Standard Operating Procedure

Methods for Sampling Benthic Macroinvertebrate Communities in Wadeable Streams

Commonwealth of Kentucky
Energy and Environment Cabinet
Department for Environmental Protection
Division of Water

Version 5.0

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DOCUMENT REVISION HISTORY

Version and Effective Date	Page(s) Revised	Revision Explanation	Version Author and Reviewers
Version 5.0 March 2025	Throughout; Sections 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 9.0 and 10.0.	**Methods for Sampling Benthic Macroinvertebrate Communities in Wadeable Streams** -slight change to SOP title to clarify focus on streams. - Updated to new SOP format, including re-numbering sections and adding Summary of Method, Acronyms, Troubleshooting, and Data & Records Management elements. -Update language in Health and Safety Statement. -Copy language from other sections into Cautions & Interferences. Add some new language. -Personnel Qualifications – remove HAZWOPER. -Equipment and Supplies – add prospecting pans, remove COC, clarify net and bucket sizes. -Develop Troubleshooting elements. -Copy Data & Records Management elements from Habitat SOP.	Jacob Becraft, Jessica Shuster, Mary Rockey
	8.1	-Compile existing information into a focused "Sampling Considerations" section including index periods, scouring events, sampling reach, and selecting sampling methodology. -Copy information about scouring events from Habitat SOP. -Clarify "wadeable" determination and sampling reach determinations. -Add Figure 1 and details about selecting high versus low gradient sampling methods in bioregions and transitional zones from Habitat SOP. Transitional zones newly defined.	
	Section 8.2	-Changes to this section were all targeted at re-aligning the sampling methodology with historic methods used in MBI data collection/development and to clarify details. -Add language about what to do if high gradient streams lack riffle habitat. -Additional details on kick net sample methods and how to distribute riffle kicks across available riffles. -Clarify description of how to spread multi-habitat sample collection across mesohabitats within the full reach. -All microhabitat sampling descriptions were edited to clarify methodology. -Undercut banks roots – focus on pool run and include riffle only if other unavailable. -Focus all jab and sweep collection on runs and pools.	

Version and Effective Date	Page(s) Revised	Revision Explanation	Version Author and Reviewers
		-Depositional areas – clarify that net is an optional method (but not preferred) for either wadeable or headwater streams and clarify replicates should total 3 overall but include silt, sand, and gravel, where present. -Change "Edge" habitat to "Supplemental" to reflect that all areas should be considered.	
	Table 1	-Changes to this section were all targeted at re-aligning the sampling methodology with historic methods used in MBI data collection/development and clarify details. -Re-order target habitat rows, use new, more specific terminology "mesohabitat" and "microhabitat" to clarify what element of habitat is being referenced in text. -Combine wadeable/headwater depositional and edit replicates to 3 in each target substrate type and clarify totals. -Add "each" to Undercut banks roots wadeable replicates and clarify totals. -Add specific sampling technique to column 2. -Remove Riffle from Jab and Sweep habitats and change others to 'all available' rather than RRP.	
	Section 8.3	-Minor changes throughout, specific updates to descriptions of how to sample Undercut Banks/Roots, Snags/Woody Debris, Cobble/Gravel, and Wood Sample. Added Bedrock/Slabrock/Hardpan.	
	Section 8.0	-Remove all alternative sampling methods. These methods are not usable for 305(b) assessments.	
	Section 8.4	-Add details on sample processing and preservation.	
Version 4.0 March 6, 2015	Section 1.B. (pg. 6), Section 1.G.2 (pg.8), Section 1.G.3. (pg. 9), Section 1.G.4.1., Section 1.G.4.2.	**Methods for Sampling Benthic Macroinvertebrate Communities in Wadeable Waters** -Riffle, run, and pool are defined in the Definitions section. -Sample Reach section moved. -Sampling periods were updated with more specific directions on prioritization of streams that around the 5 square mile catchment area size. -More details on high-gradient and low-gradient stream collection are provided. -General format changes were made. -Table 2 was added for clarification.	John Brumley, Jessica Schuster
Version 3.0 January 24, 2011	Section 1.G.2. (pg. 7), Section 1.G.3. (pgs. 7-12).	**Methods for Sampling Benthic Macroinvertebrate Communities in Wadeable Waters** -Sampling periods were updated to reflect index periods used to create MBI.	Aric Payne

Version and Effective Date	Page(s) Revised	Revision Explanation	Version Author and Reviewers
		-High-gradient stream sampling and low-gradient stream sampling methodology was revised and reorganized for general content. -Table 1-1 and Table 1-2 from 2009 SOP were used to create Table 1. -Probabilistic and TMDL Monitoring sections were updated for general content and sampling protocols.	
Version 2.0 March 2009	Section 7. Macroinvertebrates	**Standard Methods for Assessing Biological Integrity of Surface Waters in Kentucky** -Collection Methods for Benthic Macroinvertebrates in Wadeable Waters was separated from preceding document and revised/updated for general content regarding macroinvertebrate field collection methods.	Jessica Bevins, Aric Payne, John Brumley
March 13, 2008		**Standard Methods for Assessing Biological Integrity of Surface Waters in Kentucky** -General Content -Document was re-formatted to maintain headers, section titles, etc. in a consistent style. -All references to detailed water chemistry sampling were removed, and a reference inserted directing the reader to the 'Standard Operating Procedures for Sampling and Monitoring Surface Waters for Kentucky', in draft.	
July 2002		**Methods for Assessing Biological Integrity of Surface Waters in Kentucky** original document	

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1.0 SCOPE & APPLICABILITY

This manual has been developed by the Kentucky Division of Water (DOW) as guidance for the uniform and accurate collection, field processing, field handling, and quality assurance/quality control (QA/QC) of benthic macroinvertebrate samples collected from the wadeable streams of Kentucky. The methods defined herein are required for all benthic macroinvertebrate sampling, field processing, field handling, and QA/QC activities resulting in information that could be used for 305(b) water quality assessments. Any data submitted to DOW for review will undergo QA/QC review and those identified as not following the methods set forth in this document will be flagged and discarded.

2.0 SUMMARY OF METHOD

This procedure outlines the collection methods for benthic macroinvertebrates in wadeable and headwater streams of Kentucky. Sampling procedures differ based on the gradient of the stream, which closely aligns with the bioregions of Kentucky. High gradient streams are sampled with four semi-quantitative 0.25 m^2 riffle kicks and a qualitative multi-habitat sample collection. Low gradient streams are sampled with 20 proportional semi-quantitative jab samples across specified habitat types. In addition, adherence to sampling index periods is very important for accurate bioassessments using macroinvertebrates since Kentucky biocriteria are calibrated for seasonality. Index periods are determined by the catchment area of the stream reach, with headwater streams (< 5 mi^2) being sampled from March to May and wadeable stream sampling occurring from May through September.

3.0 DEFINITIONS & ACRONYMS

BG – Bluegrass Bioregion
BMP – Best Management Practices
DOW – Kentucky Division of Water
EPA – United States Environmental Protection Agency
KDEP – Kentucky Department for Environmental Protection
MT – Mountain Bioregion
MACS – Mid-Atlantic Coastal Plain Streams
MVIR – Mississippi Valley-Interior River Bioregion
PR – Pennyroyal Bioregion
PPE – Personal Protective Equipment
QA – Quality Assurance
QC – Quality Control
SOP – Standard Operating Procedure
TKM – Traveling Kick Method
TMDL – Total Maximum Daily Load

Aufwuchs – The macroinvertebrates adhering to submerged, attached filamentous algae, moss, and macrophytes.

Benthic Macroinvertebrate – DOW defines benthic macroinvertebrates as organisms large enough to be seen by the unaided eye, retained by a U.S. Standard No. 30 sieve (28 mesh/inch, 600µm openings) and live at least part of their life cycle within or upon available substrates of a water body.

Microhabitat – Habitat unit type applied to stream features that are targeted for macroinvertebrate sample collection.

Mesohabitat – Habitat unit type applied to riffles, runs, and pools.

Pool – An area of a stream characterized by deep (usually > 0.5 m), slow velocity and a variety of substrate types. Because of slower velocities, sediment deposition can occur over pool substrate. Pools may have a higher diversity of permanent microhabitat types.

Riffle – An area of a stream with an observable decrease in gradient characterized by shallow (<0.5 m), fast velocity and **stable, layered** rock substrate. The surfaces of some substrate may be exposed above the waterline.

Run – An area of a stream characterized by deep (usually > 0.5 m), fast velocity and a variety of substrate types. Runs are commonly found downstream of riffles. In low-gradient streams, runs (also called glides) are the dominant habitat where velocity is faster than the surrounding habitats.

Thalweg – Path of deepest thread of water.

4.0 HEALTH & SAFETY STATEMENT

All field staff should review *Worksite Hazard Assessment Guidance Document* (DOW, 2025; or latest revision). In addition, each employee will be individually trained by his/her supervisor, or designee, to perform assigned job tasks safely, prior to his/her performing the task.

Field staff working in and around potentially contaminated surface waters should receive immunization shots for Hepatitis A in accordance with Kentucky Department for Environmental Protection (KDEP) Departmental Policy Memorandum SSE-708 (revision, 2007). In addition, staff should receive immunization for Hepatitis B and tetanus to aid in the prevention of contracting those pathogens. All field staff should also be trained in CPR, First Aid, and Blood Borne Pathogens in accordance with KDEP Departmental Policy Memorandum SSE-711 (2001). Members of a field crew should familiarize themselves with the nearest hospital, doctor's office, or urgent medical care provider prior to leaving for site visits.

Personal protective equipment (PPE) should be used when sampling. This includes but is not limited to: site-appropriate wading boots, personal floatation device, nitrile gloves, and appropriate field clothing for sampling conditions (i.e. cold weather clothing). Waders and

specialized wading boots should be utilized when conducting in-stream biological sampling to remain dry, but also to provide a barrier from potential in-stream contaminants, and natural irritants (i.e. biting insects, poison ivy). It is recommended that a wading belt be used to reduce the chances of water filling waders during a fall. Boots should have studded soles to reduce the chances of slipping or falling. Investigators should exhibit caution around stream bank mud, boulders, bedrock, or large woody debris to reduce the threat of a falling injury.

During high flow or runoff events, biological sampling should be postponed until baseline conditions exist. When these specific events are targeted, field crews shall use best professional judgment to obtain samples (i.e. postponement, high flow equipment, etc.). If stream conditions are determined unsafe by any field staff, do not sample during that time.

5.0 CAUTIONS & INTERFERENCES

The sampling procedures herein require specific training and a demonstration of competency due to the expert judgment exercised during field sampling. It is recommended that individuals conducting benthic macroinvertebrate collections should train with DOW staff (via workshops and/or participating in field sampling) to demonstrate competence.

Benthic macroinvertebrate sampling should be performed using appropriate sampling methodologies, during base flow conditions, and in the appropriate index period (see Section 8.0). If very low/no flow, high flow, or flooding conditions are present, data collection should be postponed. If a scouring rain event has occurred in the last 14 days, data collection should be postponed (scouring event is defined in Section 8.1.2). Failure to collect data during the appropriate season, under the appropriate conditions, and using the appropriate method can invalidate the data and make it not usable for biological assessment. If a potential monitoring reach has backwater from a lake, dam, or large river, this reach should not be used for biological assessment.

It is important to keep in-stream habitat intended for benthic macroinvertebrate sampling intact and undisturbed until samples have been collected. Therefore, field personnel should avoid walking through areas designated for collection of benthic macroinvertebrates until sampling has been completed. Failure to use caution could result in sample degradation.

When performing impact assessment in a stream, the contaminant plume from a point source or tributary may flow along one bank of the stream for some distance. Make sure benthic macroinvertebrates are sampled within the influence of the plume.

For special studies, any deviation from the procedures in this document must be noted in study documentation and approved by DOW biologists prior to sampling.

6.0 PERSONNEL QUALIFICATIONS

All biologists will meet minimum job classification requirements as specified by the Personnel Cabinet. Biologists will be certified in first aid, CPR, and blood borne pathogens and recertification will be completed as required. Biologists may continue training by reviewing numerous peer reviewed journals and attending professional conferences. In order to stay current with changing biological methods and taxonomy it strongly benefits each biologist to attend a minimum of one professional conference annually, as funds allow. As available, workshops in biological methods and taxonomy should be attended. A macroinvertebrate biologist will instruct any other personnel in sample protocol before sampling. At least one macroinvertebrate biologist will be present on all sampling events. New training requirements that are identified will be discussed with the Water Quality Branch Manager and/or section supervisors.

7.0 EQUIPMENT & SUPPLIES

The following supplies are needed to complete benthic macroinvertebrate sampling.

- High or Low Gradient Stream Datasheet (DOW 2025; DOWSOP03024)
- Sample Labels
- 600µm mesh, 0.5 meter wide rectangular net or kick seine
- 500-600 µm D-frame dip net
- U.S. Number 10 sieve
- U.S. Number 30 sieve
- 500-600µm mesh wash buckets (2)
- SE stackable prospecting pans (recommended with mesh wash bucket)
- 2.5-5gal white bucket
- Fine-tipped forceps
- White picking pans or similar containers
- Wide-mouth sample jars
- 95% Ethanol

8.0 STEP-BY-STEP PROCEDURE

Division of Water samples macroinvertebrate communities to establish reference conditions within the state, to assess aquatic life use-support, and to gain understanding of trends for streams in each bioregion. In addition, samples are acquired for comparative purposes for total maximum daily loads (TMDL) and best management practice (BMP) implementation evaluation. Standardized, semi-quantitative collections are made at all sampling locations. However, more precise quantitative data may be collected on a case-by-case basis or if biological monitoring requires more rigorous statistical analyses. Qualitative multi-habitat sweep collections are also made at most sites. In general, collection methods used by DOW are similar to those discussed in Lenat (1988), Plafkin et al. (1989), Klemm et al. (1990), Eaton and Lenat (1991), EPA (1997b), and Barbour et al. (1999).

8.1 Sampling Considerations

Seasonal and site condition criteria are critical in the determination of appropriate sampling methodologies and timing.

8.1.1 Index Periods

Collection of benthic macroinvertebrate samples within the designated index periods is critical for accurate assessments.

- Headwater streams (<5 mi^2 drainage area) are sampled from March 1 to May 31.
- Wadeable streams (≥5 - 200 mi^2 drainage area) are sampled from May 1 to September 30.

While preparing for the monitoring season, biologists will prioritize when certain streams will be sampled based upon catchment size, flow, rainfall, and geology. Within each index period, it is particularly important to prioritize sampling smaller streams earlier. DOW project coordinators are responsible for establishing a sampling plan that meets these index period requirements and the unique sampling needs of each project year. In order to target appropriate flow levels, DOW staff typically make an effort to sample streams with a catchment area of <2 mi^2 in March, 2-4 mi^2 in April, 5-10 mi^2 in May, and >10 mi^2 from May to September with very large streams being sampled in late summer when water levels are low enough to provide sampleable riffle habitat. However, this is not always possible and biological samples collected at any time within the two defined index periods (headwater and wadeable) under appropriate conditions are acceptable.

While the cutoff for using wadeable collection methods is 200 mi^2, there may be instances where streams with drainages of <200 mi^2 are considered non-wadeable. A stream must be shallow enough to be sampled using the methods described in this SOP and shallow enough to safely cross by wading. Streams with a drainage area of 150 – 200 mi^2 should be evaluated prior to sampling in order to determine if successful collection efforts are attainable.

In some cases, sampling outside these index periods is permissible to assess immediate impacts (e.g. chemical spills, leaks, etc.). For routine assessments or baseline data collection, samples collected outside of these index periods are considered unacceptable.

8.1.2 Scouring Events and Stream Flow

It is imperative that biologists monitor rainfall and flow conditions of streams within watersheds that will be sampled during a monitoring season. Benthic macroinvertebrate samples should not be collected during periods of excessively high or low flows or within two weeks of a known scouring flow event.

Determination of a scouring event is based upon the biologist's best professional judgment but is typically considered 2 inches or more of rainfall within a watershed in a 24-hour period. In addition, observations of recent high water such as signs that the stream has recently exceeded its banks, obvious removal of filamentous algae, signs of recently shifted substrate, new bank scarring, turbid waters, or a lack of macroinvertebrates on large instream rocks should be used

in making this decision. If a scouring event is suspected or identified, the full data collection event should be postponed for 14 days to allow for re-colonization of the target fauna.

8.1.3 Sample Reach

A representative reach of stream should be selected for sampling. Sample reach length may be determined using a fixed-distance or proportional-distance (e.g. 40x stream width) approach. No matter the method used, the stream reach should be no less than 100 meters and no more than 300 meters in length.

Tributary streams have the potential to greatly influence the biological communities of a sampling reach. It is desirable to either sample above or below the confluence of tributaries if they are deemed to be contributing significant flow to the receiving stream. Watershed features directly influencing the stream reach should be evaluated using GIS tools to make this determination.

8.1.4 Selecting Sampling Methods

It is critical that the appropriate sampling methodology be selected prior to the collection of any data. Streams in Kentucky are characterized as high gradient or low gradient streams and as headwater or wadeable streams. This stream classification is based on flow, presence or absence of particular types of habitat, and watershed size. An understanding of the differences in high gradient and low gradient streams is important as each gradient type has its own distinct sampling methodology. In addition, within the high gradient methodology, qualitative sampling methods differ based upon whether the stream is headwater or wadeable. DOW must approve any deviation from the methods described below.

High gradient

High gradient streams are defined as streams that have velocities greater than 0.013 m/sec (0.5 ft/sec), exhibit rapid changes in stream gradient, and have a high frequency of riffle habitat. These streams are found in the Mountain (MT), Bluegrass (BG), and Pennyroyal (PR) bioregions of Kentucky and any transition areas between those bioregions (Figure 1).

Low gradient

Low gradient streams are defined as streams that have velocities less than 0.013 m/sec (0.5 ft/sec) and naturally lack riffle habitat with stable rocky substrate. The most productive habitats of these streams are typically root wads, woody snags, undercut banks, and aquatic vegetation. These streams are found in the Mississippi Valley-Interior River (MVIR) Bioregion (Figure 1).

Streams lying within the transition area between the PR and MVIR bioregions may be difficult to classify as high gradient or low gradient (Figure 1, Inset 2). Assessors should use best professional judgment and the following guidelines to determine gradient within the PR-MVIR transition area:

- If stream gradient is not obvious, assessors should walk the sampling reach, make notes, and take photographs of shallow, fast areas of the stream and determine if

these mesohabitats are composed of stable riffles with prominent cobble and/or boulder substrate (which would indicate high gradient).

- Historical sampling information may also aid in making gradient determinations. If a station has been classified as high or low gradient in the past, the classification should carry forward in future assessments.

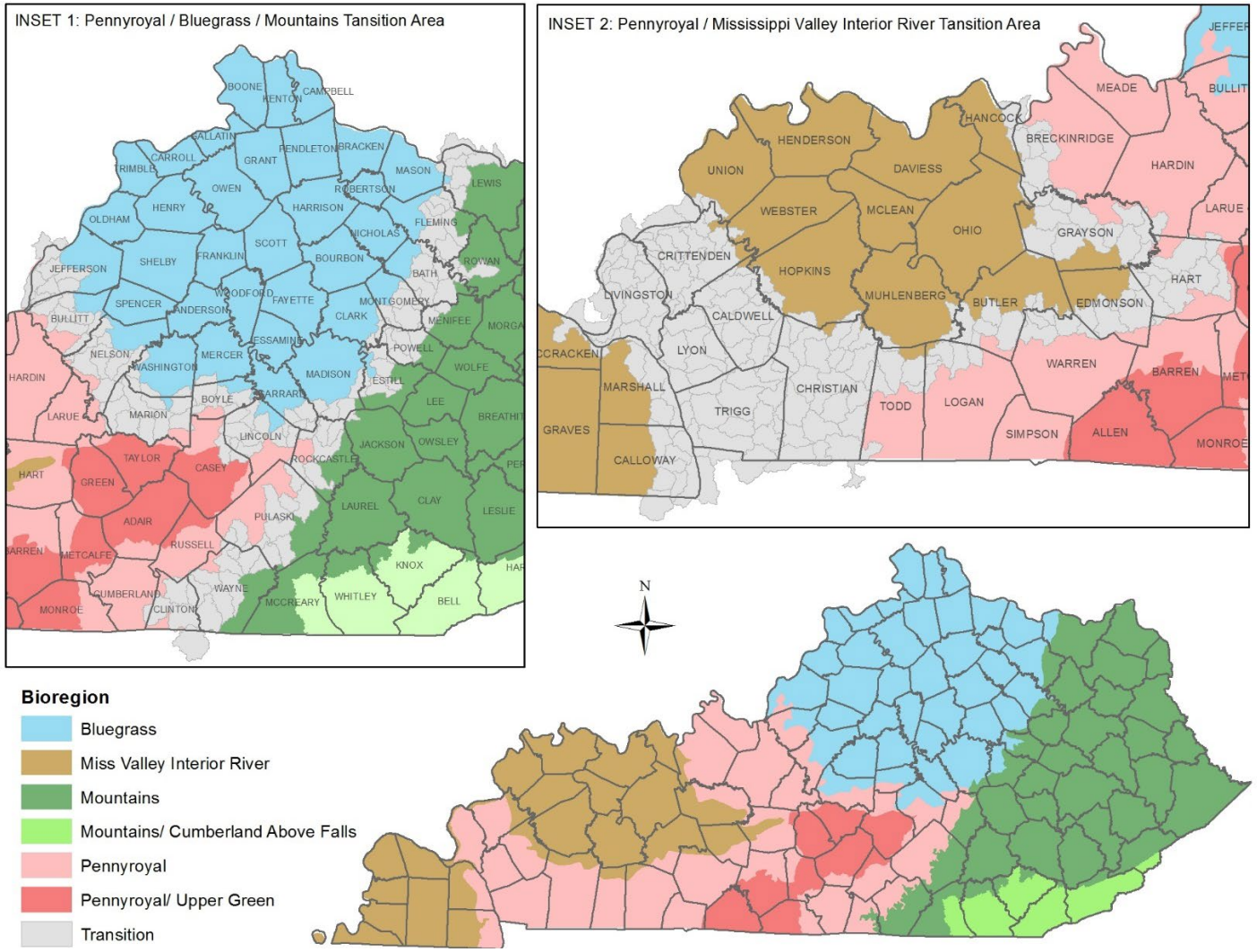

Figure 1. Bioregions of Kentucky and the transition areas between the bioregions. **Pennyroyal/Bluegrass/Mountains Transition Areas** require consideration of a secondary bioregion in station documentation (see Inset 1 and DOW 2025). **Pennyroyal/Mississippi Valley Interior River Transition Areas** require consideration of data collection methods (see Inset 2 and Section 8.1.4 Selecting Sampling Methods) and consideration of a secondary bioregion in station documentation (DOW 2025).

8.2 High Gradient Stream Sampling Methodology

The methods described in this section are modifications of the single and multi-habitat approaches outlined by Barbour *et al*. (1999).

In high gradient streams, riffle habitat predominates and is the primary targeted habitat. A collection consists of two types of samples:

1) a composited semi-quantitative riffle sample, and
2) a composited multi-habitat sample.

These two sample types must be kept separate. A summary of these collection techniques is shown in Table 1.

Special Considerations

There may be situations in which high gradient streams lack true riffle habitat. Under these circumstances, it is appropriate to use best professional judgment to identify where a riffle might be as determined by changes in stream bed slope and/or stream flow. For example, if the stream is dominated by sediment, a riffle can be defined as the area in which shallow water is moving quickly over an area that may have unstable gravel/cobble substrate. In streams dominated by bedrock, a riffle can be defined as the area in which a noticeable change in slope causes turbulence of the water surface. If a high gradient stream reach is one large pool or deep run, the reach should either be shifted or not sampled depending on project requirements. These considerations only apply to high gradient streams and should not be included in decisions of gradient determination in transition areas. To determine gradient of streams in transition areas, see section 8.1.4.

Table 1. Summary of sampling methods for headwater and wadeable high-gradient streams.

Microhabitat	Technique/Sampling Device	Targeted Mesohabitat	Replicates (composited)	
			Headwater	Wadeable
1m² stable riffle with layered substrate[1]	kick net or kick seine and mesh wash bucket	Riffle	4 – 0.25m²	4 – 0.25m²
Multi-habitat				
Undercut Banks/Roots	Jab and sweep; D-frame dip net and mesh wash bucket	Run-Pool	3 total, dispersed across targeted mesohabitat	6 total, 3 from each available mesohabitat
Sticks/Wood	Jab and sweep; D-frame dip net and mesh wash bucket	Run-Pool	3 total, dispersed across targeted mesohabitat	N/A
Marginal Emergent Vegetation	Jab and sweep; D-frame dip net and mesh wash bucket	Run-Pool	N/A	3 total, dispersed across targeted mesohabitat
Water Willow Beds	Jab and sweep; D-frame dip net and mesh wash bucket	Run-Pool	N/A	3 total, dispersed across targeted mesohabitat
Bedrock/Slab Rock/ Hardpan Clay	Scrape and sweep; D-frame dip net and mesh wash bucket	Run-Pool	N/A	3 total, dispersed across targeted mesohabitat
Leaf Packs	Collect by hand or jab and sweep with D-frame dip net; rinse in white or mesh wash bucket	Riffle-Run-Pool	3 total, dispersed across targeted mesohabitat	3 total, dispersed across targeted mesohabitat
Aufwuchs Sample	Collect by hand or sweep with D-frame dip net; rinse in white bucket	Riffle-Run-Pool	N/A	3 total, dispersed across targeted mesohabitat
Supplemental Habitat	D-frame dip net, fine-tipped forceps, and mesh wash bucket	All available	3 total	3 total
Silt, Sand, Fine Gravel	US No. 10 or 30 Sieve (or D-frame dip net); elutriate in white bucket	All available	3 total, dispersed across each available substrate	3 from each available substrate, up to 9 total
Rock Pick (large cobble/small boulder)	Wash into white bucket, then pick with fine-tipped forceps	Riffle-Run-Pool or Pool only	5 total (pool only)	15 total (5 from each Riffle-Run-Pool)
Wood Sample (5-15 cm diameter)	Wash into white bucket, then pick with fine-tipped forceps	All available	2 linear meters	3-6 linear meters

[1]Sample contents kept separate from other habitats.

8.2.1 Riffle (Semi-Quantitative) Sample

The semi-quantitative, riffle sample is comprised of four samples collected from four 0.25 m² quadrat kick net samples, also known as riffle kicks. Ideally, the riffle kicks should be portioned across four separate riffles. A sample reach may be extended up to 300 m to meet this goal, if the stream reach designation method allows. If riffle habitat is lacking in a stream, it is appropriate to sample fewer than 4 riffles. In this case, riffle kicks should be portioned across all available riffles. If only one riffle exists within the reach and extension of the reach is not possible due to habitat or programmatic restrictions, sampling effort can be limited to one riffle.

Riffles composed of stable rocks of varying sizes that provide a diverse habitat for macroinvertebrates should be prioritized for sampling, as should riffles that exhibit a variety of flow patterns. Water depth and flow within the riffle should be enough to allow for organisms to be swept into the kick net. Riffle kicks should, ideally, occur within the thalweg of the riffle.

The thalweg is targeted to ensure the highest species richness and abundance of benthic macroinvertebrates. The thalweg of a riffle also guarantees the most flow permanence and substrate stability.

A kick net sample is collected from a stationary position. The rectangular kick net should be placed in the riffle such that the mouth of the net is facing upstream, the bottom of the net is flush to the substrate, flow is directed into the net from the immediate upstream area, and there is sufficient flow through the net to pull dislodged organisms into the net bag. This placement and arrangement of the net should happen with little to no disturbance of the substrate to be sampled (i.e. the quarter meter square immediately upstream of the net) before sampling begins. However, removing some of the substrate underneath the net bag, especially boulders, may help ensure sufficient flow through the net.

Once the net is situated appropriately, larger substrate within the sampling area should be picked up and rubbed by hand with crevices inspected and picked to remove any attached organisms, such that the organisms drift into the net. These large substrate items can then be placed outside of the quarter meter square sampling area. Next, the sampler should stand upstream of the kick net, facing the net and thoroughly, but carefully use the toe and heel of the boot to shift the upper layer of cobble or gravel to the underlying bed within the quarter meter square.

After every kick, wash the collected material into the point of the net, and then empty the sample into a white bucket before proceeding to the next kick location. It is permissible if some organisms inadvertently remain in the net between kicks since they are combined, and the net can be thoroughly inspected after the final kick.

The four composited kick net samples should be processed following the methods outlined in Section 8.5. In addition, use forceps to remove organisms from the interior kick net surface and place them in the sample container.

8.2.2 Multi-Habitat (Qualitative) Sample

The qualitative sample is collected from multiple habitats that are present within the stream reach. The types of microhabitats targeted for multi-habitat sampling vary depending on stream size. Table 1 provides the sampling guidelines that should be followed when collecting qualitative samples in headwater and wadeable streams. When collecting samples from riffles, runs, and/or pools (mesohabitat), effort should be made to diversify the sampling among available mesohabitats and along the entire reach. If certain microhabitats are not available at a reach or within specific mesohabitats, these absences shall be recorded in the field notes. In such absences, samples may be collected from other mesohabitats such that the total number of sampling replicates listed in Table 1 is achieved (e.g. if emergent vegetation is only available in runs, all three replicates can be collected in runs). Descriptions of the microhabitats that are targeted and the methods for sample collection are found below. For samples collected with a jab and sweep method, care should be taken to avoid sample loss when sweeping through the disturbed area.

Undercut Banks/Root Mats *(Run-Pool; Headwater and Wadeable)*

If large, loose root wads are available, place the root wad into the dip net and shake vigorously. Otherwise, the D-frame dip net is placed under the root wad or undercut bank, jabbed for approximately 1m (or use foot to shake/jab stiff roots), and then swept through the area to collect dislodged organisms. The contents are removed from the dip net and placed into a mesh wash bucket. In headwater streams, 3 total samples are collected within the reach. In wadeable streams, at least three samples are collected within the reach. However, if undercut banks/root wads are present in more than one mesohabitat (i.e. runs and pools), then each is sampled separately with three (3) replicates each (for a total of 6 samples). If undercut roots are not sufficiently present in runs and/or pools, but are present in riffles, replicates can be supplemented with samples from riffles. If undercut banks and roots are not present in any mesohabitat, cuts under large boulders may be sampled if they are at banks of channel.

Sticks/Wood *(Run-Pool; Headwater only)*

This habitat is sampled by jabbing, kicking, or otherwise stirring up loose submerged wood piles, where present, and then sweeping through the area to collect dislodged organisms. Alternatively, material may be collected by hand and placed directly into sieve or net. Sticks/wood may be collected from piles on streambeds or snags under surface and in fast or slow flow. Material is then rinsed in the mesh wash bucket and any sticks or wood are thoroughly washed and inspected before discarding. A total of three samples will be collected. If debris piles are limited but contain both sticks/wood material and leaf packs, these collections can be counted as both sticks/wood and/or leaf pack sampling, but an effort should be made to still achieve target sample numbers for both.

Marginal Emergent Vegetation *(excluding Justicia americana beds) (Run-Pool; Wadeable only)*

This microhabitat is sampled by thrusting (i.e. “jabbing”) the dip net into the vegetation for approximately 1 m, and then sweeping through the area to collect dislodged organisms. Alternatively, sampling may be done by collecting vegetation by hand and placing directly into net. Material is then rinsed in the mesh wash bucket and any sticks, leaves and vegetation are thoroughly washed and inspected before discarding. A total of three samples are collected within the reach.

Water Willow (Justicia americana) Beds *(Run-Pool; Wadeable only)*

Water willow is a very common aquatic plant associated with marginal areas in wadeable streams. These beds are sampled by working the net through a 1 m section in a jabbing motion followed by sweeping or by sweeping through the area after kicking. The material is then emptied into a mesh wash bucket and any willow stems are thoroughly washed, inspected, and discarded. A total of three samples are collected within the reach.

Bedrock, Slab Rock, or Hardpan Clay *(Run-Pool; Wadeable only)*

Bedrock and Slab Rock (i.e. large broken slabs of bedrock) should be given preference over hardpan clay. If bedrock or slab rock are completely absent, hardpan clay can be sampled if it exists. These microhabitats are sampled by placing the edge of the dip net flush on the substrate and disturbing approximately 0.1 meter square of area to dislodge attached organisms. Material is emptied into a mesh wash bucket, rinsed, inspected for organisms, and discarded. A total of three samples are collected within the reach.

Leaf Packs *(Riffle-Run-Pool; Headwater and Wadeable)*

Leaf packs are to be sampled from riffles, runs, and pools of both headwater and wadeable streams. Leaf packs are preferably collected from "conditioned" material (i.e. not new-fall material) when possible. Conditioned leaf packs may be found in debris piles with sticks/wood not otherwise targeted in wadeable streams. Leaf packs can be collected by hand and placed directly into the mesh wash bucket (or white bucket). Alternatively, they can be sampled by jabbing and sweeping the net through the disturbed area. The collected material is thoroughly rinsed to dislodge organisms, inspected and cleaned of organisms, and then discarded. Three leaf packs will be sampled for each sampling reach.

Aufwuchs Sample *(Riffle-Run-Pool; Wadeable only)*

Aufwuchs sampling targets submerged, attached filamentous algae, moss, and/or macrophytes on which small macroinvertebrates adhere. Aufwuchs may be sampled by placing the edge of a dip net flush to the substrate downstream of the aufwuchs, thoroughly disturbing the area in front of the net (preferably by hand) and allowing streamflow to wash organisms into the net. In areas with slow to no flow, the dip net should be swept forward through the disturbed area 2-3 times to capture organisms suspended in the water column. This microhabitat may also be sampled by placing the sample directly into a white bucket half filled with water and thoroughly rinsing and elutriating the aufwuchs into the mesh wash bucket before inspecting for macroinvertebrates and discarding. A total of three samples are collected within the reach.

Supplemental Habitat *(All Available; Headwater and Wadeable)*

Supplemental habitat collections are meant to account for any microhabitat or substrate that may not be addressed in other listed habitat types but are observed within the sampled reach (e.g. stream margins, or "edge' habitat). Locations within the reach that have not been previously sampled for another habitat should be targeted. Collection procedures are based on what is most appropriate for the habitat chosen and can include selective picks, collection by hand, net grabs, or a jab and sweep. A total of three samples are collected within the reach.

Depositional Areas *(All Available; Headwater and Wadeable)*

Depositional areas are commonly associated with stream margins. A U.S. No. 10 and/or No. 30 sieve are used to scoop the substrate to an approximate depth of 5 cm to capture larger invertebrates (e.g. mussels, burrowing mayflies, dragonfly larvae) from silt, sand, and fine gravel. Optionally, a D-frame dip net can be used to sample by kicking and/or stirring up silt, sand, and fine gravel and then sweeping through the area to collect dislodged organisms. Material should be placed in a bucket and washed (larger pieces) and/or elutriated into the mesh wash bucket. In headwater streams a total of three (3) replicate samples are collected, distributed across all available substrates (silt, sand, and/or fine gravel). In wadeable streams, up to three (3) replicate samples are collected from each available substrate resulting in a total of up to nine (9) samples.

Rock Pick *(only Pool in Headwater; Riffle-Run-Pool in Wadeable)*

Benthic macroinvertebrates are picked from rocks (large cobble-small boulder size) in both headwater and wadeable streams. Selected rocks are washed in a white bucket half filled with water and then carefully inspected to remove organisms by hand or with fine-tipped forceps. Individuals are picked from 5 pool rocks in headwater streams and 15 rocks from each wadeable stream mesohabitat (5 each from riffles, runs, and pools).

Wood Sample *(All Available; Headwater and Wadeable)*

Submerged wood will be sampled in both headwater and wadeable streams. Less conditioned pieces of wood are individually rinsed into the white or mesh wash bucket and inspected for burrowers and crevice dwellers, which should be removed and placed in the sample container. Large diameter and/or well-aged logs, not recent deadfall, should be inspected and handpicked with fine-tipped forceps. Any individuals picked from logs are placed in the sample container. In headwater streams, a minimum of 2 meters (6 linear feet) of wood ranging from 5-15 centimeters (2-6 in) in diameter will be washed/picked, while 3 to 6 meters (10 to 20 linear feet) of wood ranging from 5-15 cm (2-6 in) in diameter will be washed/picked in wadeable streams. When the minimum targeted length of acceptable wood is not available in a stream, field collectors should sample from as much acceptable wood as possible and note this on the appropriate form.

8.3 Low Gradient Streams (MACS 20 Jab) Sampling Methodology

This sampling method follows, in part, the Mid-Atlantic Coastal Plain Streams Workgroup (MACS) protocol (EPA 1997a) and Barbour et al. (1999). The 20 jab method is considered proportional sampling, where some predetermined number of sample units (20 in this case) is allocated among major microhabitats in relation to their proportion found within a stream reach. Table 2 lists the major microhabitats that should be targeted in low gradient streams.

A sample unit is called a “jab” in which a D-frame dip net is thrust into the targeted habitat in a jabbing motion for approximately 0.5 meter and then swept with the net two or three times to

collect the dislodged organisms. If a jab becomes heavily clogged with debris and sediment, discard the material making sure that the net is free from any macroinvertebrates and repeat the jab. Under no circumstances should more or less than 20 jabs be collected per sample. All material is composited into a mesh wash bucket for further processing. Large leaves and twigs can be washed, inspected, and discarded to reduce the volume of the debris in the sample. Sand and sediment should be elutriated using a bucket and 600 µm sieve. As an example, if the stream reach is determined to be half undercut banks/roots, a quarter cobble/gravel, and a quarter leaf packs, the biologist would complete 10 jabs in the undercut banks/root wads, 5 jabs in the cobble/gravel, and 5 jabs in leaf packs, achieving a total of 20 jabs that were completed in the appropriate proportion.

Table 2. Major microhabitats targeted for the MACS 20 jab sampling method.

Microhabitat	Sampling Device	Targeted Mesohabitat
Proportional Jabs		
Undercut Banks/Roots	D-frame dip net and mesh wash bucket	All Applicable
Emergent Vegetation	D-frame dip net and mesh wash bucket	All Applicable
Snag/Woody Debris	D-frame dip net and mesh wash bucket	All Applicable
Cobble/Gravel	D-frame dip net and mesh wash bucket	All Applicable
Bedrock/Slab Rock/Hardpan Clay	D-frame dip net and mesh wash bucket	All Applicable
Supplemental Habitat	D-frame dip net and mesh wash bucket	All Applicable
Silt, Sand, Fine Gravel	D-frame dip net and mesh wash bucket	Margins
Leaf Packs	D-frame dip net and mesh wash bucket	Run-Pool
Pick		
Wood Sample (5-15 cm in diameter)	Fine-tipped forceps and mesh wash bucket	Run-Pool

Descriptions of the microhabitats that are targeted and the methods for sample collection are found below. For samples collected with a jab and sweep method, care should be taken to avoid sample loss when sweeping through the disturbed area.

Undercut Banks/Roots *(All Applicable Mesohabitats)*

These are sampled by placing a large root wad into a D-frame dip net and shaking vigorously or jabbing under the bank (or use foot to shake/jab stiff roots) for approximately 0.5m, and then swept through the area 2-3 times to collect dislodged organisms. The contents are removed from the dip net and placed into the mesh wash bucket. If undercut banks are present in both run and pool areas, each is allotted jabs separately.

Emergent Vegetation *(All Applicable Mesohabitats)*

In deep water, this habitat is sampled by drawing the dip net through the vegetation from the bottom to the surface (no more than 0.5 m). In shallower water, jab the net along the bottom in the rooted area, then sweep through the area to collect dislodged organisms. Material is then rinsed in the mesh wash bucket and any sticks, leaves and vegetation are thoroughly washed and inspected before discarding.

Snags/Woody Debris *(All Applicable Mesohabitats)*

Snag samples are preferably collected from "conditioned" (i.e. not new-fall) material. Submerged woody debris is sampled by jabbing medium sized snags (sticks and branches) and sweeping the net through the submerged area. Snags can be kicked first to dislodge organisms, but only after the net has been placed downstream of the disturbance. If possible, avoid large material (e.g. logs) as they are difficult to sample. If large logs are the only submerged woody debris, they can be sampled by scraping the net along the surface. Woody material is emptied into a mesh wash bucket, rinsed, inspected for organisms, and discarded.

Cobble/Gravel *(All Applicable Mesohabitats)*

Shallow cobble/gravel areas are sampled by placing the dipnet flush against the substrate and kicking the substrate starting at 0.5 meters above the net and working downstream to the net. The net should remain stationary for at least 30 seconds after kicking has stopped to allow organisms to drift into the net. In a single motion, the net is lifted just off the substrate and swept through the area that was kicked to capture any remaining organisms floating in disturbed area. The material is then emptied into a white bucket and thoroughly washed. The organisms in the sample can be elutriated away from the gravel into a mesh wash bucket, after which the gravel is inspected and discarded.

Bedrock, Slab Rock, or Hardpan Clay *(All Applicable Mesohabitats)*

Bedrock and Slab Rock (i.e. large broken slabs of bedrock) should be given preference over hardpan clay. If bedrock or slab rock are completely absent, hardpan clay can be sampled if it exists. These microhabitats are sampled by placing the edge of the dip net flush on the substrate and scraping the net against the substrate for approximately 0.5m, and then swept through the area 2-3 times to collect dislodged organisms. Material is emptied into a mesh wash bucket, rinsed, inspected for organisms, and discarded.

Supplemental Habitat *(All Applicable Mesohabitats)*

Supplemental habitat collections are meant to account for any microhabitat or substrate that may not be addressed in other listed habitat types but are observed within the sampled reach (e.g. stream margins, or "edge' habitat). Locations within the reach that have not been

previously sampled for another habitat type should be targeted. Supplemental habitat should be given the same consideration as other microhabitats when proportioning jabs within a reach.

Silt, Sand, and Fine Gravel *(Margins)*

This habitat is sampled with a bumping motion as opposed to jabbing, to reduce the amount of fines/debris in the net. The material collected is treated similarly to the cobble/gravel sample.

Leaf Packs *(Run-Pool)*

Leaf packs are collected from conditioned material, whenever possible. Samples are taken from runs and pools and placed into the mesh wash bucket. Leaf material is thoroughly rinsed to dislodge organisms, inspected, and discarded.

Wood Sample *(Run-Pool)*

Submerged wood will be sampled in both headwater and wadeable streams. Less conditioned pieces of wood are individually rinsed into the white or mesh wash bucket and inspected for burrowers and crevice dwellers, which should be removed and placed in the sample container. Large diameter and/or well-aged logs, not recent deadfall, should be inspected and handpicked with fine-tipped forceps. Any individuals picked from logs are placed in the sample container. In headwater streams, a minimum of 2 meters (6 linear feet) of wood ranging from 5-15 centimeters (2-6 in) in diameter will be washed/picked, while 3 to 6 meters (10 to 20 linear feet) of wood ranging from 5-15 cm (2-6 in) in diameter will be washed/picked in wadeable streams. When the minimum targeted length of acceptable wood is not available in a stream, field collectors should sample from as much acceptable wood as possible and note this on the appropriate form.

8.4 Sample Processing and Preservation

Composited sample material in white buckets is gently swirled and poured (elutriated) into a 500-600 µm mesh wash bucket topped with a green prospecting pan such that large stones and sand remain in the white bucket. These large stones and sand are then repeatedly elutriated into the mesh wash bucket until cleaned of all organic material and visible organisms. Handfuls of this gravel/sand mixture are then inspected in white picking pans to ensure all macroinvertebrates have been collected before being discarded back into the stream. Large organic debris including leaves and sticks held in the green prospecting pan are individually rinsed and inspected for organisms and then discarded. The sample held in the mesh wash bucket is then gently transferred to the sample jar. The mesh wash bucket and prospecting pan should be inspected, and any organisms picked and placed in the sampling jar.

Special consideration should be given to samples containing mats of filamentous green algae (*Cladophora*). As these mats often contain numerous, clinging macroinvertebrates, field processors should take extra care to wash this material several times until all unique taxa are

collected. Alternatively, *Cladophora* may be directly placed in a preservation container and processed more thoroughly in a laboratory environment, if space allows.

Samples should be preserved in 95% ethanol in a wide mouth sample container. Upon returning to the laboratory, all sample preservative is replaced with fresh 70% ethanol solution. While at the sampling location, all macroinvertebrate sample jars will receive a label. The label may be placed in the sample jar or written directly on the jar with a permanent marker. The label will include the site number, stream name, location, county, date sampled, and the collector's initials.

After sampling has been completed, all sampling gear will be thoroughly cleaned to remove all benthic organisms so that specimens are not carried to the next site. The equipment shall be examined prior to sampling at the next site to ensure that no benthic macroinvertebrates are present.

9.0 TROUBLESHOOTING

Table 3. Suggestions for troubleshooting issues encountered in the field.

Problem	Solution
(1) Preservative (95% Ethanol) is forgotten at lab.	• If preservation with Ethanol will be possible within 4 hours of sample collection, sample can be collected and kept in stream water in the sample container until preservation can occur. • If preservation with Ethanol is not possible within 4 hours of sample collection, sampling should be postponed until preservative can be obtained.
(2) Supplies for concurrent sampling (water chemistry or habitat assessment) are unavailable on sampling day.	• Determine critical concurrent sampling based upon project objectives and QA documentation. Postpone sampling until all required equipment and supplies are available.
(3) Interruption in the stream reach (tributary confluence, man-made structure, etc.) is identified *after* sampling has begun.	• Consult project manager or supervisor. Sampling across such interruptions in stream reach units may impact data usability for some purposes.
(4) Part or all of kick net sample, multi-habitat, or 20 jab sample is lost after collection. Examples: bucket containing samples is tipped, net is dropped into stream, etc.	• Discard all compromised portions of the collected sample and begin again. Do not collect samples from the same locations as previously sampled. If sufficient habitat is not available for immediate repeat sampling, sampling should be delayed for at least 14 days to allow for re-colonization. Full sampling must be repeated (kick net & multi-habitat or 20 jab) and concurrent water chemistry should be repeated on new sampling date.
(5) Part or all of a preserved sample is lost after collection.	• Discard all of sample (do not pour ethanol directly into the stream) and re-sample. Do not collect samples from the same locations as previously sampled. If sufficient habitat is not available for immediate repeat sampling, sampling should be delayed at least 14 days to allow for re-colonization. Full sampling must be repeated (kick net & multi-habitat or 20 jab) and concurrent water chemistry should be repeated on new sampling date.
(6) Inclement weather interrupts sampling.	• Cease sampling, do not retain collected material or organisms. Re-schedule sampling. Consider re-colonization needs for sampled habitat.

10.0 DATA & RECORDS MANAGEMENT

A High or Low Gradient Habitat Assessment Datasheet must be used to document macroinvertebrate sampling efforts. The 'Invertebrate Activity' in the Field Activities section of the datasheet must be filled out with the correct information: collectors' initials and sampling method used. The microhabitats sampled during Multi-Habitat or 20-jab sampling efforts should be identified with either a Y or N on high gradient datasheets and the total number of jabs administered in each microhabitat should be recorded on low gradient datasheets. Datasheets shall undergo an initial data review for accuracy and completeness. Datasheets should be promptly scanned and filed in project folders. Data entry procedures should follow project guidance outlined in project QA documentation. Digital photos should be downloaded to project folders and named according to project QA guidance. All records, including hardcopy and electronic files, that are collected by DOW staff or that are collected for the explicit use by DOW must be kept according to KDEP record retention policy (KDLA 2013).

11.0 QUALITY CONTROL & QUALITY ASSURANCE

Quality assurance and quality control measures that will be utilized to demonstrate confidence in collection procedures may include, but are not limited to, field audits, annual recertification in collection methods, annual review of standard operating procedures, and specified training for new personnel. If more specific QA/QC requirements are needed for a project, the project coordinator will address those specific needs in the Quality Assurance Project Plan.

12.0 REFERENCES

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