will be flushed directly into the stationary net. At lower velocities, capture dislodged macroinvertebrates by repeatedly sweeping the net directly above or adjacent to the 18x18-inch disturbed area. Always sweep in an upstream direction.

- When sampling fine-particle streambed substrates (e.g., silt/mud, sand), disturb the upper 1-inch of streambed in an 18x18-inch area by repeatedly bumping the leading edge of the net along the streambed surface. Complete the dip by capturing macroinvertebrates that are suspended over the sampling area by repeatedly sweeping upstream through the water column.

- Large pieces of wood or boulders may be sampled if they occupy the 18x18-inch sampling area **AND** if their dimensions would allow fitting these objects into the dipnet. When sampling these objects, wash, brush, or pick surface-clinging organisms into the dipnet and include them as part of the sample; do not retain the object in the sample.

- Between dips (as needed), combine the dipnet contents into a standard #30 (600-micron) sieve bucket (i.e., sample container). Before transferring dipnet contents to the sample container, excess debris or sediment may be removed only after first retaining all attached organisms. Removal of excess debris and sediment at this step greatly facilitates laboratory subsampling and sorting of the preserved sample. If necessary, transfer dipnet contents to a different sieve bucket or other suitable container and vigorously agitate, rinse, brush, or pick (as needed) to remove organisms from the debris; discard the debris only after ensuring that organisms have been removed. After "rinsing" and removing debris, transfer contents to the sample container.

- After performing all 20 dips and combining the contents into the sample container, transfer sample-container contents to an appropriate leak-proof jar(s) and preserve it with 95% ethanol. Label the container appropriately. If a sample contains large amounts of organic debris, check for sufficient preservation within five days (or sooner) of initial “fixing”; decant old fluid and add more 95% ethanol as needed to ensure sufficient preservation. Thereafter, periodically check the sample and re-preserve as needed.

- If the 11-transect habitat method was used, record from which of the ten stream segments each dip was taken.

# B. Methods for Collecting Stream Macroinvertebrates for Determining Impacts of a Point-Source Discharge (i.e., Facility-Related Stream Survey)

**[[pending]]**

**[[SOP for laboratory processing and subsampling of macroinvertebrate samples is needed here]]**