STANDARD OPERATING PROCEDURE FOR AQUATIC BENTHIC MACROINVERTEBRATE COLLECTION IN STREAMS

WATER QUALITY

State of Utah
Department of Environmental Quality
Division of Water Quality

Revision 2.0
Effective April, 2021

Foreword

Utah Division of Water Quality (DWQ) Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical experts. This document is intended primarily for internal DWQ use. This SOP should not replace any official published methods.

Any reference within this document to specific equipment, manufacturers, or supplies is only for descriptive purposes and does not constitute an endorsement of a particular product or service by DWQ. Additionally, any distribution of this SOP does not constitute an endorsement of a particular procedure or method.

Although DWQ will follow this SOP in most instances, there may be instances in which DWQ will use an alternative methodology, procedure, or process.

The methodology detailed below is the protocol followed by DWQ's monitoring staff and verified by DWQ's Quality Assurance officer.

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REVISION PAGE

Date	Revision #	Summary of Changes	Sections	Other Comments
5/1/14	0	Not applicable	Not applicable	New SOP adapted from USEPA EMAP protocol. Began document control/revision tracking.
12/4/2020	1.0	Changed name to SOP_StreamBMI_2021_v0	All	Previous name: SOP_StreamBMI_5.1.14_Rev0
3/23/21	2.0	Updated edge habitat to 1m linear sweeps.	9.3.2	
3/23/21	2.0	Updated language, grammar, and structure	All	Clarified and revised sentence structure and grammar throughout the entire document.
3/23/21	2.0	New field form created	Appendix 2	

Table of Contents

1.0 SCOPE AND APPLICABILITY

This document presents the Utah Division of Water Quality's (DWQ) Standard Operating Procedure (SOP) for the collection of aquatic benthic macroinvertebrates (BMI) within running waters (rivers and streams). Benthic macroinvertebrates are also commonly referred to as benthos, inverts, macroinverts, macroinvertebrates, or simply "bugs". Collection of BMI is routinely performed during DWQ's state-wide river and stream surveys. An example is the Utah Comprehensive Assessment of Stream Ecosystems (UCASE), see **Section 12**. BMI data is collected because:

1. BMI are relatively quick and inexpensive indicators for identifying a wide variety of pollutants

2. BMI typically exhibit a predictable community composition under natural conditions

3. BMI are a temporally-integrated water quality indicator versus water chemistry samples (which are essentially a snapshot of current conditions)

4. Some BMI are especially useful for targeted sampling due to their high sensitivity to environmental changes (e.g., impacts of remediation or pollutant discharges)

This SOP is applicable to rivers and streams. For collection of BMI in wetlands, refer to DWQ's SOP for Collection of Macroinvertebrates in Wetlands.

Note: If BMI samples are intended for regulatory purposes by outside (non-DWQ) entities, samples must be analyzed by an accredited laboratory with documented QA/QC and analytical procedures approved by DWQ. Please first contact DWQ for questions about specific details.

2.0 SUMMARY OF METHOD

Biological measures require sampling an extended length of waterway for a representative picture of the ecological community. To determine the representative sampling area a reach length of 40 times the channel wetted width (at base flow) is established. Riffle habitat is targeted when sampling running waters because of greater BMI diversity, ease of collection, and consistency. However, if a site is devoid of riffles, then glide or edge habitat is targeted. **Further explanation on habitat designations can be found in the Procedure, Section 9.2.**

The collection technique consists of a semi-quantitative benthic macroinvertebrate composite sample using a D-frame net. A composite sample is performed by collecting 8 subsamples at different locations within the reach. The sampler carries a sieve-bucket as they move through the reach to composite the benthic material collected in the D-net at each subsample location. At each of the 8 subsample locations, the sampler attempts to collect all available BMIs located in a one square-foot area upstream of the D-net opening. BMI are collected from all substrates in the one square-foot area to a depth of approximately 3 inches. The sampler rinses the material to the bottom of the net and then empties the contents of the net into the sieve-bucket. This process is repeated

at the remaining seven subsample locations. The result is a composite BMI sample in the sieve-bucket.

Sample processing is required for the composite sample to remove inorganic material including gravel and cobbles which are not targeted and can damage the BMI in transport. The BMI in the sample also must be concentrated into small jars for transfer to the analytical laboratory. Processing involves using a regular 2.5-gallon bucket and water to separate out heavy inorganic material from lighter organic material (where the BMI are most likely located). This separation process results in a much smaller volume of material which is then placed into 1 L plastic jars (it is acceptable to use a different container size, but a ~1L jar is most desirable) and preserved with 95% ethanol. Multiple jars may be required for one sample. Jars are then sealed, labeled, and stored until delivery to the laboratory.

Field data and other sampling details during BMI collection is recorded on a Sample Collection Form (**Appendix 2**).

3.0 DEFINITIONS

BMI: benthic macroinvertebrates

Bucket: A closed bottom bucket that differs from the sieve-bucket.

Sieve-bucket: Bucket with a sieve screen instead of a solid bottom.

ft^2: square foot

L: liter

mm: millimeter

MSDS: Material Safety Data Sheet

QA/QC: Quality Control and Quality Assurance

µm: micrometer

Reach Length: Overall distance of the sampling area along a river or stream

4.0 HEALTH AND SAFETY WARNINGS

Field personnel should be aware that hazardous conditions potentially exist at every waterbody. If unfavorable conditions are present at the time of sample collection, it is recommended that the sample collection be rescheduled. If hazardous conditions arise during sampling, such as lightning,

high winds, rising water, or flash flood warning, personnel should cease sampling and move to a safe location.

Take appropriate precautions when operating equipment and working on, in, or around water, as well as possibly steep and unconsolidated banks, bridges, or edges of ponds/lagoons. All field crews should follow DWQ health and safety procedures and be equipped with safety equipment such as proper wading gear, personal flotation devices (PFDs), gloves, first aid kits, cellular phone, etc.

Wear gloves or be sure to wash hands after sampling, especially when sampling wastewater discharges or ponds, lagoons, or other potentially contaminated sampling points at regulated facilities.

When working in Utah and other warm climates, precautionary steps should be taken to avoid heat induced illnesses such as heat stroke or heat exhaustion.

Use caution when working in waders as drowning hazards exist.

Prior to sampling be sure to review the MSDS for the preservation chemical. Pure ethanol (200-proof, 95% ethyl alcohol) is preferred for sample preservation. However, denatured alcohol may be used with caution. Wear gloves and wash off any denatured alcohol that comes in contact with skin. Denatured alcohol contains hazardous components and should not be inhaled, ingested, or come into contact with skin.

5.0 CAUTIONS

Use caution when working in streams where swift currents are possible that could cause equipment damage. Rocks and obstructions can interfere with the flow and sample collection. Be sure the net is properly placed to allow the sample to flow into the net.

Alcohol is flammable. Keep alcohol containers away from heat, sparks, flame, and all other sources of ignition. Do not smoke in the vicinity of alcohol or fumes.

6.0 INTERFERENCES

Field personnel should scout the potential sampling reach to make sure it is clear of obstacles that would prohibit sampling and data collection activities. Make every effort to avoid walking within the proposed stream reach prior to sampling to ensure biological organisms remain unaffected.

Samples must be stored and handled appropriately. Samples must be collected in the appropriate sample containers with the appropriate preservative; failure to preserve a sample properly can lead to inaccurate results, sample degradation, or invalidation of the sample by the laboratory.

Ensure preservative is adequately mixed within the sample. If the sample is not properly preserved, microbes will persist and may result in the degradation of macroinvertebrate bodies. Additionally, ensure the samples are submitted to the laboratory no later than 1 year after collection to reduce likelihood of sample decomposition.

Refer to all instructions within this SOP for setting up the sampling reach and targeting the proper sample habitat in order to collect a representative sample.

For representative data, it is best to collect BMIs during the growing season, which can vary depending on elevation and latitude, but is generally limited to the months of May through October.

7.0 PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

DWQ personnel performing BMI water sampling must be familiar with sampling techniques, safety procedures, proper handling, and record keeping. Samplers are responsible for attending refresher meetings held each spring/summer to review procedures and techniques. New staff will be trained in the field and lab by DWQ personnel.

Cooperators are required to read this SOP annually and acknowledge they have done so via a signature page (see **Appendix 1**) that will be kept on-file at DWQ along with the official hard copy of this SOP.

8.0 EQUIPMENT AND SUPPLIES

The following equipment and supplies are required for benthic macroinvertebrate collection at wadable sites:

For collecting samples (Figure 1)

- ❑ Flagging for marking reach boundary
- ❑ Kick net (D-frame with 500 µm mesh) with at least a 4-foot long handle or a modified surber with at least a 4-foot long handle.
- ❑ Stopwatch (or watch, phone, tablet, etc.)
- ❑ Plastic bucket (8 or 10 quart size)
- ❑ Sieve-bucket with 500 µm mesh openings
- ❑ Plastic forceps
- ❑ Spray bottle

- ❑ 1-Liter HDPE Nalgene sample bottles
- ❑ 95% ethanol (ETOH) in proper container
- ❑ Rangefinder or Measuring Tape

For recording measurements

- ❑ Sample Labels (**Figure 3**) – labels for jar interior must be printed on waterproof paper; labels for jar exterior can be printed on regular paper. Both labels MUST be filled out in pencil.
- ❑ Lead pencils
- ❑ Fine tipped markers
- ❑ Clipboard, Sample Collection Form (**Appendix 2**) and field notebook
- ❑ Clear tape strips to cover sample labels

9.0 PROCEDURE

9.1 Pre-Sampling Preparation

Prior to visiting a site, inspect the D-net/modified surber and sieve-bucket for holes or tears and replace or repair.

At the site, find an area downstream of the reach and wash all equipment with stream water. Fill the spray bottle with stream water.

9.2 Setting up the Sampling Reach

At the sampling location, measure the channel width at various points to determine the average wetted width, and then calculate the reach length by multiplying 40 times the average channel wetted width (during base flow). Mark each end of the reach using stakes/flags. The ideal length of sub-reaches is the reach length divided by 10. It is best to spread these sub-samples out evenly along the entire reach.

Note: There may be instances where the full reach is unattainable because of private property or obstructions. If this is the case, ensure 8 sub-samples are collected targeting riffles.

While within the boundary of the reach (preferably mid-reach), collect and record GPS coordinates (in decimal degrees) as well as the reach length. Record these on the Sample Collection Form (**Appendix 2**).

Determining Target Habitat and Substrate type

Substrates:

- Fine/sand (F): Not gritty (silt/clay/muck <0.06 mm diam.) to gritty, up to lady bug-sized (2 mm)
- Gravel (G): Fine to coarse gravel (ladybug to tennis ball-sized; 2 mm to 64 mm)
- Coarse (C): Cobble to boulder (tennis ball to car-sized; 64 mm to 4000 mm)
- Other (O): Bedrock (larger than car-sized; >4000 mm), hardpan (firm, consolidated fine substrate), wood of any size, aquatic vegetation, etc. Note type of "other" substrate in comments on field form.

Habitats:

- Pool (P): Still water; low velocity; smooth, glassy surface; usually deep compared to other parts of the channel.
- Glide (GL): Water moving slowly, with smooth, unbroken surface; low turbulence.
- Riffle (RI): Water moving, with small ripples, waves, and eddies; waves not breaking and surface tension is not broken; "babbling" or "gurgling" sound.
- Rapid (RA): Water movement is rapid and turbulent; surface with intermittent "white water" with breaking waves; continuous rushing sound.
- Edge (ED): Habitat: The margin of the stream along the bank, look for locations with macrophytes, woody debris, overhanging vegetation to disturb where habit for bugs is present.
- Run**:** Very similar to a riffle with a larger space between riffle peaks and higher velocity. DWQ considers riffle and run synonymous.

Note: Target coarse substrates such as large gravel (pea-size and larger) to small boulders (basketball-size and smaller) rather than substrates at either spectrum. If coarse substrates are lacking, woody debris, macrophytes, or leaf packs could be targeted; please, identify and document these situations.

Sampling edge habitat along the stream banks provides the best alternative to riffle samples (due to BMI diversity, ease of collection and consistency). Often, overhanging vegetation, sticks, and other material will offer protection and stability for BMI to colonize. In many cases, Utah streams may be absent of riffles and coarse sediments (e.g. desert streams in Southern Utah are predominantly characterized by glides and fine/sandy sediments). It is important to target edge habitat in these cases to get a representative BMI sample.

When making the determination regarding habitat type it is important to keep in mind riffles, glides, etc., often appear different in different sized streams. A large stream riffle would appear as a rapid in a small stream, so evaluate these habitats with the stream size in mind.

9.3 General Sample Collection at Sub-reaches

1. Gather the clipboard and Sample Collection Form, stopwatch, D-net, and both buckets (see **Figure 1**)

2. Begin walking upstream along the reach. **Sampling always begins at the downstream end of the reach targeting riffles.** However, it is acceptable to sample downstream if 8 riffles are not found on the first pass.
3. Collect 8 subsamples within the reach. See procedures below for habitat specific sample collection. **If 8 riffles are not present, a second kick could be repeated within one riffle. However, perform no more than 2 kicks per riffle** (see **Figure 2**).
4. To reduce human bias, alternate locations in the stream (e.g. left-25% of channel width, center- 50% of channel width, right- 75% channel width).
5. Start randomly with one of these locations, and then consistently follow the pattern of left (L), center (C), right (R) and repeat until 8 subsamples are collected (see **Figure 2** as an example).
6. At each of the 8 collection points in the reach: (See form in **Appendix 2**)
    a. Record habitat type (pool, glide, riffle, etc.)
    b. Record dominant substrate (fines/sand, gravel, etc.)
7. Follow the sample collection instructions specific to habitat type as listed in **9.3.1 (Riffle/Run Habitat)** and **9.3.2 (Edge and Pool/Glide Habitat)**.

9.3.1 Procedures for Riffle Habitats (Preferred)

1. With the net opening facing upstream, quickly and firmly position the net securely in the stream bottom to eliminate gaps under the frame. This will ensure flow space is limited (i.e. flow is directed into the net, not under). Avoid large rocks that prevent the net from sitting properly on the stream bottom.

Note: It can be difficult to keep the net flush with the bottom, especially in high gradient cobble-dominated streams. Make an attempt to get the net as flush with the substrates as possible, and if there are issues or concerns, record them on the Sample Collection Form.

2. Holding the net in position on the substrate, visually define an area that is one net-width wide and one net-width long upstream of the net opening. This is a one square-foot sampling area.

3. Check the sampling area for heavy organisms, such as mussels and snails. Remove these organisms by hand and place them into the bucket. Pick up loose rocks or large substrates that would reduce the effectiveness of kicking. and use your hands to dislodge organisms from their surfaces and wash them into the net. Ensure that the substrate remains in front of the net opening and flows are directed into the net. Large substrates that fall partially in the sampling area should be included in this process. Following this process, discard larger substrates outside of the sampling area.

4. Use your boot to disturb the remaining sediments. If you are kicking in soft sediment, ensure that you do not exceed a depth of 3 inches. Disturb the sediments for approximately 30 seconds.

5. 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 (dip the net material only, not the mouth).

6. Empty the contents of the net into the bucket and repeat seven more times as you move upstream. Alternatively, you do not need to empty the net if it has only very little material at this point.

Note: The purpose of this procedure is to disrupt the sediment and dislodge all macroinvertebrates. Depending on the site, this may look different. Refer to the field coordinator for any confusion.

7. Go to the next sampling location.

9.3.2 Procedures for Edge/Pool Habitats

Note: Sample edge habitat if you are at a site where beaver ponds are common or the site lacks riffles and coarse substrates.

1. Visually identify an area along the margin of the stream with potential habitat (leaves, sticks, debris, roots).

2. Agitate or disturb any overhanging vegetation, sticks, or other material on the stream bed and bank area with your hands or feet. Immediately follow this by making a 1-meter linear sweep with the bug net through the disturbed area. Be careful not to sweep the bank or bottom of the stream to prevent excess sediment from entering the net.

3. Make an effort to sweep with the net in the direction of flow, if there is any, to prevent anything from being washed back out of the net.

4. When doing 1-meter sweeps empty the net into a bucket each time to protect sample integrity.

Note: If flow is too little or slow to sweep organisms into the D- kick net, stir up the substrate with your hands and sweep the water through the fixed net.

9.4 Sample Processing and Preservation

1. After sampling is complete, separate the organic material from the heavier, inorganic material in the bucket. Gently, dump the composited material from the net into the plastic bucket. Inspect the net for any remaining bugs that may still be clinging to it. Using a wash bottle with stream water and/or forceps, flush/pick them off the net and into the bucket.

2. Next, fill the plastic bucket with stream water a few inches above the material line. Slowly swirl the contents in the bucket for about 7 seconds so that lighter (organic) material (sticks, leaves, organisms) comes to the surface and heavier material (inorganic substrates) stay at the bottom. While the material in the bucket is suspended and swirling, slowly pour the water into the sieve-bucket making sure not to dump any of the heavier material at the bottom of the bucket with it. Repeat this step several times until no more bugs are seen crawling around in the plastic bucket. This process is known as sieving.

Note: If there is an abundance of pebbles or cobbles in your sample, you will need to rinse (scrub if necessary) these off in the bucket with ample amounts of site water and then discard them.

Note: Do not attempt to sieve samples with an abundance of filamentous algae. If this is the case, simply include all of the algae into the sample jar since it is difficult to effectively process these kinds of samples in the field.

3. Ultimately, you will end up with a bucket containing coarse gravel and sand, and a sieve-bucket containing organisms and detritus (it is okay if some fine sediment is present in the sieve-bucket). Be sure to inspect the bucket with coarse substrates for caddisfly cases as they will appear as fine gravel, and could be missed. Once the sieving process is complete, you can dump the heavy material left in the 2.5-gallon bucket into the stream or on the ground.

4. Place the material in the sieve-bucket into a 1-L jar making sure not to fill it more than 40% full with sample material; use multiple jars if necessary.

Note: Be sure not to grab a large handful where material will become dislodged on the mouth of the bottle when you are filling it. Keep in mind some material will stick to your hands during each transfer. It is a good idea to rinse your hands in the sieve-bucket each time you put material in a jar.

5. As the volume of material becomes less abundant at the bottom of the sieve-bucket you will need to wash the remaining contents to one side of the sieve-bucket in order to get the rest of the sample by gently agitating the bottom outside portion of it.

6. Once you think you have removed everything from the sieve-bucket, carefully examine it for any remaining organisms. If there are still visible organisms use a pair of forceps to pick the bugs out.

7. Place a completed sample label (**Figure 3**) inside each jar (each label for the site should be filled out exactly the same except for the Jar # of total Jars, e.g., Jar 1 of 3). *Labels to be placed **inside** the jar must be printed on waterproof paper and filled out by hand using a **pencil**. Ink will fade eventually due to the ethanol.*

8. Completely fill the jar with 95% ethanol (no headspace). It is very important that sufficient ethanol be used or the organisms will not be properly preserved. Existing water in the jar should not dilute the concentration of ethanol below 70%.

Note: Samples can be transported back to the vehicle before adding ethanol if necessary. However, if the site is >15 mins walk from the vehicle (e.g. hike into site) a liter of ethanol should be taken to the site with you.

9. Replace the cap on each jar. Gently mix the sample. After mixing, seal each jar lid with electrical tape.

10. Place a sample label (**Figure 3**) on the outside of each jar making sure it coincides with the interior label. Fill it out in pencil and cover it with clear tape.

11. Transport sample bottles in an upright location and store bottles on a shelf until shipment. Samples do not need to be refrigerated or stored on ice.

9.5 Decontamination

Before leaving the site, sampling equipment and personal gear (boots, waders, etc.) need to be thoroughly decontaminated in order to prevent the spread of aquatic invasive species. For additional information for decontamination and aquatic invasive species see the Utah Division of Wildlife Resources AIS information site.

Before leaving the site or the parking area (if you hiked in):

1. Thoroughly wash the sampling equipment with stream water to remove debris. This is usually performed at the truck prior to leaving the location. If it is a hike in site, decontaminate upon return to the truck.

2. Spray sampling equipment with *Sparquat 256®*.

3. It is recommended that you wear latex gloves and eye protection when using *Sparquat 256®*. This product is an industrial cleaner and standard safety precautions should be followed.

4. Do not discard the *Sparquat 256®* solution or the rinse water in the field! Dispose of the liquid down a drain that is routed to a wastewater treatment plant.

10.0 DATA AND RECORDS MANAGEMENT

Before leaving the field site, be sure that all required samples have been collected, labeled, and that all appropriate field sheets, field notes, and sample tracking forms have been filled out completely and accurately.

Send samples to the lab at the National Aquatic Monitoring Center within one year of collection.

Reach out to the specific lab if you need to store samples for longer. General information suggests sample storage within 1 year is ok. Degradation can occur between years one and five. Procedures for handling sample results can be found in the appropriate project-specific SAP.

11.0 QUALITY ASSURANCE AND QUALITY CONTROL

BMI samples should be analyzed by an approved laboratory with documented QA/QC and analytical procedures provided to DWQ.

Frequency and type of quality control samples, particularly field replicates, will be described in project-specific Sampling and Analysis Plans (SAPs).

12.0 REFERENCES

Peck, D.V., J.M. Lazorchak, and D.J. Klemm (editors). Unpublished draft. Environmental Monitoring and Assessment Program -Surface Waters: Western Pilot Study Field Operations Manual for Wadeable Streams. EPA/XXX/X-XX/XXXX. U.S. Environmental Protection Agency, Washington, D.C.

USEPA. 2017. National Rivers and Streams Assessment 2018/19: Field Operations Manual – Wadeable. EPA-841-B-17-003a. U.S. Environmental Protection Agency, Office of Water Washington, DC.

Related DWQ Documents:

Utah Division of Water Quality: Quality Assurance Program Plan for Environmental Data Operations
Revision # 1.0, Effective September 5, 2014

Utah Comprehensive Assessment of Stream Ecosystems (UCASE) Field Operations Manual, Version 2, 2018

13.0 FIGURES

Figure 1. **Sampling equipment: Sieve-bucket, bucket, net, sample form, stopwatch (tablet, phone, watch, etc.)**

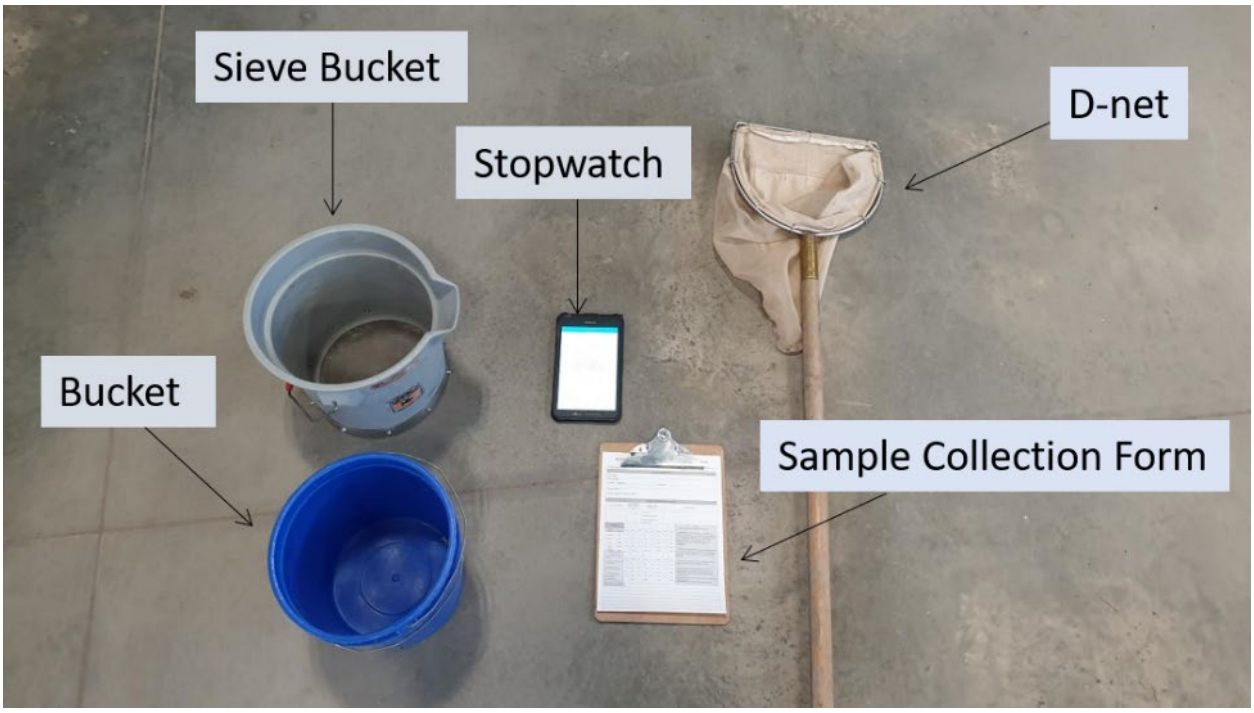


Figure 2. **Sampling locations within a reach.**

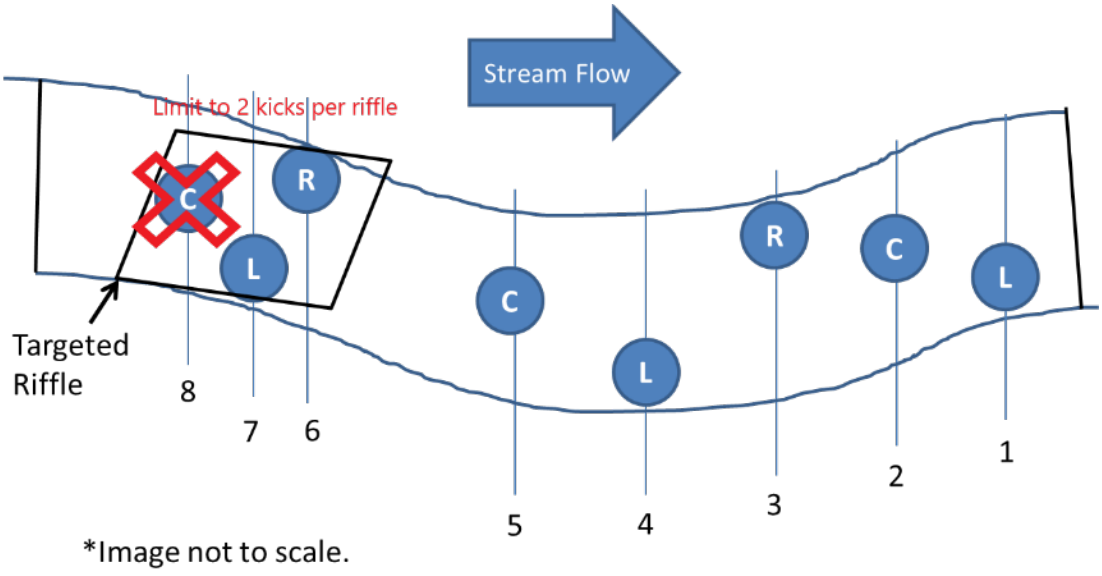
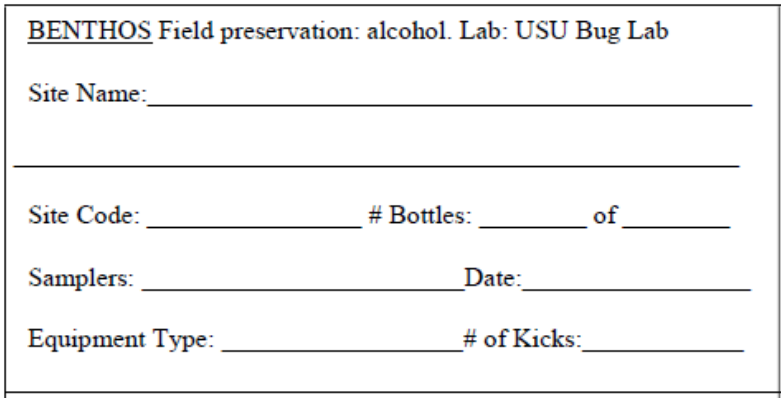


***Figure 3*. Example of a properly filled-out sample label.** Template location is U:\WQ\PERMITS\MONITORS\Labels\UCASE Labels



14.0 APPENDICES

Appendix 1 - SOP Acknowledgement and Training Form

SOP Acknowledgement Form

This SOP must be read and this form signed annually. This form must be kept with the current version of the SOP.

Document Title:	
Document Revision Number:	
Document Revision Date:	

Please sign below in accordance with the following statement: "I have read and understood the above referenced document. I agree to perform the procedures described in this SOP in accordance with the document until such time that it is superseded by a more recent approved revision."

Printed Name	Signature	Date

SOP Acknowledgement and Training Form (continued)

Trainee: Sign below to acknowledge that training on this SOP was received, understood, and all questions/concerns were addressed by the trainer.

Trainer: Sign below to acknowledge that training on this SOP was completed for the individual listed and that trainee is competent to perform the procedures described within.

Date of Training	Trainee Printed Name	Trainee Signature	Trainer Printed Name	Trainer Signature

Appendix 2 – Sample Collection Form

BENTHIC MACROINVERTEBRATE SAMPLE COLLECTION FORM FOR WADEABLE STREAMS

Reviewed by (initial): _____

SITE/STORET ID: ___ ___ ___ ___ ___ ___ ___ ___ **DATE:** ___ ___/___ ___/ 2 0 ___ ___

WATER CHEMISTRY

Site Name:

Site Location:

NAD83 Longitude: ___ ___. ___ ___ ___ ___ ___ **Longitude:** ___ ___ ___. ___ ___ ___ ___ ___

Wetted Width:

Reach Length (40x wetted width):

TARGETED BENTHOS SAMPLE

No. of Jars (Primary)	Was a Replicate Sample Taken (If Yes, record no. of jars?	Collection Method (Choose one)	Comments/Flags
___ ___	Y / N ___ ___	○D-net ○Modified surber ○Other (indicate in comments)	

Sub-sample		1		2		3		4	
Dom. Substrate	Channel	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.
Fine/Sand	Pool	○F	○P	○F	○P	○F	○P	○F	○P
Gravel	Glide	○G	○GL	○G	○GL	○G	○GL	○G	○GL
Coarse	Riffle	○C	○RI	○C	○RI	○C	○RI	○C	○RI
Other: Note in Comments	Rapid	○O	○RA	○O	○RA	○O	○RA	○O	○RA
	Edge		○ED		○ED		○ED		○ED
SUBSTRATE SIZE CLASSES F/S – ladybug or smaller (<2 mm) G – ladybug to tennis ball (2 to 64 mm) C – tennis ball to car sized (64 to 4000 mm) O – bedrock, hardpan, wood, vegetation, leaf litter, undercut, macrophytes, etc.		5		6		7		8	
		Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.
		○F	○P	○F	○P	○F	○P	○F	○P
		○G	○GL	○G	○GL	○G	○GL	○G	○GL
		○C	○RI	○C	○RI	○C	○RI	○C	○RI
		○O	○RA	○O	○RA	○O	○RA	○O	○RA
			○ED		○ED		○ED		○ED

Note:
- Always perform 8 kicks at every site.

- Target riffle habitat primarily. If riffles are scarce or absent (e.g. low gradient, beaver ponds, etc.), target edge habitats (e.g. overhanging veg., undercut banks, etc.) and mark "O" in the substrate column and explain situation in comments.

- If riffles are present, but scarce, multiple kicks can be performed at the same riffles throughout the reach. NOT MORE THAN 2 KICKS PER RIFFLE.

- If kicks are made in beaver influenced areas, please explain in the comments section.

Process: Fill a clean wide-mouth bottle with ~40% of composite sample (use multiple bottles if necessary).
Preservation: Fill rest of bottle with denatured alcohol (EtOH) and seal lid with electrical tape. Keep secure in upright position.
Lab: Utah State University Bug Lab; submit in large batch at end of field season.
Shop storage: Store on shelf with other bug samples.

BENTHOS COMMENTS: