Benthic Macroinvertebrate Sampling Standard Operating Procedure

Water Quality Control Division • Environmental Data Unit

1.0 Introduction

The use of benthic macroinvertebrates for assessing and monitoring the condition of lotic systems has become increasingly widespread and acceptable in the domain of Colorado's water quality standards setting. Macroinvertebrates are particularly suitable indicators of the condition of lotic systems as they are found in almost all freshwater environments. They are easy to sample and identify and different taxa show varying degrees of sensitivity to pollution and other impacts (Boothroyd & Stark 2000). The recent advent of statewide multi-metric indices or the "bioassessment indicator tool" necessitates supplementary macroinvertebrate data from which to support the use of this indicator tool within the Water Quality Control Division's assessment methodology.

2.0 Scope and Applicability

This standard operation procedure describes semi-quantitative methods for collecting a single aquatic benthic macroinvertebrate sample from perennial, wadeable streams.

Perennial is defined as a well-defined channel that contains water year round during a year of normal rainfall with the aquatic bed located below the water table for most of the year. A perennial stream exhibits the typical biological, hydrological, and physical characteristics commonly associated with the continuous conveyance of water.

Wadeable is defined as a waterbody that can be safely traversed when collecting samples. Separate protocols are provided for hard-bottomed and soft-bottomed streams. Benthic macroinvertebrate data collected on un-wadeable large rivers or intermittent type streams are beyond the scope of this procedure.

3.0 Index Period

The index period is the period of time that samples shall be collected to minimize seasonal variation. The standard index period utilized by the Water Quality Control Division ("division") in Biotypes[1] 1 and 2 shall be late June to November 30. For Biotype 3, the index period shall be May 1 to November 30.

These periods are congruent with the central tendency of sample dates of macroinvertebrate replicates used to regionally calibrate the multi-metric indices.

4.0 Sampling Frequency

This protocol recommends that benthic macroinvertebrate samples be collected once per year per site and within the standardized index periods provided in Section 3.0.

[1] Biotype – biological similar regions in Colorado as established during multi-metric index development.

5.0 Site Selection

The study reach length shall be one of the following: 1) 40 times the average wetted width of the wadeable waterbody or 2) long enough to encompass multiple riffles (for hard-bottomed sites) or glides/pools/microhabitats (for soft-bottomed sites) from which to produce a single, representative sample from the predominant habitat type. The study reach should be representative of the typical habitat conditions that occur at or immediately above and below the greater stream segment.

Riffle habitat refers to the portions of the stream where moderate velocities and substrate roughness produce turbulent conditions which break the surface tension of the water and may produce whitewater (Bain and Stevenson, eds. 1999). A glide generally refers to a calm stretch of shallow water flowing smoothly.

Although riffle areas with hard-bottomed substrates are generally the most diverse and productive habitat type in mountainous streams, these may not be entirely representative of the overall types of habitat present within the study reach. Alternately, although glide/pool areas with soft-bottomed substrates are generally the most diverse and productive habitat type in plains and plateau streams, these may not be entirely representative of the overall types of habitat present within the study reach.

There are some advantages to taking samples in or near the thalweg. Especially in small streams, the thalweg portion of the riffle usually has larger and cleaner substrate, better food supply and more reliable flow. When the thalweg is not or cannot be sampled, attention must be paid to the recent history of flow. Many Colorado streams are subject to flow variation on a short time scale due to flow regulation. Substrate that has been inundated only recently or that is inundated only occasionally should not be sampled because it is unlikely to support many specimens.

5.1 Hard-Bottomed Streams

A hard-bottomed stream is one where the stream substrate is dominated by particles gravel size or larger. Riffle habitats are common in these high to moderate gradient streams. Gravel, cobble and boulder sized substrate are frequent in these streams. These types of streams are conducive to the single habitat approach described in Section 6.0.

5.2 Soft-Bottomed Streams

Soft-bottomed streams are usually low gradient, often found in the Eastern Plains and in the far western xeric plateaus of Colorado, and are dominated by glide/pool habitats. The dominant substrate is sand, silt, clay or mud. Gravel, cobble and boulder sized substrate are naturally rare or entirely absent in these low gradient streams. These types of streams are conducive to the multi-habitat approach described in Section 6.0.

6.0 Sample Collection Information

There are two sampling procedures used to collect the required single representative sample within a stream reach:

- Semi-quantitative sample collection of hard-bottomed streams that focuses collecting macroinvertebrates from riffle habitats. These samples are collected using a modified kick net.

- Semi-quantitative sample collection of soft-bottomed streams that focuses collecting macroinvertebrates from non-riffle habitats, such as vegetated bank margins, submerged woody debris or snags and aquatic macrophytes. These samples are generally collected using a jab or sweeping technique that utilizes the same modified kick net.

Semi-quantitative sample collection methods are designed to collect the widest variety of aquatic macroinvertebrates available at the study reach. For these methods, it is not necessary to know the exact area sampled. Both procedures are suitable for use with both relative abundance and fixed count processing protocols from which a variety of species richness and relative abundance metrics (Stark et al 2001) and multi-metric predictive model analysis can be calculated.

In hard-bottomed streams or those streams predominated by substrate greater than gravel size, a single sample shall consist of a one-minute timed sample collected over an area of one square meter (1 m^2). The investigator shall select a single riffle or run from within the study reach that represents the predominant velocity and substrate type.

In soft-bottomed streams or those streams predominated by substrate smaller than sand size, a single sample shall consist of several individual sweeps or jabs collected from a fixed area of approximately 1 m^2. The multi-habitat sampling effort is limited to 1 minute. Time spent traversing from one habitat type to another is not included in the total time.

If the predominant habitat type expected to occur at the site does not occur along the defined study reach, then the investigator should specify some other stable and productive habitat type to sample. In circumstances where hard-bottomed or cobble substrates represent less than 20% of the study reach, multi-habitat(s) will need to be sampled. Alternatively, in circumstances where hard-bottomed or cobble substrates represent greater than 80% of the study reach in plains/xeric streams, then a riffle sample will need to be collected.

6.1 Equipment and Supplies

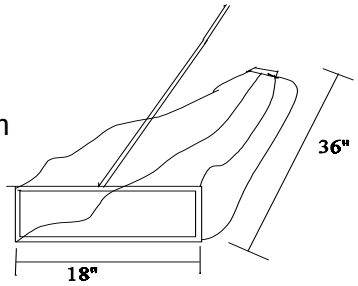
- Kick-net (see specifications in Section 6.1.1)
- Sieve dolphin bucket (504 µm mesh)
- 1-liter wide-mouth sample jars with screw tight lids
- 95% ethanol stored in sealed and labeled polyurethane carboys or bottles
- 1-liter rinse bottle
- Fine-tip forceps
- Number 30 (600 µm) **or** 35 (500 µm) standard sieve
- 5-gallon bucket with handle
- Dissecting tray
- Pre-printed, rectangular "*Rite in the Rain"* labels
- Standard #2 pencil(s)
- Waterproof stop watch
- Tape measurers (100 ft or more)
- Galvanized metal stakes or nails

- 48 quart or larger ice chests or sealable Rubbermaid totes
- Safety glasses
- Rubber or nitrile gloves
- Hip or chest waders with wading boots
- Applicable field sheets

6.1.1 Kick Net Specifications

The kick net is comprised of the following components:

- 18" x 8" rectangular frame
- 500 to 600 µm mesh nylon bag with canvas reinforced bottom and shroud reinforced opening (≈ 36" long)
- 1 or 2 piece long handle (≈ 70" long)
- Sieve dolphin bucket (504 µm mesh)
- Sieve dolphin bucket adaptor



7.0 Sample Collection

The following section discusses procedures used to collect benthic macroinvertebrate samples in perennial, wadeable streams.

7.1 Riffle Habitat Method

- Ensure that the sampling net and sieve bucket are clean prior to usage.

- Select the dominant riffle habitat within the study reach according to Section 5.0.

- Place the net frame flush to the streambed with the frame open to the upstream flow. Check that the nylon bag and sieve bucket are freely floating immediately downstream of the net frame. This will ensure that once the substrate is disturbed that specimens will be directed through the nylon bag and into the capture sieve bucket.

- Carefully lower the handle forward in an upstream direction until the sampling net is nearly horizontal to the water surface but the net frame is still flush to the streambed. The point at which the tip of the handle extends along the streambed is the point at which the kicking activity will cease. This distance multiplied by the width of the net frame equals one square meter. Return the handle to its vertical position.

- Position yourself next to sampling net and begin to disturb the substrate immediately upstream of the net. Disturb the substrate using the heel of your boot or entire foot by kicking to dislodge the upper layer of cobbles or gravel and to scrape the underlying bed. The area disturbed should extend no further than the point delineated and not exceed 1 minute. Approximately 0.25 meters should be disturbed for every 15 seconds.

- Larger cobble may be scraped by hand, if necessary, to remove specimens. Cobble should be scraped clean quickly and efficiently as the scraping is counted within the one minute time frame.

- Transfer material (matrix of specimens and insubstantial amount of stream substrate/detritus) from the interior of the net and sieve bucket into the sample jar and

wash or pick all specimens off the net interior. Specimens that cling to the exterior of the net are not considered part of the sample. They may be removed and placed back into the stream.

- Release back into the stream any fish, amphibians, reptiles or crayfish/rusty crayfish caught in the net.

- If excessive or large debris items are present refer to Section 7.4.1.

- The kick-net should be rinsed clean by backwashing with site water before collecting additional samples.

- At this point refer to Section 7.4 for sample processing.

7.2 Multi-Habitat Method

- Ensure that the sampling net and sieve bucket are clean prior to usage.

- Sample multiple habitats, as defined below, using the following procedures. The design is to sample an equivalent of a 1 meter sweep across multiple non-riffle habitats. Avoid dredging the kick net through mud or silt and clumps of leafy detritus or algal material. Also avoid hard-bottomed substrates as those habitats will be sampled separately according to Section 6.0.

  Woody Debris or Snag

  Jab the kick net into an area of submerged and partially decayed woody debris to dislodge specimens, followed by 1-2 "cleaning" sweeps through the water column to capture specimens in the water column. Scrub larger debris by hand over the opening of the kick net. The area of the larger debris should be included in the one meter unit effort.

  Bank Margins

  Locate an area of bank within the study reach. Jab the kick net vigorously into the bank for a distance of 1 meter to dislodge specimens, followed by 1 to 2 "cleaning" sweeps to collect specimens in the water column.

  Aquatic Macrophytes

  Sweep the kick net through submerged or emergent vegetation for a distance of 1 meter to loosen and capture specimens, followed by 1 to 2 "cleaning" sweeps to collect specimens in the water column.

- Transfer material (matrix of specimens and insubstantial amount of stream substrate/detritus) from the interior of the net and sieve bucket into the sample jar and wash or pick all specimens off the net interior. Specimens that cling to the exterior of the net are not considered part of the sample. They may be removed and placed back into the stream.

- Release back into the stream any fish, amphibians, reptiles or crayfish/rusty crayfish caught in the net.

- If excessive or large debris items are present refer to Section 7.4.1.

- The kick-net should be rinsed clean by backwashing with site water before collecting additional samples.

- At this point refer to Section 7.4 for sample processing.

7.3 Field Duplicates

One out of ten (10%) sample events shall include a duplicate field sample to ensure quality control (QC). For example, when a biological survey consists of collecting benthic macroinvertebrates at 10 stations, then 1 out of the 10 stations shall include a duplicate field sample. It is acceptable to increase the rate of duplicate field samples (QC>10%). However, it is unacceptable for the rate to fall below 10%.

The duplicate field sample shall be collected within the same habitat type and in close proximity to the standard field sample and in a manner consistent with procedures set forth in Sections 7.1 or 7.2.

7.4 Sample Processing (On-site)

Sample processing is characteristically conducted in the field. Sample processing consists of excessive material or large debris item removal and rinsing, elutriation (if necessary), preservation, and storage.

7.4.1 Removing Excessive and Large Debris Items

Picking and rinsing should be performed in a Number 30 (600 µm) or 35 (500 µm) standard sieve. Rinse off and remove any excessive debris such as algal clumps or large debris items such as leaves, sticks, or rocks that will not fit into a 1-liter sample jar or will lessen the effectiveness of the preservative. Calmly rinse the debris with stream water over the sieve opening using care not to cause unnecessary splattering of material. Examine larger debris to ensure that all specimens have been thoroughly rinsed or scraped into the sieve. Discard the material.

Transfer the remaining sample matrix in the sieve to a 1-liter wide-mouth polyethylene sample jar. Each sample jar should be no more than half full of sample material. Consequently, splitting the sample into two or more sample jars is acceptable. See Section 7.4.4 for labeling split samples.

7.4.2 Elutriation

Elutriation is a technique used to extract specimens from excessive substrate that has been captured during the sample collection process. This technique works best when the substrate is comprised of fines, sands and pebbles and should be used in circumstances when the amount of substrate is disproportionate to the amount of the detritus/specimen matrix. This step follows the removal of large debris items detailed in Section 7.4.1.

Keeping the sample in the 5-gallon bucket, add stream water to the bucket. Gently swish the sample around in the bottom of the bucket to liberate organic material and macroinvertebrates from the substrate. Pour the water and all floating material and specimens into a Number 30

(600 µm) or 35 (500 µm) standard sieve. This process may not work for heavy invertebrates such as snails, larger annelids or case-building caddis flies that use sand. Continue rinsing in a similar fashion 2-3 more times to maximize retention of specimens collected. If it appears that the heavy invertebrates are not being separated from the substrate, pour the remaining sample in the bucket into a tray and spread the sample homogenously across the bottom of the tray. Use forceps to remove remaining specimens and place them into the sieve.

Transfer the remaining sample matrix in the sieve to a 1-liter wide-mouth polyethylene sample jar. Each sample jar should be no more than 1/2 full of sample material. Consequently, splitting the sample into two or more sample jars is acceptable. See Section 7.4.4 for labeling split samples.

7.4.3 Sample Preservation

Sample preservation is very important to ensure the integrity of the benthic organisms collected from the site. The sample is preserved by decanting as much remaining water as possible and then filling the jar with 95% ethanol (ETOH) so the ETOH is 1" above the detritus/specimen matrix. Gently invert the sample jar several times to thoroughly homogenize the sample and preservative. This will make certain that the entire sample is preserved. Poorly preserved specimens can impede the identification and enumeration process. Any liquid leaking from the jar lid with the bottle inverted indicates an incomplete seal.

Allowing for dilution with water remaining in the sample container, the minimum ethanol concentration should always be greater than 70%. If in doubt, or with samples containing a large amount of organic material, the ethanol should be decanted after initial preservation and replaced with fresh 95% ethanol. In general, the volume of the container should contain no more than 50% of the sample.

7.4.4 Labels

Add pre-printed, moisture resistant labels to both the inside and outside of the sample container. Affix the label to the outside using transparent packaging tape. Pull back a corner of the packaging tape prior to affixing the label so the tape/label can be easily removed later once the taxonomist returns the 1-liter jars.

The following information should be recorded with a pencil on each label and placed in each sample container:

- Site number
- Stream name
- Stream description
- Date
- Check if kicknet was used
- Check habitat type sampled
- Collector's initials
- Indicate if sample is a duplicate
- Indicate if sample is split

If splitting the sample among several containers, label appropriately to indicate that the sample has been split (e.g., Sample 1 of 2 and Sample 2 of 2). If not, simply label sample as 1 of 1.

7.4.5 Storage

Place the sample jars in a hard-cased ice chest or equivalent container for transport to the laboratory. Ensure that jar lids are thoroughly tightened to eliminate leakage and fumes from developing inside vehicle cargo holds or truck beds.

8.0 Invasive Species

Precautions must be taken to avoid collecting invasive species from areas where the Colorado Parks and Wildlife ("CPW") has issued urgent closures to the taking of those species. Those species captured incidentally and prohibited under order of the Director of the Colorado Department of Natural Resources must either be immediately returned to the waterbody, where it was captured, or immediately killed.

Further precautions must be taken to avoid inadvertently transferring invasive species from one waterbody to another waterbody. This is best accomplished by following appropriate disinfection procedures of personal gear (i.e. waders/boots) or sampling equipment, as instructed by CPW.

9.0 References

Bain, M. B., and Stevenson, N. J., eds. 1999. Aquatic habitat assessment: Common methods. American Fisheries Society, Bethesda, MD.

Boothroyd, I. K. G; Stark, J. D. 2000: Use of invertebrates in Monitoring In: New Zealand Stream Invertebrates: Ecology and implications for management. Collier K. & Winterbourn, M. J. eds. New Zealand Limnological Society, Hamilton. Pp. 344-373.

Stark, J. D.; Boothroyd, I. K. G; Harding, J. S.; Maxted, J. R.; Scarsbrook, M. R. 2001: Protocols for sampling macroinvertebrates in wadeable streams. New Zealand Macroinvertebrate Working Group Report No. 1. Prepared for the Ministry for the Environment. Sustainable Management Fund Project No. 5103. 57p.

10.0 Document Version

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11.0 Approval Signatures

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11/17/2016

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