



## **ACKNOWLEDGEMENTS**

(Place to acknowledge peer reviewer)

THIS PAGE LEFT INTENTIONALLY BLANK

## TABLE OF CONTENTS

1.0 SCOPE AND APPLICABILITY .....	2
2.0 SUMMARY OF METHOD .....	2
3.0 HEALTH AND SAFETY WARNING .....	3
4.0 CAUTIONS.....	3
5.0 INTERFERENCES .....	3
6.0 FIELD PERSONNEL QUALIFICAITONS/RESPONSIBILITIES .....	3
7.0 EQUIPMENT AND SUPPLIES .....	4
8.0 FIELD PROCEDURE .....	5
9.0 DATA AND RECORD MANAGEMENT .....	11
10.0 QUALITY ASSURANCE AND QUALITY CONTROL .....	12
11.00 REFERENCES.....	12
APPENDIX A. Field Collection Data Recording Form	
APPENDIX B. Macroinvertebrate Sample Jar Labels (both inside and outside)	
APPENDIX C. Macroinvertebrate Sample Log	
APPENDIX D. SOP Acknowledgement and Training Form	

## 1.0 SCOPE AND APPLICABILITY

This document presents the North Dakota Department of Environmental Quality, Division of Water Quality's (DWQ) Standard Operating Procedure (SOP) for collecting a macroinvertebrate sample from wadable rivers and streams. This SOP applies to all DWQ field staff, non-DWQ cooperators, and citizen volunteers.

## 2.0 SUMMARY OF METHOD

Benthic macroinvertebrates inhabit the sediment or live on the bottom substrates of streams. This group of organisms is an important component for measuring the overall biological condition of an aquatic community. Populations in the benthic assemblage respond to a wide array of stressors in different ways. By monitoring assemblage status, it is possible to detect trends in ecological condition and often determine the type of stress that has affected a macroinvertebrate community (e.g., Klemm et al., 1990). Because many macroinvertebrates have life cycles of a year or more and are relatively immobile, the structure and function of the macroinvertebrate assemblage is a response to exposure of past or present conditions.

This benthic macroinvertebrate protocol is intended to evaluate the biological condition of wadable streams in North Dakota for the purpose of detecting stresses on community structure and assessing the relative severity of these stresses. It is based on the updated Rapid Bioassessment Protocols (RBPs) and Wadable Streams Assessment (WSA), both published by the U.S. Environmental Protection Agency (Barbour et al., 1999), and adopted for use by many states. The protocol's use of a D-frame kick net (Figure 7.17.1) for benthic macroinvertebrate collection is versatile for varying habitat type and is the preferred macroinvertebrate collecting method for streams with flowing water.

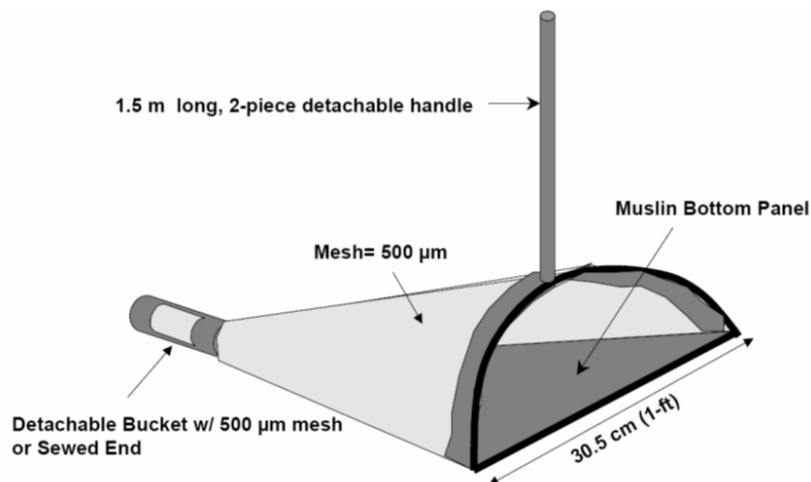


Figure 7.17.1. Modified D-frame Kick Net (Not drawn to scale)

### **3.0 HEALTH AND SAFETY WARNING**

Field personnel should take appropriate precautions when working on, in, or around the water. All sampling crews should be equipped with personal protective equipment (PPE). This equipment would include non-breathable waders, rubber gloves, eye protection, etc. When operating a boat, the North Dakota's boating laws and rules shall be followed by all field personnel. Field personnel should be aware that hazardous conditions potentially exist at every waterbody. If unfavorable conditions are present at the time of sampling, the sample visit is recommended to be rescheduled. If hazardous weather conditions arise during sampling, such as lightning or high winds, personnel should cease sampling and move to a safe location.

### **CAUTIONS 4.0**

Completely fill the jar(s) with 95% ethanol (no headspace). It is very important that sufficient ethanol be used, or the organisms will not be properly preserved.

### **5.0 INTERFERENCES**

If large rocks prevent the sampler from seating the net properly on the stream bottom, spend 30 seconds hand picking a sample from about 0.09 m<sup>2</sup> (1 ft<sup>2</sup>) of substrate at the sampling point. For vegetation-choked sampling points, sweep the net through the vegetation within a 0.09 m<sup>2</sup> (1 ft<sup>2</sup>) quadrat for 30 seconds. Place the contents of this hand-picked sample directly into the sampling container. If there is too little water to collect the sample with the D-frame net, randomly pick up 10 rocks from the riffle. Wash anything attached to the rocks (e.g. sand, insects, vegetation, etc.) into the closed or sieve bottomed bucket and pick any remaining organisms off and place them into the bucket. For samples located within dense beds of long, filamentous aquatic vegetation (e.g., algae or moss), use a knife or scissors to remove only the vegetation that lies within the quadrat (i.e., not entire strands that are rooted within the quadrat) and place it into the net.

### **6.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES**

All personnel collecting a macroinvertebrate sample from wadable rivers and streams must read this SOP annually and acknowledge they have done so via a signature page (see Appendix B). New field personnel must also demonstrate successful performance of the method. The signature page will be signed by both trainee and trainer to confirm that training was successfully completed and that the new monitor is competent in carrying out this SOP. The signature page will be kept on-file at DWQ along with the official hard copy of this SOP.

## 7.0 EQUIPMENT AND SUPPLIES

### Field Equipment and Supplies

- \_\_\_\_\_ Copy of this SOP
- \_\_\_\_\_ Surveyor's rod
- \_\_\_\_\_ Tape Measure
- \_\_\_\_\_ Laser Range Finder
- \_\_\_\_\_ Modified Kick Net (D-Frame with 500 $\mu$ m mesh) and 4ft handle (Wildco #425-C50)
- \_\_\_\_\_ Buckets, 8-to10-qt capacity
- \_\_\_\_\_ Hand sieve with 500 $\mu$ m mesh openings
- \_\_\_\_\_ Watchmakers' forceps
- \_\_\_\_\_ Wash bottle, 1-L capacity labeled "STREAM WATER"
- \_\_\_\_\_ Small spatula, spoon, or scoop to transfer sample
- \_\_\_\_\_ Funnel, with large bore spout
- \_\_\_\_\_ Sample Jars, HDPE plastic with screw caps, 500-mL and 1-L capacity, suitable for use with ethanol
- \_\_\_\_\_ 95% Ethanol, in a proper container
- \_\_\_\_\_ Rubber gloves, heavy rubber
- \_\_\_\_\_ Cooler (with suitable absorbent material) for transporting ethanol and samples
- \_\_\_\_\_ Composite Benthic sample labels, with preprinted ID numbers (barcodes)
- \_\_\_\_\_ Composite Benthic sample labels without preprinted ID numbers
- \_\_\_\_\_ Blank labels on waterproof paper for inside of jars
- \_\_\_\_\_ Sample collection form for site
- \_\_\_\_\_ Clear mailing tape (strips or roll)
- \_\_\_\_\_ Pocket knife
- \_\_\_\_\_ Scissors
- \_\_\_\_\_ Pocket-sized field notebook (optional)
- \_\_\_\_\_ Field operations and methods manual
- \_\_\_\_\_ Laminated sheets of procedure tables and/or quick reference guides for benthic macroinvertebrates
- \_\_\_\_\_ Spare net(s) and/or spare bucket assembly for end of net
- \_\_\_\_\_ Sieve-bottomed bucket, 500  $\mu$ m mesh openings
- \_\_\_\_\_ Soft (#2) lead pencils
- \_\_\_\_\_ Fine-tip indelible markers

\_\_\_\_\_ Stopwatch

## 8.0 FIELD PROCEDURE

### Lay Out of the Sampling Reach

1. Use a surveyor's rod, tape measure, or laser range finder to determine the wetted width of the channel at 5 places of "typical" width within approximately 5 channel widths upstream and downstream from the X-site. Average the 5 readings together and round to the nearest 1 m. If the average width is <4 m, use 150 m as a minimum reach length. If the average width is >12.5 m, use 500 m as a maximum reach length. *For channels with "interrupted flow", estimate the width based on the unvegetated width of the channel (again, with a 150 m minimum and 500 m maximum).*
2. Check the condition of the stream about the X-site by having one team member go upstream and one downstream. Each person proceeds until they can see the stream to a distance of 20 times the average channel width (equal to one-half the sampling reach length) determined in Step 1.
3. Determine if the reach needs to be adjusted about the X-site due to confluences with higher order streams (downstream), a change to a lower order streams (upstream), impoundments (lakes, reservoirs, ponds), physical barriers (e.g., falls, cliffs), or because of access restrictions to a portion of the initially-determined sampling reach.
4. Starting at the X-site (or the new midpoint of the reach if it had to be adjusted as described in Step 3), measure a distance of 20 channel widths down one side of the stream using a GPS unit, laser rangefinder, or tape measure. Be careful not to "cut corners". Enter the channel to make measurements only when necessary to avoid disturbing the stream channel prior to sampling activities. This endpoint is the downstream end of the reach and is flagged as Transect "A".
5. At Transect A, use the seconds display on a digital watch to select the initial sampling station for standard transect samples: 1-3= "Left", 4-6="Center", 7-9=Right. Mark "L", "C", or "R" on the transect flagging; the 3 potential collection points are roughly equivalent to 25%, 50%, and 75% of the channel width, respectively.
6. Measure 1/10 of the required reach length upstream from transect A. Flag this spot as transect B. Assign the sampling station systematically after the first random selection (Figure 4-6 & Table 4-6).
7. Proceed upstream with the tape measure and flag the positions of 9 additional transects (labeled "C" through "K" as you move upstream) at intervals equal to 1/10 of the reach length. Continue to assign the sampling stations systematically.



## Sample Collection

The transect sample design for collecting benthic macroinvertebrates is shown in Figure 7.17.2. This design was used in the Environmental Monitoring & Assessment Program (EMAP) - Western Pilot stream study in the western U.S., which provides continuity for nationwide assessments.

A sample is collected from **1 meter downstream** of each of eleven cross-section transects (Transects “A” through “K”) at an assigned area (Left, Center, or Right in the stream). The sampling point at Transect “A” is assigned at random using a die or other suitable means (e.g., digital watch). Once the first sampling point is determined, points at successive transects are assigned in order (Left, Center, Right). At transects assigned a “Center” sampling point where the stream width is between one and two net widths wide, pick either the “Left” or “Right” sampling point instead. If the stream is only one net wide at any transect, place the net across the entire stream width and consider the sampling point to be “Center”. If a sampling point is located in water that is too deep or otherwise unsafe to wade, select an alternate sampling point on that transect.

The procedure for sample collection at each transect is described below. For each sampling site, first establish that the majority of the river reach is wadable. Next determine if the habitat is a “riffle/run” or a “pool/glide”. Any area where water current is not sufficient to extend the net is operationally defined as a pool/glide habitat. As sampling proceeds upstream from transect to transect, combine all samples into a bucket or similar container.

**Do not fill out the collection form or labels until wadability is confirmed at the site and samples are collected.**

1. At **1 m downstream** of each cross-section transect, beginning with Transect “A”, locate the assigned sampling point (Left, Center, or Right facing downstream) at 25%, 50%, and 75% of the wetted width, respectively. If a sample cannot be collected at the designated point because of deep water or unsafe conditions, relocate to another random point on the same transect.
2. Make sure that the D-frame net is securely tightened to the pole as the net may become twisted in a strong current, causing the loss of sample materials.
3. Determine if there is sufficient current in the area at the sampling point to fully extend the net. If so, classify the habitat as “riffle/run” and proceed to Step 4. If not, use the sampling procedure described for “pool/glide” habitats, Step 9.

**NOTE:** If large rocks prevent the sampler from seating the net properly on the stream bottom, spend 30 seconds hand picking a sample from about 0.09 m<sup>2</sup> (1 ft<sup>2</sup>) of substrate at the sampling point. For vegetation-choked sampling points, sweep the net through the vegetation within a 0.09 m<sup>2</sup> (1 ft<sup>2</sup>) quadrat for 30 seconds. -Place the contents of this hand-picked sample directly into the sampling container.

#### **Riffle/Run Habitats:**

4. With the net opening facing upstream, position the net quickly and securely on the stream bottom to eliminate gaps under the frame. Avoid large rocks that prevent the sampler from seating the net properly on the stream bottom.

**NOTE:** If there is too little water to collect the sample with the D-frame net, randomly pick up 10 rocks from the riffle. Wash anything attached to the rocks (e.g. sand, insects, vegetation, etc.) into the closed or sieve bottomed bucket and pick any remaining organisms off and place them into the bucket.

5. While holding the net in position on the substrate, visually define a rectangular quadrat that is one net width wide and one net width long upstream of the net opening. The area within this quadrat is 0.09 m<sup>2</sup> (1 ft<sup>2</sup>).

6. Check the quadrat for heavy organisms, such as mussels and snails. Remove these organisms from the substrate by hand and place them into the net. Pick up any loose rocks or other larger substrate particles in the quadrat. Use hands or a small scrub brush to dislodge organisms so that they are washed into the net. Scrub all rocks that are golf ball-sized or larger and which are over halfway into the quadrat. Large rocks that are less than halfway into the sampling area should be pushed aside. After scrubbing, place the substrate particles outside of the quadrat.

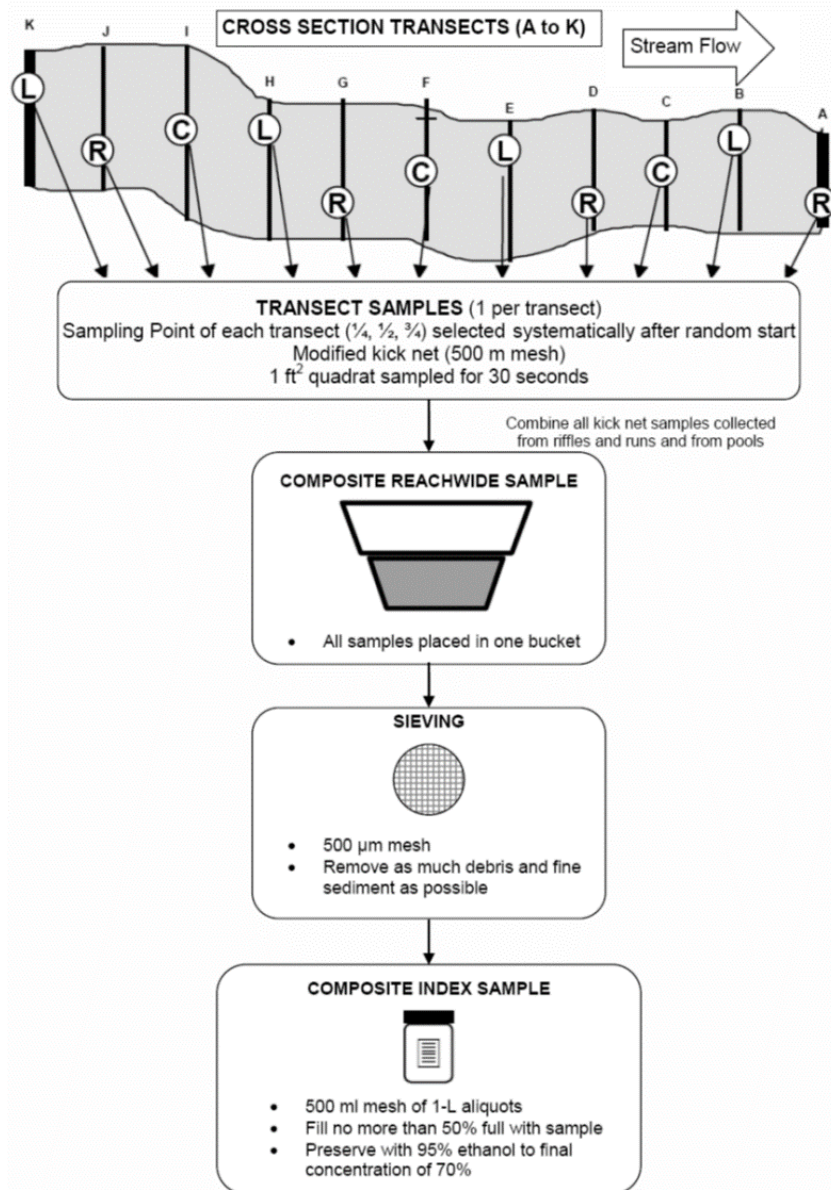
7. Keep holding the D-net securely in position. Start at the upstream end of the quadrat; vigorously kick the remaining finer substrate within the quadrat for 30 seconds (use a stopwatch).

**NOTE:** For samples located within dense beds of long, filamentous aquatic vegetation (e.g., algae or moss), kicking within the quadrat may not be sufficient to dislodge organisms in the vegetation. Usually these types of vegetation are lying flat against the substrate due to current. Use a knife or scissors to remove only the vegetation that lies within the quadrat (i.e., not entire strands that are rooted within the quadrat) and place it into the net.

8. Pull the net up out of the water. Immerse the net in the stream several times to remove fine sediments and to concentrate organisms at the end of the net. Avoid having any water or material enter the mouth of the net during this operation. Go to Step 13.

**Pool/Glide habitats:**

9. Visually define a rectangular quadrat that is one net width wide and one net width long at the sampling point. The area within this quadrat is -0.09 m<sup>2</sup> (1 ft<sup>2</sup>).



**Figure 7.17.2.** Transect Sampling Design for the Benthic

## Macroinvertebrate Sample

10. Inspect the stream bottom within the quadrat for any heavy organisms, such as mussels and snails. Remove these organisms by hand and place them into the net or bucket. Pick up any loose rocks or other larger substrate particles within the quadrat and hold them in front of the net. Use hands (or a scrub brush) to rub any clinging organisms off of rocks or other pieces of larger substrate (especially those covered with algae or other debris) into the net. After scrubbing, place the larger substrate particles outside of the quadrat.

**NOTE:** If the water is too deep to effectively “kick” the substrate in front of the net, face upstream and jab/sweep the net through the 0.09m<sup>2</sup> quadrat. So that individuals from previous jabs do not escape the net after each sequence, remove the net completely from the water and replace it at the start of the quadrat. Three series of jabs/sweeps through the quadrat should be sufficient to obtain a sample at each transect.

11.—Vigorously kick the remaining finer substrate within the quadrat while dragging the net repeatedly through the disturbed area just above the bottom. -Keep moving the net all the time so that the organisms trapped in the net will not escape. -Continue kicking the substrate and moving the net for 30 seconds.

**NOTE:** If there is too little water to use the kick net, stir up the substrate with gloved hands and use a sieve with 500 µm mesh size to collect the organisms from the water in the same way the net is used in larger pools.

12. After 30 seconds, remove the net from the water with a quick, net surfacing motion to wash the organisms to the bottom of the net.

### All samples:

13. Invert the net into a bucket and transfer the sample. Inspect the net for any organisms clinging to the mesh and use forceps to deposit them into the bucket. -Carefully inspect any large objects (e.g. rocks, sticks, or leaves) in the bucket and wash any organisms into the bucket. Discard as much rocks, sediments and detritus as possible without losing any organisms.

14. Thoroughly rinse the net before proceeding to the next sampling location. Proceed upstream to the next transect (including Transect K, the upstream end of the sampling reach) and repeat Steps 1 through 14. Combine all kick net samples from riffle/run and pool/glide habitats into the bucket.

## Sample Processing

Use a closed bottom or sieve bottom bucket while sampling to carry the composite sample to the next transect. -Alternatively, place each sample in a five-gallon bucket and use a soil sieve (500 $\mu$ m = 0.5 mm) to cull-down the sample before it is packed and preserved in a Nalgene container(s) upon completion (Figure 7.17.2). Record the composite sample tracking information on a field recording form shown in (Figure 7.17.3).

1. Pour the entire contents of the bucket through a sieve (or into a sieve bucket) with 500 $\mu$ m mesh size. Remove any large objects and wash any clinging organisms back into the sieve before discarding.
2. Use a wash bottle filled with stream water to rinse all the organisms from the bucket into the sieve. -This is the composite reach-wide sample for the site.
3. Estimate the total volume of the sample in the sieve and determine how large a jar will be needed for the sample (500-mL or 1-L) and how many jars will be required.
4. Wash the contents of the sieve to one side by gently agitating the sieve in the water. Wash the sample into a jar using as little water from the wash bottle as possible. Use a large-bore funnel if necessary. If the jar is too full pour off some water through the sieve until the jar is not more than  $\frac{1}{2}$  full or use a second jar if a larger one is not available. Carefully examine the sieve for any remaining organisms and use watchmakers' forceps to place them into the sample jar.
5. Completely fill the jar(s) with 95% ethanol (no headspace). It is very important that sufficient ethanol be used, or the organisms will not be properly preserved.

**NOTE:** Prepared composite samples can be transported back to the vehicle before adding ethanol if necessary. Fill the jar with stream water, which is then drained using the net across the opening to prevent loss of organisms and replaced with ethanol.

6. Place a waterproof label (Figure 7.17.4), with the following information, inside of each jar. Use a lead pencil when writing waterproof label as ink will deteriorate in Ethanol.

- Project Code
- Type of sample
- Sample container size
- Date of collection
- Sampler Name
- Site Identification number
- Number of transect samples composited (Jar N of X)
- Preservation fixative
- Time of collection

**NOTE:** Write “Jar N of X” on each sample label using a waterproof marker (“N” is the individual jar number, and “X” is the total number of jars for the sample).

7. Replace the cap on each jar. -Slowly tip the jar to a horizontal position, and then gently rotate the jar to mix the preservative. -Do not invert or shake the jar.

8. Fill in outside jar labels with the stream information listed in step 6. -Attach the completed label to the jar and cover it with a strip of clear mailing tape. Record the sample information for the composite sample on the Sample Log Form (Figure 7.17.5). For each composite sample, make sure the number on the form matches the number on the label.

9. Store labeled composite samples in a container with absorbent material that is suitable for use with 95% ethanol until transport or shipment to the laboratory.

## 9.0 DATA AND RECORDS MANAGEMENT

Macroinvertebrate data will be recorded on the field form 7.17.3 (Appendix A). Once personnel reach the office, data recorded on the field form are entered into the DWQ Ecological Data Application System (EDAS). Field notes should be used to record any quality control activity performed such as measurements taken by more than one sampler, or to record any sampling conditions that may have interfered with the data collected. Field forms and notes should be stored in the appropriate project folder at DWQ.

## 10.0 QUALITY ASSURANCE AND QUALITY CONTROL

Quality assurance and quality control (QA/QC) procedures will be followed as explained above. Individuals will have to follow the field and laboratory standard operating procedures to comply with the QA/QC for collecting and processing macroinvertebrate samples.

## 11.0 REFERENCES

- Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish*. Second Edition. EPA/841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Assessment and Watershed Protection Division, Washington, D.C.
- Klemm, D.J., P.A. Lewis, F. Fulk, and J.M. Lazorchak. 1990. *Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters*. EPA/600/4-90/030. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.
- Klemm, D.J., J.M. Lazorchak, and P.A. Lewis. 1998. Benthic Macroinvertebrates. pp. 147- 182 IN: J.M. Lazorchak, D.J. Klemm, and D.V. Peck (Eds.). *Environmental Monitoring and Assessment Program-Surface Waters: Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams*. EPA/620/R-94/004F. U.S. Environmental Protection Agency, Washington, D.C.
- Peck, David V., James M. Lazorchak, and Donald J. Klemm. 2001. *Environmental Monitoring And Assessment Program-surface Waters: Western Pilot Study field Operations Manual For wadeable Streams*. EPA/XXX/X-XX/XXX. U.S. Environmental Protection Agency, Washington D.C.
- Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. *Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish*. EPA/440/4-89/001. U.S. Environmental Protection Agency, Assessment and Watershed Protection Division, Washington, D.C.

### Related DWQ SOPs

7.18 Laboratory Processing of Macroinvertebrate Samples

7.21 Selection of Reference and Disturbed Sites for Biological Monitoring in North Dakota

**APPENDIX A**

Field Collection Data Recording Form



**SITE ID:** \_\_\_\_\_ **DATE:** \_\_\_\_\_ / \_\_\_\_\_ / \_\_\_\_\_

**FIELD NUMBER** \_\_\_\_\_ **SAMPLERS:** \_\_\_\_\_

**STATION DESCRIPTION:** \_\_\_\_\_  
\_\_\_\_\_

**LATITUDE:** \_\_\_\_\_ **LONGITUDE:** \_\_\_\_\_

**ECOREGION** (circle one): 43 42 46 48

**INVERTEBRATE COLLECTION METHOD** (circle one): D-NET OTHER \_\_\_\_\_

**REACH LENGTH:** \_\_\_\_\_ meters

FIELD WATER CHEMISTRY	SITE PHOTOS
TEMP:	UPSTREAM:
DO:	DOWNSTREAM:
pH:	COMMENTS:
COND:	

**WEATHER CONDITIONS** (Temp., Wind, etc.):

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

**COMMENTS:**

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Figure 7.17.3. Macroinvertebrate Field Collection Data Recording Form.

**SITE DRAWING** (Show direction of water flow and north)

**COMMENTS:**

**Figure 7.17.3 ctd.** Macroinvertebrate Field Collection Data Recording Form (reverse).

Checked by: \_\_\_\_\_ Date: \_\_\_\_\_

09/23/2020

## **APPENDIX B**

Macroinvertebrate Sample Jar Labels (both inside and outside)

<p>Project Code _____ Site ID#: _____</p> <p>Site#: _____ Field#: _____</p> <p>Analysis: Macroinvertebrates Jar _____ of _____</p> <p>Container: 1L Preservation: 95% Ethanol</p> <p>Date ____/____/____ Time ____:____</p> <p>Sampler:</p>
<p>Project Code _____ STORET#: _____</p> <p>Site#: _____ Field#: _____</p> <p>Analysis: Macroinvertebrates Jar _____ of _____</p> <p>Container: 1L Preservation: 95% Ethanol</p> <p>Date ____/____/____ Time ____:____</p> <p>Sampler:</p>
<p>Project Code _____ STORET#: _____</p> <p>Site#: _____ Field#: _____</p> <p>Analysis: Macroinvertebrates Jar _____ of _____</p> <p>Container: 1L Preservation: 95% Ethanol</p> <p>Date ____/____/____ Time ____:____</p> <p>Sampler:</p>
<p>Project Code _____ STORET#: _____</p> <p>Site#: _____ Field#: _____</p> <p>Analysis: Macroinvertebrates Jar _____ of _____</p> <p>Container: 1L Preservation: 95% Ethanol</p> <p>Date ____/____/____ Time ____:____</p> <p>Sampler:</p>

**Figure 7.17.4.** Macroinvertebrate Sample Jar Labels (both inside and outside).

**APPENDIX C**  
Macroinvertebrate Sample Log



Field Number	Station ID and Description	Date/Time	Collection Method	Observer(s)	Comments

Figure 7.17.5. Macroinvertebrate Sample Log.

## **APPENDIX D**

SOP Acknowledgement and Training Form





