

Standard Operating Procedure 1:

Annual Workflow and Field Season Preparation

(Version 1.0)

Summary

This Standard Operating Procedure (SOP) includes:

1. An overview of the Eastern Rivers and Mountains Network (ERMN) benthic macroinvertebrate (BMI) monitoring annual workflow;
2. Step-by-step preparation procedures that should be conducted by the ERMN Aquatic Ecologist, Hydrologic Technician and other field staff (as needed) prior to March 1 of every calendar year; and
3. Data sheets, supply lists, gear construction instructions, and other information related to BMI monitoring necessities.

All supplies and equipment must be in hand (i.e., constructed or purchased) by March 1st because that is the earliest potential sampling start date in the two-month index period (March-April). Most procedures and designs in this SOP are directly from U.S. Geological Survey protocols for field (Moulton et al. 2002) and laboratory (Moulton et al. 2000) procedures.

Revision Log

Original Version #	Date of Revision	Revised By	Changes	Justification	New Version #
	4/27/2016			Protocol Implementation Plan publication	1.0

Annual Review

The ERMN Aquatic Ecologist (AE) and Hydrologic Technician (HT) are primarily responsible for BMI monitoring; however, other people (e.g., ERMN Vegetation Technician) may assist with sampling after they have been trained. Every January, the AE, HT, and other staff (as needed) must review the benthic macroinvertebrate (BMI) monitoring protocol implementation plan (PIP) and SOPs prior to field season preparations. Review must include field and laboratory procedures, data management, analysis and reporting, as well as fundamental design issues. The adequacy of sampling effort and careful consideration of the resulting analyses and overall confidence in results must be evaluated. Changes to this PIP should be minimal and infrequent; however, any necessary changes must be made to the PIP and SOPs with enough time for those alterations to be implemented prior to field seasons.

Scheduling and Organizing Field Work

The AE is responsible for planning and conducting field work so that it is completed according to the schedule (Figure 1). Field work is conducted during the March and April index period in as short an amount of time as safely possible. A more detailed timeline that includes staff responsibilities is provided in Table 1.

The timing of events can differ slightly from year to year and is dictated primarily by environmental conditions (i.e., weather, stream flow) during field seasons. It is important to begin sampling as early as possible in the index period because unnecessary temporal variability in BMI community metrics (e.g., due to aquatic insect emergences) is undesirable for biomonitoring; however, sampling dates cannot always be repeated from year-to-year because conditions differ from year to year. The best that can be done to minimize this variability is to consistently start as soon as conditions allow after March 1st and follow the ideal sampling order (Table 2). Sampling is not conducted outside of the index period (March 1 – April 30).

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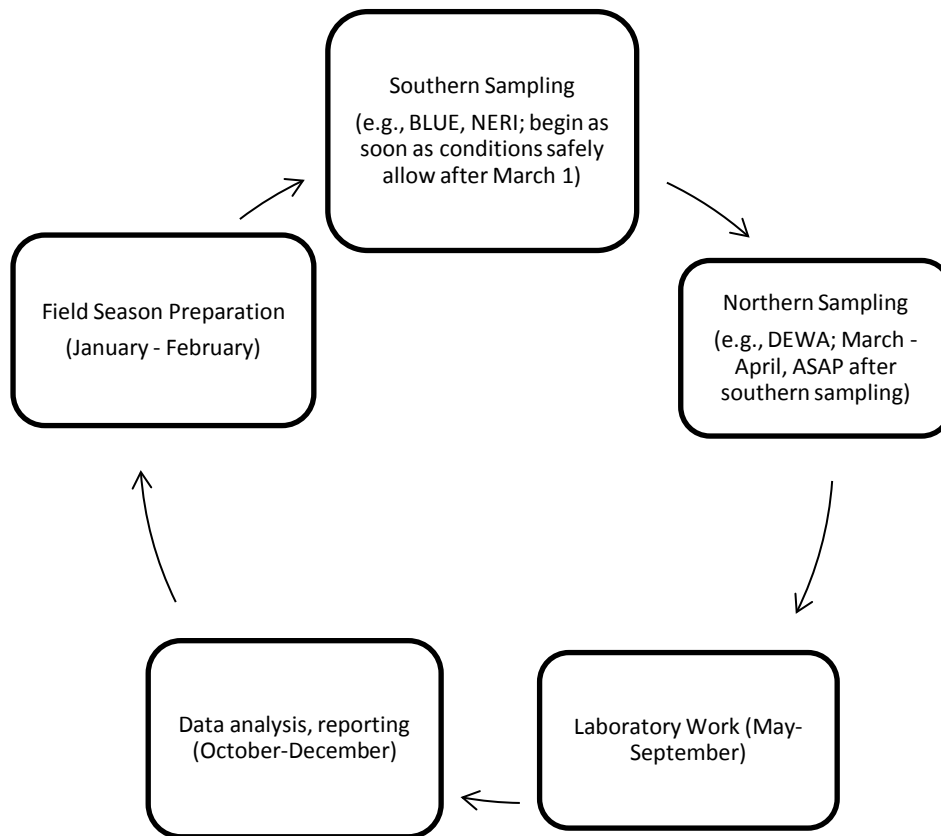


Figure 1. Flow chart of ideal benthic macroinvertebrate monitoring schedule. Sampling start dates may differ depending on stream flow and weather conditions.

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Table 1. Annual benthic macroinvertebrate sampling plan that identifies tasks by project stage, indicates who is responsible for the task, and establishes the timing of execution. Actual timing may differ from year to year due to weather and hydrologic conditions. AE=Aquatic Ecologist, DM=Data Manager, HT=Hydrologic Technician.

Project Stage	Task	Responsibility	Timing
Preparation	Prepare and print field maps and field forms.	AE/HT	Feb
	Provide database/GPS training as needed and upload most current sampling waypoints to GPS units.	AE/DM	Feb
	Train/review field work SOPs.	AE/HT	Feb
	Contact park managers and inform them of sampling plan.	AE	Feb
	Ensure vehicles, equipment, and supplies are ready for use.	HT	Feb
	Use equipment and supply lists to pack vehicle.	HT/AE	Feb
Data acquisition	Collect benthic macroinvertebrate (BMI) samples.	AE/HT	Mar and Apr
	Process/subsample BMI samples.	HT	Apr - Sep
	Identify BMI samples.	AE/HT	
Data entry & processing	Scan and file field and laboratory datasheets and site photos and archive them electronically on the ERMN server.	HT	Mar – Sep
	Enter field and laboratory data into the BMI database.	HT	
Quality review	Quality review and data validation.	AE	Monthly
Metadata	Identify any sensitive information contained in the data set.	AE/DM	Dec-Jan
	Update project metadata records.	AE/DM	Dec-Jan
Data certification and delivery	Certify the year's data.	AE/DM	Dec-Jan
	Deliver certified data and updated metadata to DM.	AE	Dec-Jan
	Upload certified data into master project database, store data files in ERMN Digital Library ¹ .	AE/DM	Dec-Jan
	Update project GIS data sets, layers and associated metadata records.	AE/DM	Dec-Jan
	Finalize and parse metadata records, store in ERMN Digital Library ¹ .	DM	Dec-Jan
	Status and trend analyses.	AE	Dec-Jan
Product development	Create summary or periodic trend report.	AE	Dec-Jan
	Screen all reports and data products for sensitive information.	AE	Dec-Jan
	Submit draft report to Network Coordinator for review.	AE	Dec-Jan
	Review report for formatting and completeness, notify AE of approval or need for changes.	Network Coordinator	Dec-Jan
Posting & distribution	Submit metadata to appropriate NPS database.	DM	Dec-Jan

¹ The ERMN Digital Library is a hierarchical digital filing system stored on the ERMN file server.

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Project Stage	Task	Responsibility	Timing
	Post reports to NPS clearinghouse.	DM	Dec-Jan
Archival & records management	Store finished products in ERMN Digital Library ¹ .	AE/DM	Dec-Jan
	Review, clean up and store and/or dispose of project files	AE	Dec-Jan
Season close-out	Meet to discuss the recent field season, and document any needed changes to field sampling protocols or the working database.	AE, Park Managers, and DM	Jan-Feb
	Discuss and document needed changes to analysis and reporting procedures.	AE, Park Managers, and DM	Jan-Feb

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Table 2. Ideal sampling order to visit Eastern Rivers and Mountains Network benthic macroinvertebrate sampling sites between March 1st and April 30th in even years (e.g., 2016, Panel 1) and odd years (e.g., 2017, Panel 2). Dates will vary among years depending on weather and stream flow conditions.

Panel 1			Panel 2		
Order	ID	Name	Order	ID	Name
1	NERI.3018	Keeney Creek 10	1	NERI.3082	Keeney Creek 15
2	NERI.3001	Meadow Fork 1	2	NERI.3074	Manns Creek 24
3	NERI.3038	Arbuckle Creek 2	3	NERI.3050	Ephraim Creek 08
4	NERI.3034	UNT 21 New River 01	4	NERI.3054	Arbuckle Creek 05
5	NERI.3021	Fire Creek 17	5	NERI.3065	Meadow Fork 06
6	NERI.3005	Buffalo Creek 16	6	NERI.3049	UNT to Buffalo Creek 06
7	NERI.3009	Slater Creek 20	7	NERI.3069	Buffalo Creek 04
8	NERI.3032	River Branch 06	8	NERI.3053	Slater Creek 13
9	NERI.3016	River Branch 04	9	NERI.3077	Dowdy Creek 02
10	NERI.3044	Laurel Creek 08	10	NERI.3058	Richlick Branch 17
11	NERI.3041	UNT Laurel Creek 03	11	NERI.3085	Laurel Creek 61
12	NERI.3037	Laurel Creek 47	12	NERI.3064	Batoff Creek 07
13	NERI.3042	Bucklick Branch 03	13	NERI.3048	Mill Creek 12
14	NERI.3026	Little Laurel Creek 06	14	NERI.3080	Mill Creek 01
15	NERI.3011	Meadow Creek 17	15	NERI.3072	Big Branch 09
16	NERI.3043	Meadow Creek 39	16	NERI.3052	Fall Branch 07
17	BLUE.1001	Indian Branch	17	NERI.3075	Meadow Creek 12
18	BLUE.1002	Mountain Creek	18	NERI.3059	Meadow Creek 58
19	NERI.3024	Big Branch 10	19	DEWA.3033	Dunnfield Creek 26
20	BLUE.1004	Jarrell Branch	20	DEWA.3025	Vancampens Brook 22
21	BLUE.1003	Little Bluestone River	21	DEWA.3030	Yards Creek 07
22	NERI.3036	UNT to Fall Branch 02	22	DEWA.3026	UNT to Vancampens Brook 05
23	DEWA.3001	Caledonia Creek 13	23	DEWA.3038	Vancampens Brook 76
24	DEWA.3005	Dunnfield Creek 03	24	DEWA.3028	White Brook 15
25	DEWA.3013	Little Bushkill Creek 01	25	DEWA.3022	Toms Creek 25
26	DEWA.3002	Van Campen Creek 12	26	DEWA.3034	Toms Creek 03
27	DEWA.3018	Toms Creek 07	27	DEWA.3029	Sand Hill Creek 08
28	DEWA.3006	Toms Creek 20	28	DEWA.3036	Mill Creek 12
29	DEWA.3020	Mill Creek 25	29	DEWA.3035	Hornbecks Creek 15
30	DEWA.3007	Spackmans Creek 08	30	DEWA.3031	Dingmans Creek 30
31	DEWA.3003	Deckers Creek 03	31	DEWA.3023	UNT to Dingmans Creek 07
32	DEWA.3004	Dingmans Creek 05	32	DEWA.3039	Adams Creek 21
33	DEWA.3015	Dingmans Creek 39	33	DEWA.3027	Adams Creek 03
34	DEWA.3008	Dingmans Creek 57	34	DEWA.3032	Raymondskill Creek 13
35	DEWA.3011	Adams Creek 14	35	ALPO.1001	Millstone Run
36	DEWA.3012	Adams Creek 33	36	ALPO.2001	Blair Gap Run
37	DEWA.3010	Vancampens Brook 95	37	FRHI.1001	Ice Pond Run
38	DEWA.3014	Vancampens Brook 43	38	FRHI.1002	Dublin Run
			39	FONE.1002	UNT to Great Meadows Run
			40	FONE.2001	Great Meadows Run

Field and Laboratory Equipment Maintenance and Procurement

Although it is important to inspect and maintain sampling gear during sampling seasons, most gear repair and replacement will occur in the laboratory.

Specialty items (e.g., chemicals and electronics) must be inventoried far in enough advance of field work that the AE has time to order replacements if necessary. A master list of sampling equipment and supplies is provided in Table 3 and lists are provided in respective SOPs for specific activities.

Inspect collection nets and sieves for holes. Mark small holes with a permanent pen or marker as they are noticed. Repair small holes with silicon adhesive or hot glue or melt them shut with a wood burning tool or soldering iron. Large holes and tears must be sewn shut with heavy thread. Clean and dry nets as frequently as possible and store them out of direct sunlight because dampness and ultraviolet radiation promote deterioration of net material.

Waders must be checked for leaks and boots (including studs) must be inspected and repaired or replaced following manufacturer recommendations. Water quality and flow meters must be cleaned, calibrated, and checked against calibration standards to ensure that they are operating properly.

Data sheets for benthic macroinvertebrate collection (Appendix A) and rapid habitat assessments (SOP 6) must be laser printed on waterproof paper and organized in clipboards. Sample labels (Appendix B) must also be printed on waterproof paper and placed inside and taped onto the outside of sample jars.

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Table 3. Equipment and supplies for the benthic macroinvertebrate (BMI) monitoring protocol.

<u>Benthic macroinvertebrate sampling</u> <i>Supplies</i> Slack sampler with 500-µm mesh, PVC sampling template, BMI Sampling Forms, Habitat Assessment Forms, White plastic buckets (19 L), Wire mesh sieves (20-cm and 30-cm diameter, 500-µm mesh), Knives (pocket and putty), scissors, White enamel or plastic trays), Hand rakes, Squirt bottles, Wide-mouth sample jars (750 mL) with waterproof labels on inside and outside, Clipboards, Waterproof pens, Pencils, Waterproof paper, Field data sheets (including extra sheets) laser-printed on waterproof paper
<i>Electronics and accessories</i> Global positioning system unit with preloaded sampling location waypoints, Digital camera, Current velocity meter and D batteries (Appendix C), Eureka Sub2 (or similar) to measure core water quality parameters, Batteries (various sizes), Cellular phone and charger, Radio tuned to appropriate park frequencies, LED headlamp and flashlight (per person), "spot" beacon
<i>Miscellaneous supplies</i> Safety plan/emergency phone numbers, First aid kit (up to date), Backpacks (2), Wader repair kit, Ruler and tape measure, Braided, 1.9-cm nylon rope, Tool set, Paper towels, Heavy-duty nylon thread/sewing needles, Handsaw or lopping shears, Resealable plastic bags (various sizes), plastic trash bags, duct tape
<i>Personal supplies</i> Studded wading boots (per person), Chest waders (per person and extra pair), Rain gear (per person), Polarized sunglasses, Shoulder-length PVC gloves (pair per person)
<i>Liquids</i> 95% ethanol, distilled/deionized water, multiprobe calibration standard solutions [specific conductance (10, 100, and 1000 µS/cm) and pH (4, 7, and 10)], Insect repellant, Sun screen, Antibiotic liquid soap, drinking water, GS256 disinfectant solution (10% dilution)
<u>Laboratory (sample processing)</u> <i>Supplies</i> Forceps (jewelers, lightweight, blunt), Probes (fine-tipped and blunt), Vials (4 – 6 dram), Shell vials (1/4 dram), Quantitative BMI Sample Processing Worksheet, Subsampling and Preliminary Enumeration Worksheet, Taxonomic identification labels (minimum information: taxonomic identification, taxonomist, sample identification), Scissors, Vial racks, Scrub brushes
<i>Equipment</i> Dissecting microscopes (6 – 50 X magnification), estimation trays, Fiber optic illuminators, Plastic wash basins, Desk lamps with 10X magnifying glass, Standard metal sieves (500-µm mesh), Subsampling frames, White sorting trays (15 x 20 cm, 20 x 30 cm, 40 x 50 cm)
<i>Liquids</i> 95% and 70% Ethanol, water

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Chemical Preparation

The AE is responsible for ensuring that adequate supplies of chemicals (Table 3) are purchased prior to the field season. Conductivity and pH standards are used as prepared by the vendors whereas 70% ethanol (used in the laboratory) is diluted from stock solutions.

70% Ethanol

Benthic macroinvertebrates are preserved in 95% ethanol in the field. Typically, water contained in the preserved organic material ultimately dilutes the 95% ethanol to approximately 70% ethanol; consequently, the ERMN reuses ethanol from preserved samples for temporary storage of BMI. Fresh 95% ethanol that is used for voucher/reference collections is diluted to approximately 70% using the formula:

$$V_1 * M_1 = V_2 * M_2$$

V_1 = volume of desired ethanol concentration

M_1 = desired ethanol concentration (e.g., 70%)

V_2 = stock ethanol volume

M_2 = stock ethanol concentration (e.g., 95%)

Example:

To make 1.0 L (1000 mL) 70% ethanol from 95% ethanol

$$1000 * 70 = V_2 * 95$$

$$V_2 = 70000/95 = 736.8 \text{ mL}$$

Take 737 mL ethanol (95%) and add distilled water (263 mL) until total volume = 1000 mL.

Table 3. Chemical vendors and recommended chemical stocks (Quantity) used each field season by the Eastern Rivers and Mountains Network wadeable streams monitoring program. Part numbers are provided parenthetically in the vendor column.

Chemical	Quantity (L)	Vendor
Conductivity Standards		
10 μ S	30.0	VWR [AAA44065-K7 (4L)]
100 μ S	50.0	VWR [AAA43445-K7 (4L)]
1,000 μ S	30.0	VWR [varies]
pH		
4	50.0	GSA
7	30.0	GSA
10	30.0	GSA
Ethanol (Tecsol, 95% ethanol)	50 (13 gal)	GSA; UNIVAR [624215 (208 L/55 gal)]
GS256 disinfectant	1.0	Spartan Chemical Supply

Preparation of Benthic Macroinvertebrate Sampling Equipment

Field equipment

Area template construction

Materials:

One 1.5 m (5 ft) length of 1.27 cm (0.5 in) PVC pipe, Two PVC T-connectors, Shock cord or braided nylon rope, Two spring clips, PVC primer and glue, Velcro straps (1/4" x 5")

Instructions:

Cut PVC pipe into three equal (50 cm) segments.

Connect PVC pipe sections with the two T-connectors to form two right angles. By using T-connectors (instead of elbows) and drilling several holes in the pipe, water will flow into the frame, preventing it from floating during sampling.

String the cord or rope through all pipe segments but keep some slack in it so that the frame can be collapsed, clipped, and tightened when not in use (Figure 3). Thread a Velcro strap through holes in one of the pipe sections so that the template can be folded and secured during transport.

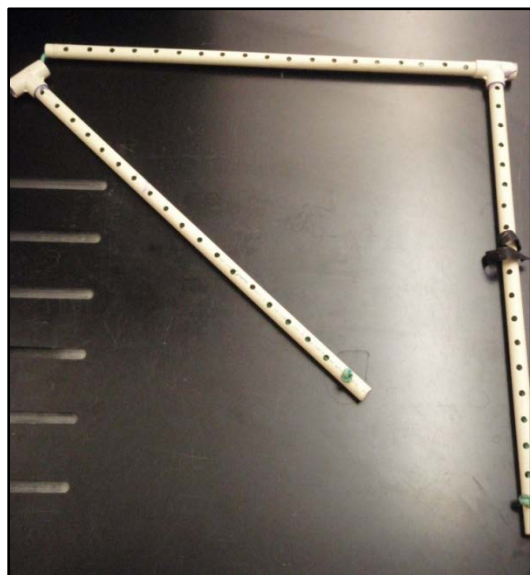


Figure 3. Area template constructed of PVC. Pipe section on the left is detached to show where 'T' fittings are glued. Note the green nylon rope threaded through the pipe and Velcro strap used to hold the template together when not in use.

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Dunking bucket construction

Materials:

10 L (2.5 gal) dunking bucket, 1.3 cm (0.5 in) wire mesh

Instructions:

Replace the bottom of a 10 L (2.5 gal) bucket with a 1.3 cm (0.5 in) wire mesh screen (Figure 4). To remove the bottom of the plastic bucket use a reciprocating saw or jig-saw - be sure to leave a ½" rim to attach the wire mesh. Cut the mesh to fit snugly around the rim in the bucket bottom. Attach the wire mesh to the bucket bottom with duct tape and then seal the tape and wire with a generous coating of silicone adhesive. The silicone waterproofs the tape and helps eliminate jagged wire ends.

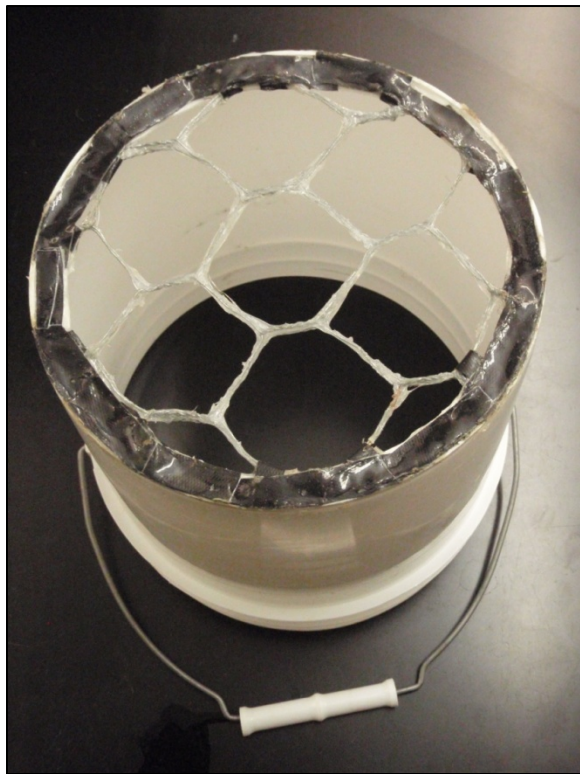


Figure 4. 2.5 gal dunking bucket designed to fit inside a 5.0 gal bucket for rinsing large quantities of leaves collected in benthic macroinvertebrate samples.

Laboratory Equipment

Estimation trays are constructed of 1.3 cm thick clear Plexiglas™ (Figure 5). Each tray is 1.3 cm deep and is etched on the bottom with grid lines at 1.3-cm intervals (Table 4). Subsampling frames are constructed of 1.3 cm thick clear Plexiglas™, 1.3- by 1.3-cm galvanized wire mesh, and 100- μ m Nitex™ mesh (Figure 6). Although samples are collected by using a mesh size >100 μ m, a fine mesh facilitates removing grids of sample matrix because the tarsal claws of insects and other fine matter do not adhere to a fine mesh as easily as they do to a coarse mesh. The 100- μ m mesh and the galvanized mesh are bonded to the bottom of the Plexiglas™ frame with silicone adhesive. The galvanized mesh supports the 100- μ m mesh and functions as a reference grid for the removal of the 5.1- by 5.1-cm subsamples. Dimensions of the estimation trays and three subsampling frames are listed in below.

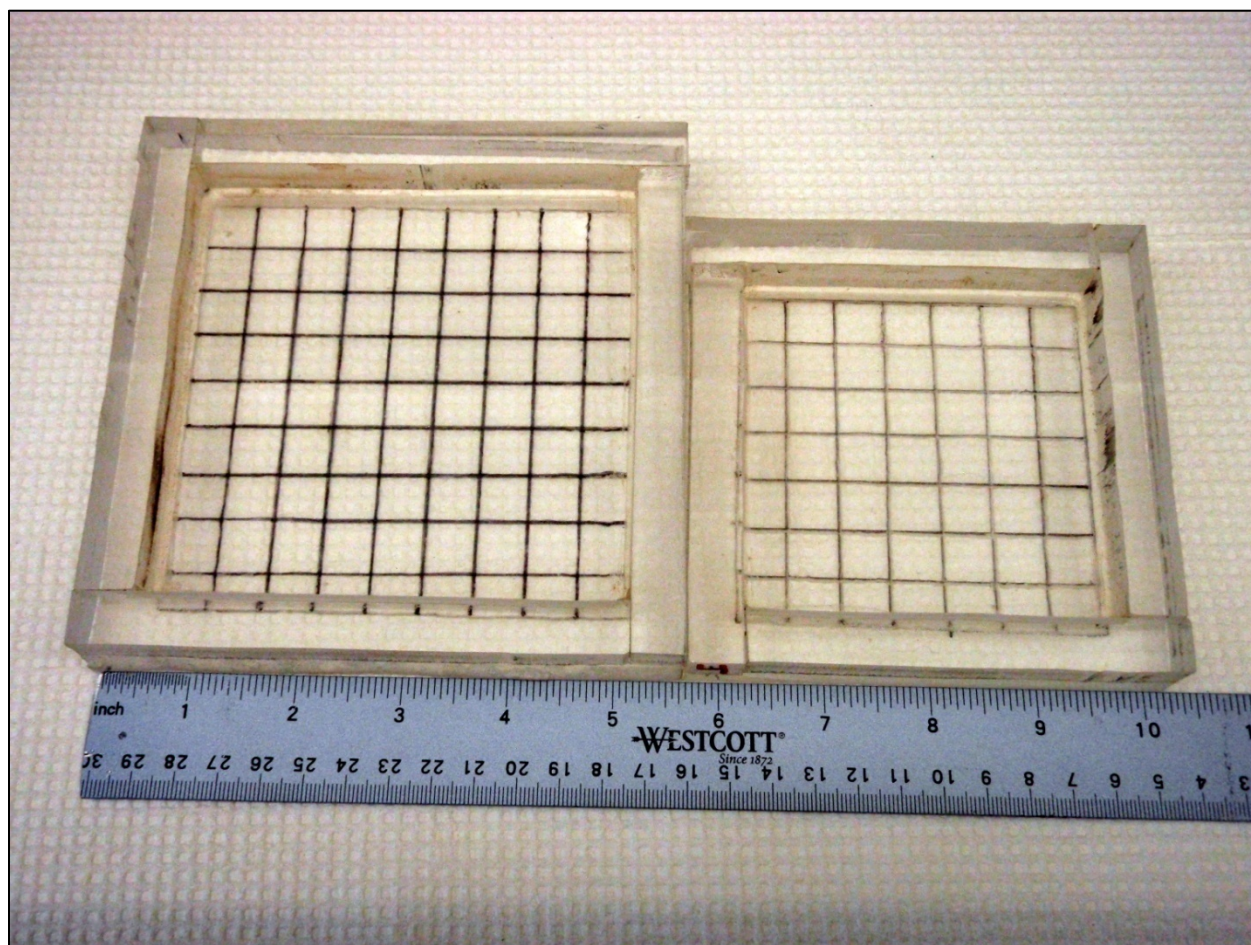


Figure 5. 81-grid (left) and 49-grid (right) estimation trays used for processing benthic macroinvertebrate samples.

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Table 4. Estimation tray and subsampling frame dimensions.

Type	Inside dimensions (cm)	Grid dimensions (cm)
Estimation tray		
49 grid	8.9 x 8.9 x 1.3	1.3 x 1.3
81 grid	11.4 x 11.4 x 1.3	1.3 x 1.3
Subsampling frame		
12 grid	15.2 x 20.3 x 3.8	5.1 x 5.1
24 grid	20.3 x 30.5 x 3.8	5.1 x 5.1
42 grid	30.5 x 35.6 x 3.8	5.1 x 5.1

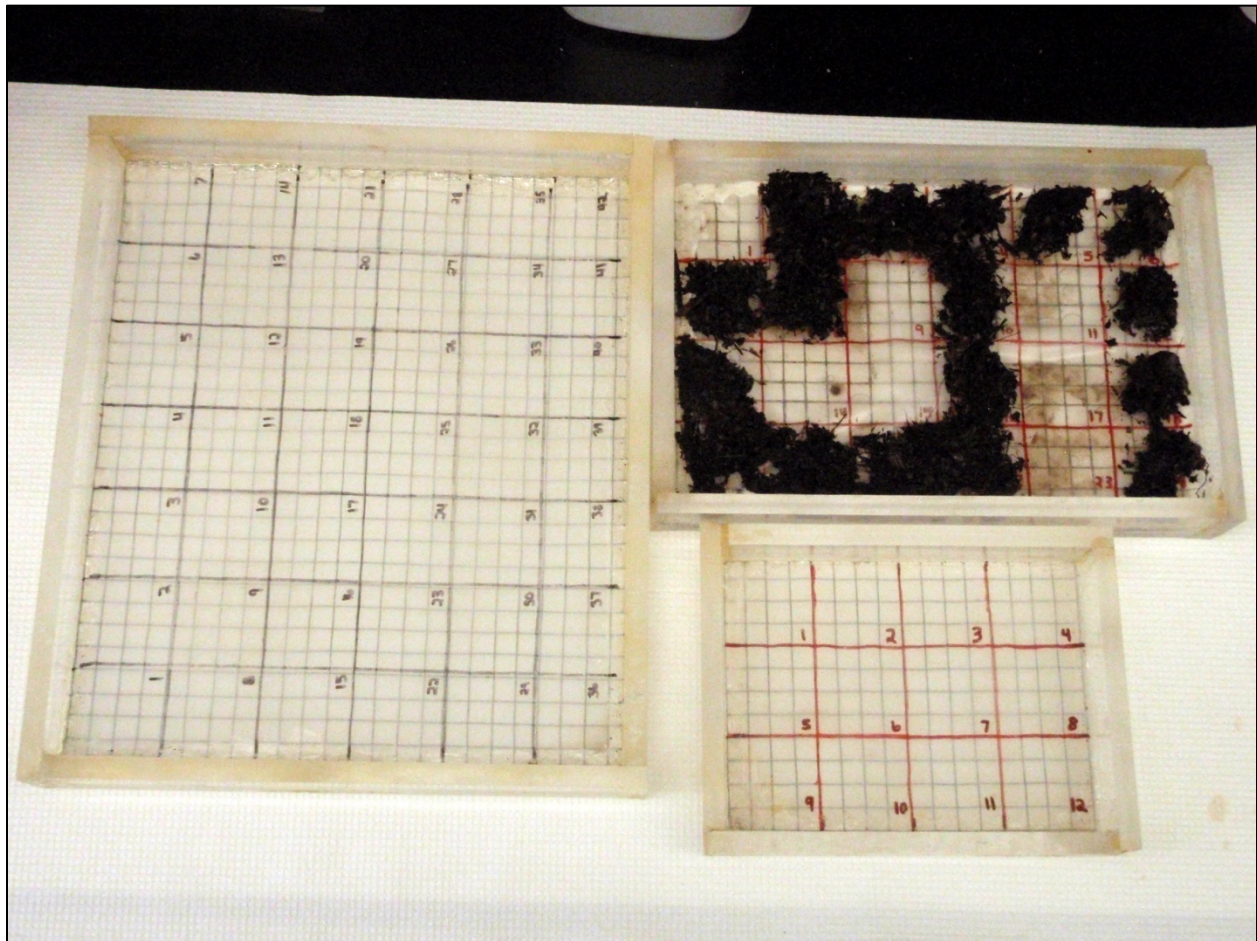


Figure 6. 12-, 24-, and 42-grid subsampling frames used for processing benthic macroinvertebrate samples. The 24-grid frame (top right) illustrates sample dispersion and removal of randomly selected portions of the sample.

Literature Cited

- Moulton, S. R., Carter, J. L., Grotheer, S. A., Cuffney, T. F., and T. M. Short. 2000. Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory – processing, taxonomy, and quality control of benthic macroinvertebrate samples. U.S. Geological Society Open-File Report 00-212, 61 p.
- Moulton, S. R. II, J. G. Kennen, R. M. Goldstein, and J. A. Hambrook. 2002. Revised protocols for sampling algal, invertebrate and fish communities as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 02-150. 87 pp.

Appendix A. Semi-Quantitative Targeted Habitat BMI Sample Field Data Sheet					
Stream Name:		Collectors:		Date (MM/DD/YYYY): / /	
		Reach ID:			
Length:		Width:		UP Lat:	
				UP Long:	
Photo #'s:		UP		DOWN	
				DN Lat:	
				DN Long:	
				Start time:	
				End time:	
Sample and photographic notes:					
RELATED SAMPLING ACTIVITIES (circle all that apply)					
Water chemistry Discharge Habitat survey Other:					
PHYSICAL SITE CONDITIONS					
Clouds: % Wind (circle): Calm Light Moderate Gusty					
Precipitation Intensity (circle): N/A Light Moderate Heavy Other weather:					
Riparian shading (circle): Shaded Partial Full sun					
Water clarity (circle): Clear Slightly turbid Turbid Very turbid					
Water color (circle): Black Brown Clear Dark green Light green Yellow Red					
Water Quality Measurements					
Time	Temperature (°C):	Specific Cond. [(µS/cm) @ 25 °C]:	Dissolved oxygen (% Sat)	Dissolved oxygen (mg/L):	pH
:	.	.	. %	.	.
Downstream End			Upstream End		

<u>Reach Name:</u>			Sampling gear (circle): <i>Slack Sampler</i> Other: _____		
<u>Number of discrete collections constituting composite:</u>			<u>Total sample area:</u> _____ cm ²		
<u>Elutriation method (circle):</u> <i>Bucket</i> Other: _____		<u>Sample-splitting method (circle):</u> <i>Not split</i> Sieve diameter _____			
INSTREAM HABITAT SAMPLED Main channel Channel Margin Island Margin					
HISTORICAL REACH CHARACTERISTICS AND ACCESS NOTES:					
SUPPORTING INFORMATION					
<i>Substrate type codes:</i> (1) smooth bedrock/concrete/hardpan; (2) silt, clay, marl, muck, organic detritus; (3) sand; (4) fine/medium gravel; (5) coarse gravel; (6) very coarse gravel; (7) small cobble; (8) large cobble; (9) small boulder; (10) large boulder/irregular bedrock/irregular hardpan/irregular artificial surface					
Velocity meter used (circle one): Flowstick Marsh McBirney Other: _____					
Substrate type					
Sample	Water depth (m)	Substrate embeddedness (%)	Velocity (m/s)	Dominant	Co-dominant
1					
2					
3					
4					
5					
<u>Physical site condition or other comments</u>					

Appendix B. Semi-Quantitative Targeted Habitat BMI Sample Labels

(Place one inside and one outside sample jars)

ERMN Benthic Macroinvertebrate Sample	
Park ID:	Site ID #:
Site Name:	
Collection date:	Time:
Collectors:	
ERMN Benthic Macroinvertebrate Sample	
Park ID:	Site ID #:
Site Name:	
Collection date:	Time:
Collectors:	
ERMN Benthic Macroinvertebrate Sample	
Park ID:	Site ID #:
Site Name:	
Collection date:	Time:
Collectors:	

ERMN Benthic Macroinvertebrate Sample	
Park ID:	Site ID #:
Site Name:	
Collection date:	Time:
Collectors:	
ERMN Benthic Macroinvertebrate Sample	
Park ID:	Site ID #:
Site Name:	
Collection date:	Time:
Collectors:	
ERMN Benthic Macroinvertebrate Sample	
Park ID:	Site ID #:
Site Name:	
Collection date:	Time:
Collectors:	