State of Wisconsin
Department of Natural Resources

Guidelines for Collecting Macroinvertebrate Samples from Wadable Streams

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Bureau of Fisheries Management and Habitat Protection
Monitoring and Data Assessment Section
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GUIDELINES FOR COLLECTING MACROINVERTEBRATE SAMPLES FROM WADABLE STREAMS

Overall Objectives and Sampling Design for Stream Macroinvertebrates

Data derived from aquatic macroinvertebrate samples provide valuable information on the physical, chemical and biological condition of streams, which along with stream habitat and fish community data permits a comprehensive assessment of stream health. Most aquatic macroinvertebrates such as immature insects live for 1 or more years in streams, integrating the affects of various environmental stressors over time. Since the majority of aquatic invertebrates have limited mobility (relative to fish), they can be good indicators of localized conditions as well as detecting upstream land impacts and water quality degradation.

Various metrics and indices are used to interpret macroinvertebrate sample data. Hilsenhoff's Biotic Index (HBI) has been used extensively by the Department as an indicator of low dissolved oxygen concentrations resulting from organic pollution (Hilsenhoff 1987). In streams where oxygen stress may not be a problem (e.g. high gradient streams with significant groundwater discharge), other metrics can be applied that assess the impacts of riparian habitat degradation, sedimentation problems, scouring, etc (Lillie et al. 2003). In general, as the level of environmental degradation increases within a stream there is a corresponding decrease in a number of environmentally sensitive macroinvertebrate species and an increase in a few environmentally tolerant species.

General Sampling Procedures:
Time of year, sampling site selection, and sampling technique are key factors to be considered when collecting macroinvertebrate samples.

In general:

1. Collect samples in the spring (March – April) or in the fall (mid -September – November).
2. Collect samples in riffles (shallow areas where water breaks over rocks).
3. Sample where the stream flow velocity is at least 0.3 meters per second.
4. Sample substrate composed of coarse gravel to larger rubble (< 0.3 meters diameter).
5. Sample similar environments and standardize protocols (points 1 – 4) at all assessment sites if possible, particularly if comparisons are being made between or among sites, or at sites over time.
6. In the absence of riffles, vegetation caught in logjams, snags, or vegetation overhanging from the stream banks can be sampled. Investigators should be aware that direct comparisons between samples collected from different habitats is undesirable.
7. For Baseline monitoring, one sample per stream assessment site is sufficient. If the macroinvertebrate data will be used in impact assessments or enforcement cases, replicate sampling should be considered. Additional samples can be collected and processed later if additional data is needed.

Equipment:

Net mesh-size can strongly influence the types and proportions of macroinvertebrate taxa collected. A 500-micron mesh net should be used for collecting baseline macroinvertebrate samples for the Department. While net frames are available in various sizes and shapes. Rectangular or D-shaped net frames are good choices. Smaller triangular-shaped net frames are also available, but their relatively small net opening may miss many of the invertebrates dislodged from the substrate when kick sampling.

Sampling Methods:

Collect samples by holding the net frame firmly against the stream bottom and disturbing the substrate upstream (approximately a full arm's length) from the net with your feet. Dig deeply into the substrate with the heel or toe to dislodge macroinvertebrates from the streambed. Avoid kicking debris into the net (let the macroinvertebrates wash downstream into the net). Make sure that the plume of silt that results from disturbing the substrate is flowing into the net, as this plume also contains the dislodged invertebrates.

In streams that lack riffles, vegetation (twigs, leaves, grass) caught in in-stream snags or logjams, or riparian vegetation overhanging into the stream can be sampled. Sample by jabbing the net into the vegetation to dislodge the clinging invertebrates. The net should be inspected often to make sure the invertebrates that are being dislodged are washing into the net. If two people are collecting the sample, one person can hold the net while the other manually removes vegetation from the snag or logjam and rinses it into the net, or shakes the snags to loosen the vegetation caught so it drifts into the net. Course debris should be removed from the net, while making sure to rinse the macroinvertebrates that are clinging to the vegetation back into the net.

Sampling Effort, Number of Macroinvertebrates / Sample Volume:

More than 100 macroinvertebrates should be collected per sample. A quantity of debris about the size of a softball should contain over 100 macroinvertebrates, and such a sample should take approximately 3 minutes or less to collect. Inspect the net contents to insure that more than 100 macroinvertebrates have been collected. If it is determined that insufficient numbers of macroinvertebrates are captured after initial sampling efforts, sampling should be extended for a second period of equal duration and noted on the field sheet. If insufficient numbers exist after completion of the second sampling effort, stop collecting and preserve the sample. Low numbers of organisms may be indicative of water quality or habitat problems and should be noted on the field sheet. Conversely, some small, highly shaded, or wetland dominated streams, naturally have relatively low numbers or diversity of aquatic macroinvertbrates.

Sample Handling and Preservation:

After the sample is collected, rinse fine sediment from the net by forcefully swishing the net through the water a few times, being careful not to lose the organisms captured. Removing fine sediment from the net makes lab picking of the sample easier and helps insure adequate preservation of the sample. Discard large sticks, rocks, and undecomposed leaves from the net after rinsing any clinging macroinvertebrates back into the net

Transfer the fine debris and macroinvertebrates to a tightly sealing plastic or glass wide-mouth jar Whirl-pac™ bag of sufficient size. Inspect the net and transfer clinging macroinvertebrates into the sample container. The sample debris should occupy less than 1/2 the sample container’s volume. Make sure that the container is properly labeled. Labels should have at least the following information: **sample ID number**, **replicate number**, **waterbody name**, **Water Body Identification Code, county, collector’s name** and a **split-sample designation** if needed. If a single sample's contents has to be placed in 2 separate containers due to large sample quantity, label the container accordingly e.g. container 1 of 2, sample 19990510-16-05. Place a label inside the sample jar, using bond paper written in pencil.

Initially preserve the sample with 80-85% alcohol (isopropyl or ethanol). Higher concentrations of alcohol make some invertebrates brittle, making taxonomic identification more difficult or impossible. Fill the sample jar to the top with the alcohol solution. Be sure to tightly seal the sample container and gently invert the container several times to thoroughly mix the sample. Samples containing large amounts (over 50%) of filamentous algae or other organic matter should be preserved and re-preserved. Within 24 hours, pour-off the alcohol solution and refill with fresh 80-85% alcohol. Poorly preserved samples are unpleasant to analyze, and decayed organisms make taxonomic identifications difficult or impossible.

Summary of Advantages and Disadvantages of Spring or Fall Sampling

Spring sampling:

Advantages. - Streams that may become isolated pools or dry-up entirely in fall can be sampled in spring. Spring samples typically contain well-developed insect larvae which are readily identifiable.

Disadvantages. - Since many aquatic insect taxa emerge over short time periods in spring, timing is critically important. Spring samples are also more likely to be affected by the catastrophic effects of spring floods. Spring samples are also less likely to reflect localized impacts resulting from organic enrichment (e.g. barnyard runoff) because intolerant macroinvertebrates can recolonize and inhabit impact areas as long as colder water temperatures reduce dissolved oxygen stress or ammonia toxicity and provide temporarily favorable conditions.

Fall sampling:

Advantages. - Fall samples are more likely to detect the true extent of organic enrichment (low D.O.)problems. Fall samples more closely follow the summer and fall stresses of warm water temperatures, low-flows, and low-D.O. concentrations associated with point source and nonpoint source pollution, while limiting the time that may elapse in which favorable conditions have existed for the downstream drift of intolerant species into impacted areas.

Disadvantages. - Small or immature larvae of some insect taxa make identification to genus or species level difficult or impossible; however, in general invertebrates collected in mid-September through November (all winter actually) should be identifiable.

Sample Site Documentation

Macroinvertebrate Field Data Report (Form 3200-081)
This form is used to record the sampling location identifiers, sample and site descriptions, stream and watershed descriptors, and provides special instructions to the laboratory for analyzing or retaining the sample. **One Macroinvertebrate Field Data Report form should be filled-out for each sampling location**. Data fields written in **bold** font are mandatory information.

***Location Identifiers*_____**

Waterbody Name The name of the waterbody as shown on the most recent U.S.Geological Survey (USGS) 7.5' topographic quadrangle map. The name reported should be identical to that used for other data sheets that may have been filled-out for habitat or fisheries assessments. Make sure that the waterbody name is identical on all other macroinvertebrate field data report forms filled-out for all other sampling sites on the waterbody.

Waterbody ID Code A unique number that identifies each stream; most streams, rivers, and lakes in Wisconsin have an assigned number. These numbers are available on the WDNR Intranet, under the listing for "DNR Tabular Database Service" for the WDNR Register of Waterbodies (ROW). As with stream name, waterbody ID code should be the same for all stations on a stream.

Site Mile The reporting of this parameter is optional. The distance along the stream channel from the mouth of the stream to the downstream end of the sampling station. This distance is a useful shorthand for indicating and identifying the location of the station. Site mile should be measured on the most recent USGS 7.5' topographic map to the nearest 0.1 mile using a map measurer (map wheel).

Station No. If a stream has two or more stations, the downstream station is 1, the next upstream station is 2, and so on. If there is only one station then the station number is 1.

Sample ID Record a sample identification number using an 8-digit format for the date the sample was collected; the 2-digit county code (e.g. Adams County is 01, and Wood County is 72); and a 2-digit field number which indicates the sample site number for the stream (YYYYMMDD-CY-FD). For example, if 1 site is sampled on a stream that date, the field number is 01 even if multiple (replicate) samples are collected at the site; if a second site is sampled on the same stream, or another stream is sampled, the field number for the second sampling site is 02.

Sampling Location A precise narrative description of the point on the stream where the macroinvertebrate sample(s) was collected. The description should include the exact distance and direction of the start from a "permanent" landmark such as a bridge, building, road marker, rock formation, etc. **Avoid using landmarks that might be lost in future years** (e.g., don't use tree or fence lines). Make the description as specific and precise as possible so that someone visiting the sampling site for the first time can easily find the starting point.

Township, Range, Section, ¼ - ¼ Section, 1/4 Section Legal description for the Starting Location of the station within the Public Lands System. These can be determined from recent USGS 7.5'

topographic maps or a detailed county map. On a topographic map, a "land locator" template is useful for determining the ¼ - ¼ and 1/4 Sections, which are indicated by a compass direction (NW, NE, SW, or SE). Note that in Wisconsin, all Townships are "N" (north), but Range can be either "E" or "W" (east or west). Make sure the appropriate letter is included for both Township and Range.

STORET If water chemistry samples are collected and processed through the Wisconsin State Laboratory of Hygiene, record the 6-digit STORET number assigned to the water chemistry sample.

Latitude and Longitude It is important that geographic coordinates of the sampling site are recorded, along with the method used to determine latitude and longitude (e.g. USGS topo map, mapping software, plat map, global positioning system (GPS) units). If a map or GPS unit are used, the geodetic datum upon which the coordinates are based (e.g. North American Datum 1983 (NAD 83)), should also be recorded. Datum for USGS topo maps are shown on the map legend. 7.5' Quad Map Name The name of the USGS 7.5' topographic quadrangle map on which the station is found.

Basin Name The name of the basin in which the stream is located.

Watershed Name The name of the watershed in which the stream is located.

County The name of the county in which the sampling site is located.

Sample and Site Descriptors _____

Sample Collector The full names of the person(s) who collected the sample(s).

Project Name The name of the project for which the sample(s) were collected.

Sampling Device Check which type of sampling device that was used to collect the sample(s). Habitat Sampled Check which type of habitat was sampled.

Total Sampling Time Record the number of minutes it took to collect each sample. Some collectors use a set amount of time to collect samples, this standardization technique may be valuable in making comparisons among sites or between sites over time, as differing numbers of macroinvertebrates collected for a given effort may be indicative of differing stream productivity or environmental stress.

Estimated Area Sampled (m^2) Record an estimate of the area of stream bottom disturbed to collect the sample(s). Like total sampling time, some collectors standardize the area of stream bottom disturbed as a way of comparing stream productivity or environmental stress among sites or at a site over time.

Number of Composites in Sample Record the number of discrete samples that are combined into 1 sample to form a composite sample. Some collectors sample multiple habitats and combine into a single sample to get a representation of the entire stream macroinvertebrate community versus sampling a single habitat such as a riffle. The collector should be aware that the HBI was developed specifically for riffle samples, and that sampling depositional or other non-riffle habitats may influence diversity measures or water quality indices like the HBI.

Replicate No. __ of __ Record the number of replicate samples collected at a sampling site. If no replicates were collected report 1 of 1.

Reason for Sampling Check the box that indicates why the sample was collected.
Water Color Record whether the water is Clear, Turbid due to suspended sediment, or Stained due to dissolved organic compounds.

Water Temperature If possible measure the water temperature in mid-channel, during the warmest part of the day to estimate maximum values.

Dissolved Oxygen (D.O.) The reporting of this parameter is optional. If reported, measure with a high-quality meter, which should be air-calibrated before every use. Follow the manufacturer's instructions for use and maintenance (e.g. the probe membrane and electrolyte should be replaced frequently during the field season). Record D.O. in milligrams per liter (parts per million).

Dissolved Oxygen Percent Saturation The reporting of this parameter is optional. If reported, measure with a high-quality meter, which calibrated before every use. Follow the manufacturer's instructions for use and maintenance.

pH (su) The reporting of this parameter is optional. If reported, measure with a high-quality meter, which is routinely calibrated. Follow the manufacturer's instructions for use and maintenance (e.g. the probe membrane and electrolyte should be replaced frequently during the field season).

Turbidity The reporting of this parameter is optional. If reported, measure with a high-quality meter, that is calibrated before every use. Follow the manufacturer's instructions for use and maintenance. Report turbidity in nephelometric turbidity units (NTUs).

Total Dissolved Solids The reporting of this parameter is optional. If reported, measure with a high quality meter, that is calibrated before every use. Follow the manufacturer's instructions for use and maintenance. Report total dissolved solids in milligrams per liter (parts per million).

Conductivity (umhos/cm) The reporting of this parameter is optional. If reported, measure with a high-quality electronic meter. Most conductivity meters have built-in automatic temperature compensation to 25 °C (77 °F), but this should be confirmed before using the meter. On some older meters the temperature compensation must be set by hand, and on others, there is no compensation. For the latter meters, conductivity at 25 °C can be calculated using procedures outlined in "Standard Methods for the Analysis of Water and Wastewater", a book available at many WDNR offices. Whatever meter is used, it should be calibrated before every use. Report conductivity in umhos/cm.

Stream Order The reporting of this parameter is optional. A qualitative measure of stream size, based on the amount of branching of the watershed upstream from the station, using Strahler's modification of Horton's original system. Generally, the higher the order, the larger the stream. Determine from USGS 7.5" topographic maps; usually requires multiple maps because the entire stream network upstream from the station must be examined. In making determinations, all "blue lines" (streams) on the maps, including intermittent streams, are included. The order system is as follows: All streams (including intermittent streams) from their source downstream to their first tributary are **First** order (stream order is "1" on data sheet). When two first order streams meet, the stream below this confluence is **Second** order (stream order is "2"). When two second order streams meet, the stream below this confluence is **Third** order (stream order is "3"), and so on.

When two streams of unequal order meet, the stream order below this confluence is equal to the higher of the two orders. For example, if a first and a third order stream meet, the stream below this confluence is third order. Stream order increases only when two streams of equal order meet. Stream Gradient The reporting of this parameter is optional. The overall decrease in elevation (on a per kilometer basis) of the stream over the entire station. Determine from USGS 7.5' topographic maps, using a map wheel. First, find the downstream and upstream ends of the station on the map. Then find the first contour line that **crosses** the stream upstream of the station and the first contour line that **crosses** the stream downstream of the station. For low gradient streams this may require going to additional maps, covering many miles of stream, and possibly including other streams. With the map wheel, determine the distance along the stream channel between these two contour line crossings. Then determine the elevation drop between these two contour lines. The drop in elevation is equal to the total number of contour lines crossing the stream within the station (often zero), plus the contour line immediately above and the contour line immediately below the station, minus one, times the elevation drop between adjacent contour lines (Note: most topographic maps have 10 ft contours, but some have 20 ft contours; check the legend at the bottom of the map). Divide the elevation drop by the distance measured by the map wheel. This is the gradient for the station. Convert feet/mile to m/km by dividing by 5.3. Estimated Stream Velocity (mps) Either an **estimated** or **measured** stream-flow velocity should be reported. Report estimated stream-flow velocity in meters per second.

Measured Velocity (mps) Report the measured velocity of the stream in meters per second. Average Stream Depth (m) Report the estimated average stream depth upstream of the riffle area sampled. If a habitat assessment was conducted at the site use the measured value from the habitat assessment.

Average Stream Width (m) Report the estimated average stream with upstream of the riffle area sampled. If a habitat assessment was conducted at the site use the measured value from the habitat assessment.

Composition of Substrate Sampled (Percent) Record the percent composition of the substrate where the macroinvertebrate sample(s) were collected, the total percentages should equal 100 percent.

Embeddedness of Substrate at Sample Site (%) Record the percent embeddedness of the substrate sampled. See: "Guidelines for Evaluating Habitat of Wadable Streams in Wisconsin" for guidance on quantifying substrate embeddedness.

Canopy Cover at Sample Site (%) Estimate the percent canopy that is shading the stream at the site where the macroinvertebrate sample(s) were collected. Stream shading reduces instream periphytic growth, which can strongly influence the community of macroinvertebrate scrapers, and grazers that feed on periphyton.

Stream and Watershed Descriptors_____

Categories of Local and Watershed-wide Biological, Physical, and Chemical, stressors that may be influencing the macroinvertebrate community, and Sources of Stream Impacts are listed. Local: land use or water resource factors that may be influencing water resource integrity within the stream reach from which the macroinvertebrate sample was collected. Watershed-wide: land

use or water resource factors that may be influencing water resource integrity within the watershed upstream of the site where macroinvertebrate sample was collected. For each factor or Sources of Stream Impacts report whether it is certain that it is: (N) Not a problem; (U) Present, but uncertain as to the degree of impact; (P) Present, and probably creating a problem; or (Leave Blank) if uncertain to the potential impacts to the macroinvertebrate community.
Comments Any and all information that is relevant to the macroinvertebrate sample(s) should be recorded here. For example if it was difficult to collect over 100 organisms, this may be of importance when interpreting the taxonomic results. **If in doubt write it down**.

Special Instructions for Laboratory Provide specific instructions to the laboratory, if for example the lab should retain samples for enforcement cases, scan the entire sample for rare or endangered species, report non-HBI taxa, etc.

Table 1. Equipment used for collecting macroinvertebrates, and equipment suppliers.

Item	Supplier
Net Used to collect macroinvertebrate samples. Turtox – Design, D - Frame Net Stock # 425 (**be sure to order 500 micron mesh size**)	Wildlife Supply Co. 95 Botsford Place Buffalo, NY 14216 800 / 799-8100 (FAX) 800 / 799-8301 www.wildco.com
Widemouth Sample Jars Used for sample storage. 16 oz. Or larger plastic or glass widemouth jars	Fisher Scientific* 4500 Turnberry Dr. Hanover Park, IL 60103 800 / 766-7000 www.fishersci.com
Alcohol Used to preserve macroinvertebrate samples. Isopropyl or enthanol can be used.	State of Wisconsin Materials Distribution Service* 2102 Wright St. Madison, WI 53704 608 / 245-2900 www.bussvc.wisc.edu/mds/mds.html
Waterproof Paper Used for making copies of the macroinvertebrate field form.	J. L. Darling Co. 2614 Pacific Hwy. E. Tacoma, WA 98421 253/922-5000 www.riteintherain.com

*The State of Wisconsin has a contract with Fischer Scientific and other vendors for substantial discounts on equipment and supplies purchases. To receive these discounts Regional WDNR staff should set-up an account with Fischer or other vendors by contacting their Region purchasing agent.Along with a discount on equipment and supplies, there are no shipping charges on regular or hazardous materials.

Literature Cited:

Hilsenhoff, W. L. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomolgist 20:31 – 39.

Lille, R. A., Szczytko, S. W. and M. A. Miller. 2003. Macroinvertebrate Data interpretation Manual. WI Dept. of Natural Resources, P. O. Box 7921 Madison, WI 53707-7921.

County Codes

Adams (01)
Ashland (02)
Barron (03)
Bayfield (04)
Brown (05)
Buffalo (06)
Burnett (07)
Calumet (08)
Chippewa (09)
Clark (10)
Columbia (11)
Crawford (12)
Dane (13)
Dodge (14)
Door (15)
Douglas (16)
Dunn (17)
Eau Claire (18)
Florence (19)
Fond Du Lac (20)
Forest (21)
Grant (22)
Green (23)
Green Lake (24)
Iowa (25)
Iron (26)
Jackson (27)
Jefferson (28)
Juneau (29)
Kenosha (30)
Kewaunee (31)
La Crosse (32)
Lafayette (33)
Langlade (34)
Lincoln (35)
Manitowoc (36)
Marathon (37)
Marinette (38)
Marquette (39)
Menomenee (40)
Milwaukee (41)
Monroe (42)
Oconto (43)
Oneida (44)
Outagamie (45)
Ozaukee (46)
Pepin (47)
Pierce (48)
Polk (49)
Portage (50)
Price (51)
Racine (52)
Richland (53)
Rock (54)
Rusk (55)
St. Croix (56)
Sauk (57)
Sawyer (58)
Shawano (59)
Sheboygan (60)
Taylor (61)
Trempeleau (62)
Vernon (63)
Vilas (64)
Walworth (65)
Washburn (66)
Washington (67)
Waukesha (68)
Waupaca (69)
Waushara (70
Winnebago (71)
Wood (72)