

Standard Operating Procedure #7:

Collection of Benthic Macroinvertebrates

Version 1.0

In Monitoring Protocol for Wadeable Streams of the Great Lakes Network

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December 2017

Please cite this publication as:

Elias, J. E., R. Damstra, D. D. VanderMeulen, and L. Kainulainen. 2017. Standard operating procedure #7: Collection of benthic macroinvertebrates. *In* D. D. VanderMeulen, J. E. Elias, S. Magdalene, R. Damstra, and J. Glase. 2017. Monitoring protocol for wadeable streams of the Great Lakes Network, version 1.0. Natural Resource Report NPS/GLKN/NRR—2017/1567. National Park Service, Fort Collins, Colorado.

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Revision History Log

The following table lists all edits and amendments to this document since the original publication date. Information entered in the log must be complete and concise. Users of this standard operating procedure will promptly notify the project manager and/or the Great Lakes Network (GLKN) data manager about recommended and required changes. The project manager must review and incorporate all changes, complete the revision history log, and change the date and version number on the title page and in the header of the document file. For complete instructions, please refer to Procedure for Revising the Protocol, SOP #16.

Previous Version #	Revision Date	Author (with title and affiliation)	Location in Document and Concise Description of Revision	Reason for Change	New Version #
Add rows as needed for each change or set of changes tied to an updated version number					

Acknowledgements

This standard operating procedure is based on the methods used by the Wisconsin Department of Natural Resources (WDNR 2006), the U.S. Environmental Protection Agency (Plafkin et al. 1989; Barbour et al. 1999), Dinger et al. (2013), and Bowles et al. (2008.)

7.0 Introduction

Benthic macroinvertebrates have been used as biotic indices of stream quality for many decades. The State of Wisconsin, for example, began using the Hilsenhoff Biotic Index (Hilsenhoff 1977) in the 1970s, and since then has incorporated numerous refinements and improvements over the original index (Lillie et al. 2003). When the USEPA developed a rapid bioassessment protocol for streams it also included benthic macroinvertebrates as an important index of stream quality (Plafkin et al. 1989; Barbour et al. 1999).

Most aquatic macroinvertebrates live for a year or more as immature stages in streams. Because of their relatively limited mobility, they serve as good integrators of water conditions in their localized area of the stream. Indices calculated from macroinvertebrate species assemblages yield information on a variety of potential stressors such as low dissolved oxygen; pollution from toxics, contaminants, heavy metals, and organics; changes in substrate embeddedness; disruption of flow; and temperature extremes (Lillie et al. 2003). In general, as the environmental quality of a stream decreases, the numbers of environmentally tolerant macroinvertebrate species increases and the number of intolerant species decreases (WDNR 2006).

The objective of monitoring macroinvertebrates is to assess current stream conditions and analyze for change over time. Quantification of metrics related to species richness, abundance, diversity will provide indicators of water quality and stream habitat condition.

This standard operating procedure describes the methods for collecting and preserving benthic macroinvertebrate samples from wadeable streams.

7.1 Timing of Sampling

Because some indices vary by season, with more tolerant species occurring in mid-summer (Hilsenhoff 1988), it is important that macroinvertebrate samples be collected at a consistent time of year, based on factors other than the calendar. For simplicity, all sampling will take place in July with actual sampling dates determined by the project and field crew leaders. Sampling during this time period is advantageous because benthic macroinvertebrates reach their maximum representation or diversity, macroinvertebrate indices should fall within the 95% confidence limits (Hilsenhoff biotic index; Hilsenhoff 1988), and the in-stream environment typically has stabilized following high spring flows.

Once a sampling date is established, subsequent samples of that reach should be timed to be within $365 \text{ d} \pm 7 \text{ d}$, if possible.

7.1.1 Sequence of Events

The general sequence of events should be as follows:

1. Prepare sample bottles and labels in advance.

2. Review checklist of field gear.
3. Prepare new data sheets for each monitoring station, printed on waterproof paper. Separate data sheets are needed for a) mapping and measuring habitat features; b) habitat sampling along transects; c) station summary, including water quality; d) flow; e) macroinvertebrate collection; and f) periphyton collection.
4. Conduct daily calibration of appropriate meters and probes.
5. Inspect motorized vehicles/boats at the beginning of every field day, including all safety and directional lights, oil, gasoline, and air pressure levels of tires.
6. Travel to monitoring site. Refer to description of monitoring station location, directions, and photos to verify correct location. Verify coordinates on GPS unit.
7. Measure water quality variables with a multiprobe and transparency with a transparency tube downstream of first transect per SOP #6; record measurements on data sheet.
8. Obtain mean stream width to calculate $40 \times \text{MSW}$ to find sample reach Length, as needed.
9. Moving from downstream to up, conduct physical habitat assessment instream and in the riparian area along transects, Map and measure habitat features as you go. Also as you move upstream, select three riffles or other appropriate habitats for collecting macroinvertebrate and periphyton samples (per SOPs #7 and #8).
10. Collect and preserve macroinvertebrate and periphyton samples, per SOPs #7 and #8, respectively.
11. Measure water quality variables with a multiprobe and transparency with a transparency tube upstream of last transect per SOP #6; record measurements on data sheet.
12. Measure flow along a cross section of the stream (per SOP #10) at an appropriate place within the sampling reach; record measurements on data sheet.
13. Download data from continuous logging devices, replace batteries or logging devices if necessary, and re-deploy.
14. Record stage reading.
15. Verify that all data sheets are completely filled out, and initial them.
16. If sampling more than one monitoring station in a day, go back to step 6, ensuring that equipment has first been decontaminated for invasive species per SOP #5.
17. Return to office or park laboratory.
18. Clean and decontaminate sampling equipment (SOP #5) and perform calibration checks, as detailed in SOP #6.

19. Download field data and check against hard copy field data sheets as soon as feasible.
20. Enter all data into NPSTORET at the end of the season.
21. This SOP contains details on mapping and measuring habitat features and conducting physical habitat assessments instream and in the riparian area.

7.2 Equipment and Supplies

Refer to the checklist of supplies and equipment needed for collecting benthic macroinvertebrate samples (Table 1) prior to each sampling trip. Plan ahead to be sure all equipment is available, clean, and ready for use on the day it is needed. Keep on hand all necessary forms, field logbooks, field data sheets, procedural manuals, and equipment instructional manuals. Copy field data sheets on waterproof paper. See Appendix A for a blank data sheet. Specific sections of the protocol narrative and this SOP that might be important to have in the field should be copied onto waterproof paper and included in the field kit.

A D-frame kick net (500 μm mesh, 30.5-cm-wide frame; Figure 1) will be used to collect macroinvertebrate samples, with a 0.093 m^2 (30.5 $\times$ 30.5cm) PVC frame (approx. 1 ft^2) to define the sampling area.

Table 1. Checklist of supplies and equipment needed for collecting macroinvertebrates.

✓	Item
	D-frame kick net, 500 μm mesh, 30.5 cm width and 0.093 m^2 (30.5 $\times$ 30.5 cm) PVC frame (approx. 1 ft^2)
	bucket, 3–5 gal capacity
	sieve bucket (500 μm mesh) or white plastic pan
	hand rake
	Forceps
	squirt bottle (to wash down net)
	scoop to transfer sieve materials to sample jar
	specimen bottles/jars and labels
	Preservative
	GPS and extra batteries
	Camera
	clipboard, data sheets, pencils, etc.
	wrist watch
	waders, hat, bug head-net
	First aid kit

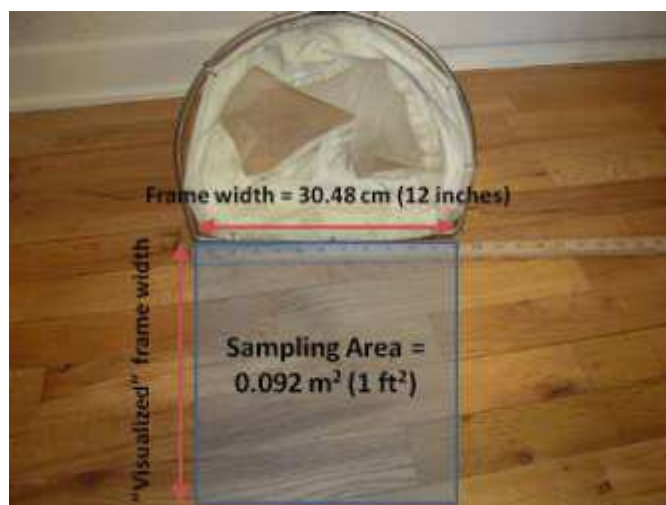


Figure 1. D-frame kick net and sampling area immediately upstream of the net opening. A PVC frame may be used to define the sampling area. Photo from Dinger et al. (2013).

7.3 Define the Sampling Area

The length of reach that comprises the sampling area at each site is defined relative to the mean stream width, and is described in SOP #4, Site Establishment. Beginning at the downstream end of the sampling reach and proceeding upstream, measure habitat features and construct a map per SOP #9.

Select three riffles that are similar to one another in bottom substrate size and composition and indicate their locations on the site map. Macroinvertebrate and periphyton samples will be collected in these three riffles. If the sampling reach does not contain three riffles, sample from as many riffles as possible (one or two) and sample coarse woody debris (e.g., log jams) for the remainder. See section 7.4.2, below, for details on sampling in habitats other than riffles.

7.3.1 In-Stream Habitat Types

The following are descriptions of major stream habitat types are taken from Barbour et al. (1999):

- Cobble (hard substrate) – Cobble is typically present in riffles and runs and will have a coarse substrate of mixed gravel, cobble, or boulder.
- Snags – Snags and other woody debris that have been submerged for a relatively long period of time are included here. Accumulated material in pool areas are considered as snag habitat. Avoid large logs, as they are difficult to sample adequately.
- Vegetated banks – Includes lower banks that are submerged and undercut banks that have roots and emergent plants associated with them.

- Submerged macrophytes – Beds of plants that are rooted in the bottom of the stream, such as *Vallesneria americana*.
- Sand (and other fine sediment) – Areas that are relatively free of the above defining characteristics and are dominated by bare, fine sediment. This habitat is usually the least productive stream habitat for macroinvertebrates.

7.4 Sampling

7.4.1 Riffle Habitats

Three macroinvertebrate subsamples will be collected from three riffles in each sampling reach. These three subsamples will be pooled into one sample per riffle, resulting in a total of three samples for each reach. If three riffles do not exist in a reach, other habitats will have to be sampled. Details on non-riffle habitat sampling are provided in section 7.4.2.

After mapping the sampling reach and selecting the riffles for sampling, begin at the downstream-most selected riffle. Determine the upper and lower boundaries of the riffle based on a visual assessment of gradient, velocity, and substrate characteristics, and divide the riffle lengthwise into three equal portions (Figure 2). Avoid the downstream portion of the riffle if it deepens and grades into a run habitat. Use the seconds indicator on a watch to randomly select the first sampling location as follows: for 0–20 seconds, the first sample will be in the right-hand third of the downstream end of the riffle; for 21–40 seconds, the first sample will be in the center of the downstream end; and for 41–60 seconds, the first sample will be in the left-hand third of the downstream end of the riffle. Three macroinvertebrate (and three periphyton, SOP #8) samples will be collected from each selected riffle. The order of the sample will always move right to left. If the first macroinvertebrate sample is on the right (1), the second sample will be in the center (2), moving upstream, and the third sample will be on the left (3) at the upstream end of the riffle. If the center is selected first, then the next sample will be taken on the left, and the third sample will be taken on the right. If the left side is selected first, the next sample will be on the right, and the third in the center. The process is repeated for the next two riffles (Figure 2).

Always begin at the downstream end of the reach and proceed upstream to avoid disturbing the substrate in areas that have not yet been sampled.

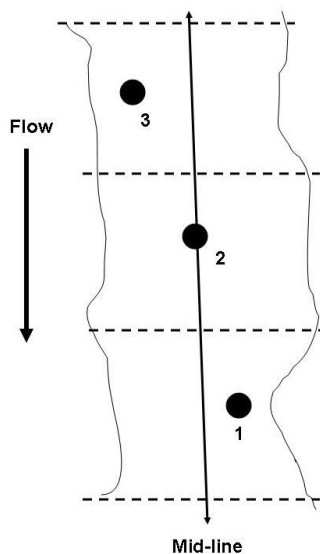


Figure 2. Placement of macroinvertebrate samples in a riffle, with sample #1 on the right, sample #2 in the center, and sample #3 on the left. Figure from Bowles et al. (2008).

Place the D-frame net frame firmly on the bottom with the opening facing upstream. While one team member holds the net firmly against the stream bottom facing the current, a second team member picks up a rock from within the sampling frame and dislodges all of the clinging invertebrates with a soft scrub brush or by hand, and lets them drift into the net. Use forceps, if necessary, to remove clinging invertebrates. Set the rock aside outside the sampling area once the scrubbing is completed. Repeat this procedure with all rocks greater than approximately golf ball size. Disturb the remaining substrate within the frame by using a hand rake for approximately 30 seconds. Dislodged macroinvertebrates should drift into the net. Try to avoid including debris into the net.

Remove the net from the water and rinse the contents down into the bottom of the net. Invert the net, and dump the contents into a bucket filled approximately half-way with stream water. Inspect the net and remove any clinging organisms with a forceps; add the organisms to the bucket. Repeat the procedure for all riffle locations (three sites each in three riffles) and composite the sub-samples into the bucket. Repeat this procedure with the two remaining riffles in the sampling reach.

7.4.2 Non-riffle Habitats

In streams without three riffles containing similar substrate to one another, or without any riffles, other habitats can be sampled. The order of sampling habitat preference is as follows:

- 1) Three riffles with similar substrate composition
- 2) Three riffles, regardless of similarity of substrate composition
- 3) Two riffles and coarse woody debris
- 4) Two riffles and most abundant other instream habitat type

- 5) One riffle and coarse woody debris
- 6) One riffle and two most abundant other instream habitat types
- 7) Coarse woody debris (if enough exists for 20 kick/jab samples)
- 8) Coarse woody debris and other instream habitat by proportion of occurrence

If after mapping the sampling reach you have determined that three riffles with similar substrate composition do not occur, estimate the proportion of the reach in each habitat type (snags, sand, vegetated banks, etc.) and record on the data sheet. Following the prioritization above, determine how many kicks or jabs to do with the kick net in each habitat type. Do a total of 20 kicks or jabs if no riffles exist. If one or two riffles are present, follow the procedures above in section 7.4.1 on how to proceed with sampling in riffles.

Consider each riffle sample equivalent to two kick net samples, so if one riffle exists in the reach, then the riffle sample would count as six kick net samples, and 14 kick net samples would be taken in the rest of the reach. If two riffles are present and, then that would mean that 8 kick net samples would have to be collected in the other habitats.

If the reach consists of 50% cobble, 20% vegetated banks, and 20% snags, then 10 kicks should be conducted in cobble habitats, 4 kicks or jabs each in vegetated bank and snag habitats, and 2 kicks or jabs in any remaining habitat. If any single habitat type comprises less than 5% of the reach, do not collect any samples from it but instead, divide the remaining samples proportionately among the more common types. Spend approximately 30 seconds on each kick and jab subsample.

To collect invertebrates from vegetated banks, submerged macrophytes, and snags, the net should be jabbed into the vegetation or roots and drawn along the vegetation. The habitats can be kicked first to help dislodge organisms, but be sure to place the net downstream. If two people are conducting the sampling, one person can hold the net while the other person takes sticks or vegetation from a snag and rinses them into the net. Check the net frequently to ensure organisms are being rinsed into it. The 30.5 × 30.5 cm PVC frame may not be able to be used directly to delimit the sampling area depending on the structure, but can be useful to help visualize the volume of habitat to be sampled with each kick or jab subsample.

To collect invertebrates from hard or soft bottom substrates, place the 30.5 × 30.5 cm PVC frame on the bottom and the kick net immediately downstream of the frame. Disturb the bottom sediments with a hand rake or by kicking and scraping for approximately 30 seconds. Watch the current to be sure the organisms are drifting into the net.

After each kick or jab, remove the net from the water and rinse the contents down into the bottom of the net. Invert the net and deposit the contents into a bucket filled approximately half-way with stream water. Inspect the net and remove any clinging organisms with a forceps; add the organisms to the bucket. Repeat the procedure for all 20 kicks or jabs and composite the samples into the bucket. For locations where non-riffle habitats are sampled, all kicks and jabs from non-riffle habitats

will be composited into a single sample for each stream reach. The three subsamples from each individual riffle will still be composited into a single sample for each riffle.

7.4.3 Composite the Samples

When sampling sites with riffles, each riffle will yield three subsamples that will be composited into a single sample for each riffle. All samples from other habitats, such as large woody debris will be composited together, regardless of the number of subsamples. For example, if there are no riffles in a reach, all kick and jab subsamples from all habitats will be composited into a single sample for the whole reach. If there is one riffle present, then the three subsamples from the riffle will be composited into a single riffle sample, and the remaining 14 kick and jab subsamples would be composited into a single non-riffle sample, yielding a total of two samples for the reach.

When all samples have been composited into the bucket, remove any large debris, making sure no organisms are clinging to it. Pour the bucket contents through a 500 μm sieve and use a scoop to transfer the material into a sample container. Use forceps if necessary to include all organisms collected. You can do this with a portion of the bucket contents at a time rather than all at once, to avoid clogging the sieve. Rinse the sample by swirling the bucket in water; use care not to submerge the bucket. Continue rinsing until all the fine silt and other sediments have been washed from the sample. Remove the sample contents from the wash bucket and place them in the sample container. Inspect the net, bucket, and sieve for any remaining organisms and carefully place them in the sample container. Excess water in the sample container can be drained into the sieve and inspected for organisms so that the preservative is not overly diluted.

More than one bottle may be necessary, depending on the amount of debris remaining in the sample. Do not fill any sample bottle more than one half full with the sample so that adequate space remains for preserving the sample. Fill the sample bottle with 95% ethanol and place a label written in permanent ink on waterproof paper inside. Invert gently several times to mix preservative throughout the sample contents; do not shake. Ensure both the external and internal labels are filled out completely, and include the project, date, stream name, site code, and collector's name. The outside label should also include "preserved with ~80% ethanol". If more than one sample container is needed for a sample, label each container accordingly (e.g., 1 of 2 or 2 of 2).

Before moving on to the next task, make sure all data sheets for macroinvertebrate sampling are filled out completely. Rinse the net in the stream to remove any lingering detritus.

7.5 Packing, Storing, and Sending Samples

Samples will be sent to a contractor, who will be hired to identify and enumerate the benthic macroinvertebrate samples.

- Before sending the sample containers to the contractor, check to be sure all containers are filled to the top with 80% ethanol to minimize damage to the specimens. When a sample jar contains a lot of organic debris, such as leaves and algae, proper preservations may be questionable. In such cases, decant the ethanol solution and refill with fresh alcohol within 24 hours of sampling to avoid specimen decay. If the preservative is too weak, the sample will decay; if the preservative is too strong, the invertebrates will get brittle.
- Carefully pack the sample containers in a box lined with a plastic garbage bag; be sure to use enough packing material to cushion the samples.
- Complete a chain of custody (COC) form, keeping the ‘client copy’ for the project files. Seal the contractor’s copies in a one-gallon plastic sealable bag and include in the box with the samples. Prepare separate COC forms for each box. An example COC form is provided in Appendix C.
- Call an appropriate courier service ahead of time to arrange pick-up. Check to be sure the courier will accept shipments containing alcohol.
- Alert the contractor when samples have been shipped and provide them with the tracking number.

7.6 Quality Assurance/ Quality Control

Avoid disturbing the substrate prior to sampling. During sampling, ensure D-frame net is positioned firmly against the substrate, with the cod end facing downstream. Ensure the sampling is conducted according to procedure. Examine the net and any discarded rocks or debris thoroughly to be sure that all organisms are included in the sample.

Prior to departure from the sampling site, check to be sure all data forms have been filled out completely. Samples must be labeled on the outside and must contain an internal label on waterproof paper. Both labels should include the same information - the project, date, stream name, site code, and collector’s name. If chain-of-custody forms are used for sending samples to a contract lab, they should also contain the same information as the sample container labels.

After sampling is completed at a site, rinse all equipment and gear, especially nets and sieve buckets. Inspect to be sure no organisms or debris remains. Follow decontamination procedures described in SOP #5 before using the equipment at a new site.

See SOP #15, Quality Assurance/Quality Control Procedures for details regarding all aspects of QA/QC for stream monitoring.

7.7 Literature Cited

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Appendix A. Macroinvertebrate and Periphyton Data Sheet

Park Code _____
 Stream Name _____ Site Code _____
 Date (DDMMYYYY) _____ Site Name _____
 Personnel _____

Macroinvertebrates

Gear Used ☐ Serber Sampler ☐ Kick Net ☐ Other

How were the samples collected ☐ From bank ☐ Wading ☐ From boat

kicks/jabs/samples taken in each habitat type

Riffles _____ Cobble (hard substrate) _____ Snags (CWD) _____

Vegetated banks _____ Submerged macrophytes _____ Sand/Silt/Mud/Muck _____

Macroinvertebrate Sample Notes:

Periphyton

Gear Used ☐ Pipette ☐ Scraping tool ☐ Toothbrush ☐ Other

How were samples collected? ☐ Wading ☐ From bank ☐ From boat ☐ Other

samples taken in each habitat type

Riffles _____ Woody debris _____ Depositional areas _____

Other _____ Estimated total area sampled (cm²) _____ Should be approx.. 108 cm²

samples taken from each substrate type

Cobble _____ Gravel/small stones _____ Woody debris _____

Sand/Silt/Mud/Muck _____ Other _____

General Comments