

Standard Operating Procedure 8:

Benthic Macroinvertebrate Sample Processing and Identification (Version 1.0)

Summary

This SOP describes the step-by-step procedures that the ERMN uses for processing and identifying BMI samples in the laboratory, which were developed by the U.S. Geological Survey National Water Quality Laboratory (Moulton et al. 2000). In addition to the “Quantitative Fixed-Count Method for Processing BMI Samples” described in this SOP, Moulton et al. (2000) provided methods for other procedures (e.g., Qualitative Processing) that are not included here. The wording and structure of this SOP differs slightly from Moulton et al. (2000) to align with ERMN objectives but the adopted procedures generally do not – the most substantive difference is that ERMN identifies chironomid midges to a higher taxonomic level (i.e., family) than USGS does (i.e., genus or species). Other differences are primarily logistical ones (e.g., ERMN must use external cooperators for QC measures) and are described in the Protocol Implementation Plan.

Revision Log

Original Version #	Date of Revision	Revised By	Changes	Justification	New Version #
NA	2/23/2016	C. Tzilkowski	NA	Protocol Implementation Plan publication	1.0

1.0. Processing and Identification Overview

The purpose of this Standard Operating Procedure (SOP) is to describe how to efficiently sort and identify benthic macroinvertebrate (BMI) samples. Five main steps are used to process a BMI sample: (1) prepare a sample for subsampling or sorting; (2) sort BMIs from the sample matrix; (3) identify and enumerate BMIs; (4) enter BMI data; and (5) apply quality-control (QC) procedures to quality assure steps 1 through 4.

The Aquatic Ecologist is responsible for ensuring that BMI sample processing and identifying equipment and supplies (Table 1) are stocked and in good working order.

Table 1. Benthic macroinvertebrate sample processing/identification equipment and supplies

Chemicals 95% and 70% Ethanol
Equipment Dissecting microscopes (6 - 50 X magnification), estimation trays (see SOP 1 for construction instructions), Fiber optic illuminators, Plastic wash basins, Desk lamps with magnifying glass, Standard metal sieves (500- μ m mesh), Subsampling frames (see SOP 1), White sorting trays (15 x 20 cm, 20 x 30 cm, 40 x 50 cm)
Supplies Forceps (jewelers, lightweight, blunt), Probes (fine-tipped and blunt), Vials (4 – 6 dram), Shell vials (1/4 dram), Taxonomic identification labels (minimum information: taxonomic identification, taxonomist, sample identification), Scissors, Vial racks, Putty knife, Scrub brushes, Subsampling and preliminary enumeration worksheet (Appendix A), Benthic Macroinvertebrate Identification and Enumeration Bench Sheet (Appendix B)

2.0. Summary of Quantitative Fixed-Count Method for Processing Benthic Macroinvertebrate Samples

Moulton et al. (2000) detail two methods for processing BMI samples - the “Quantitative Fixed-Count Method” and the “Qualitative Visual Sort Method”. The ERMN uses the Quantitative Fixed-Count Method because it allows for estimation of the abundance of each taxon sorted from a BMI sample. The method is similar to the fixed-count method described in Barbour et al. (1999). Organisms are sorted for later identification by using 10 X magnification from either the entire sample or more often from randomly selected grid subsamples of the original sample.

The quantitative method developed by Moulton et al. (2000) differs slightly from Barbour et al. (1999) in several aspects, including:

1. Instead of acquiring a fixed count of organisms with a numerical range of ± 20 percent, the goal of the method that the ERMN uses is to acquire a minimum number of organisms. For example, if a fixed-count target was 300 organisms, by using the method of Barbour et al. (1999), the number of organisms sorted could range from 240 to 360 (300 ± 20 percent). In contrast, the method used by the ERMN consists of sorting at least 300 organisms. Although these methods are similar, randomly sorting a minimum number of organisms provides a more uniform data set indexed to the fixed-count goal from which a rarefied, unbiased index of richness might be determined (see references in Moulton et al., 2000).
2. When estimates of abundance are based on subsamples of the original sample, large-rare organisms are visually sorted from the unsorted portion of the sample for an additional 15 minutes. Sorting large-rare organisms from the unsorted portion of the sample provides a biased but more representative estimate of the taxa present in a sample (Vinson and Hawkins, 1996).
3. The USGS method limits sorting effort to a maximum of 8 hours. In agreement with a previous finding by Moulton et al. (2000), the ERMN has found that approximately 100 organisms can be sorted from BMI samples in 1 hour. However, samples that contain excessive amounts of detritus and that have organism densities near or less than a given fixed-count goal are extremely time-intensive to sort (for example, greater than 50 hours).
4. The ERMN (and USGS) sorts all quantitative BMI samples using 10 X magnification. Other laboratories that use a similar fixed-count method might sort without magnification.

The fixed count is based on a minimum number (i.e., 300) of organisms sorted from the sample. Samples containing more organisms than the fixed-count target are subsampled by using a subsampling frame partitioned into 5.1 x 5.1 cm grids; however, uniformly distributing a sample in a subsampling frame is often difficult, and organisms in the sample matrix tend to have a clumped distribution (Moulton et al. 2000). Therefore, subsampling by simply acquiring a single, very small portion from a subsampling frame could lead to extreme errors in estimating the

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abundance of taxa in the sample. The method described in this SOP uses multiple, randomly selected 5.1 x 5.1 cm portions of the original sample (stage-1 grids) to estimate abundance accurately. Large-rare organisms are sorted from any remaining portion(s) of the sample after the random subsampling is complete.

Total sorting time is limited to a maximum of 8 hours. The time limitation has been implemented to avoid spending too much time on samples that contain few organisms (e.g., ≤ 300) or have exceedingly difficult detritus to sort (e.g., filamentous algae).

A generalized processing procedure (Figure 1) is as follows:

- The sample is uniformly distributed in a subsampling frame (stage-1 subsampling frame).
- An estimate of the average number of organisms per stage-1 grid is obtained.
- An appropriate processing strategy is selected by using the average number of organisms per stage-1 grid.
- The grids are randomly selected from either a stage-1 or a stage-2 subsampling frame, and organisms are sorted from each grid.
- Large-rare organisms are sorted from any remaining unsorted portion(s) of the sample.

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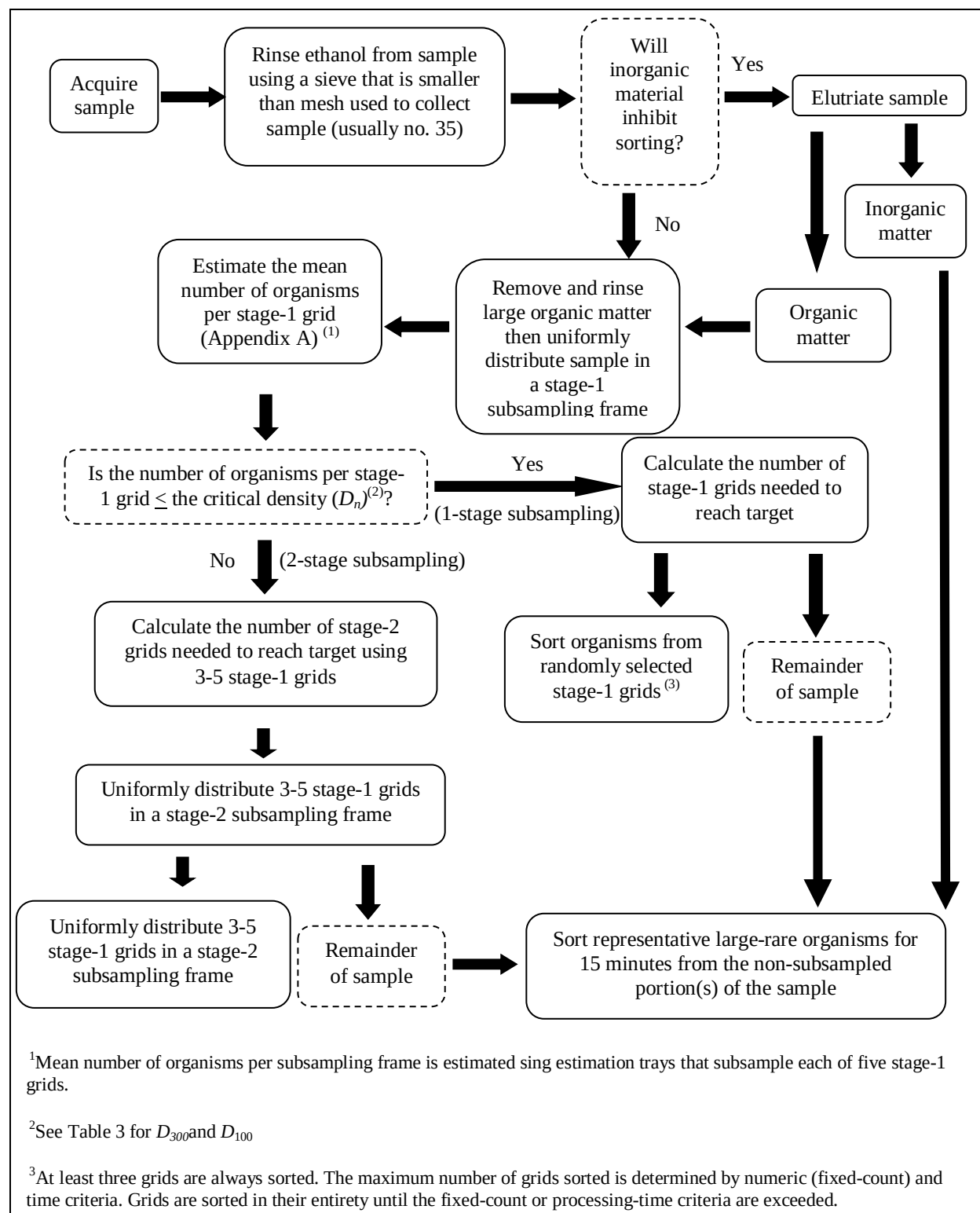


Figure 1. Overview of the quantitative, fixed-count method for processing benthic macroinvertebrate samples in the laboratory.

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2.1. Choosing a Subsampling Frame

Three sizes of subsampling frames (Figure 2) are used and the size of the frame depends on the total sample volume and organism density. The frame size increases with sample volume and density. If the volume of a sample is very low but the density of BMIs is high, the subsampling frame size is dictated by the density of organisms in the sample. It is possible that the volume of detritus could be so small and BMIs could be so depauperate that the use of a subsampling frame is unnecessary. The primary objective is to choose a frame size for uniform dispersal of the sample.

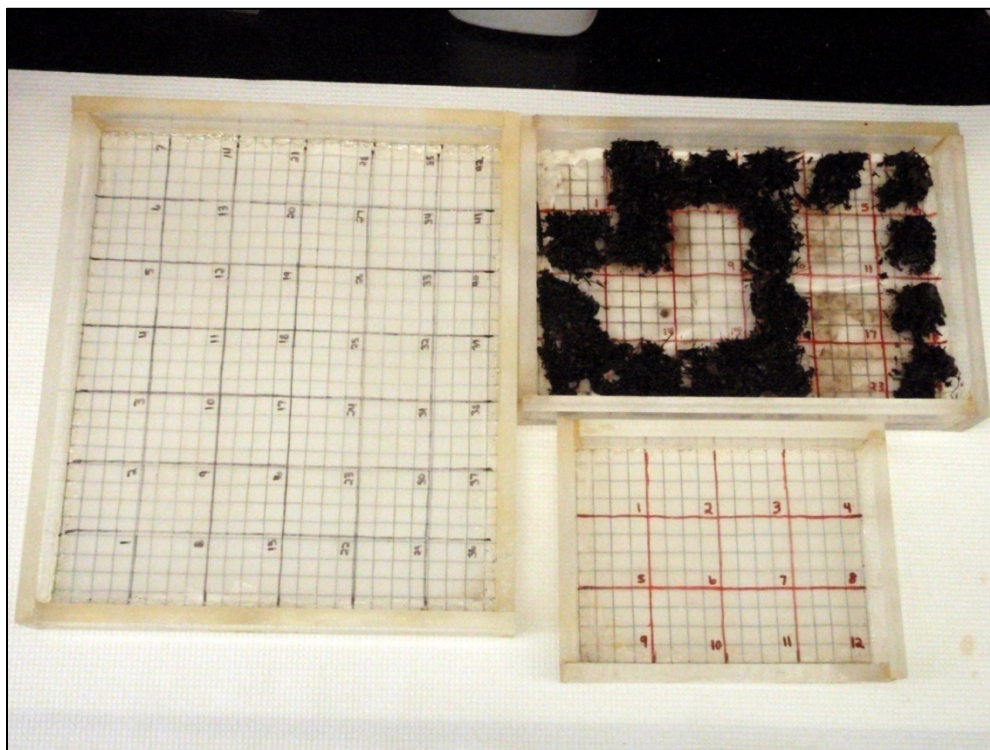


Figure 2. Subsampling frames (12, 24, and 42-grid) that are used to subsample benthic macroinvertebrate samples. Note that seven of 24 grids were removed (for processing) from the subsampling frame in the top right of the figure.

2.2. Estimating the Mean Number of Organisms per Stage-1 Grid

The mean number of organisms per stage-1 grid is used to determine the appropriate subsampling strategy. The mean is estimated by randomly selecting five grids from the stage-1 subsampling frame and uniformly distributing the material from each grid into separate, appropriately sized, estimation trays. Estimation trays with either 49 or 81 grids (Figure 3) can be used to obtain a uniform distribution and density of sample material – estimation tray size determination depends primarily on the amount of material in each grid of the subsampling frame. Organisms in each of three randomly chosen estimation tray grids are counted and used to estimate the number of organisms in each estimation tray, and hence, each stage-1 grid. Separate estimates are made from each of the five estimation trays. The resulting five estimates are averaged to give a final estimate of the number of organisms in each stage-1 grid.

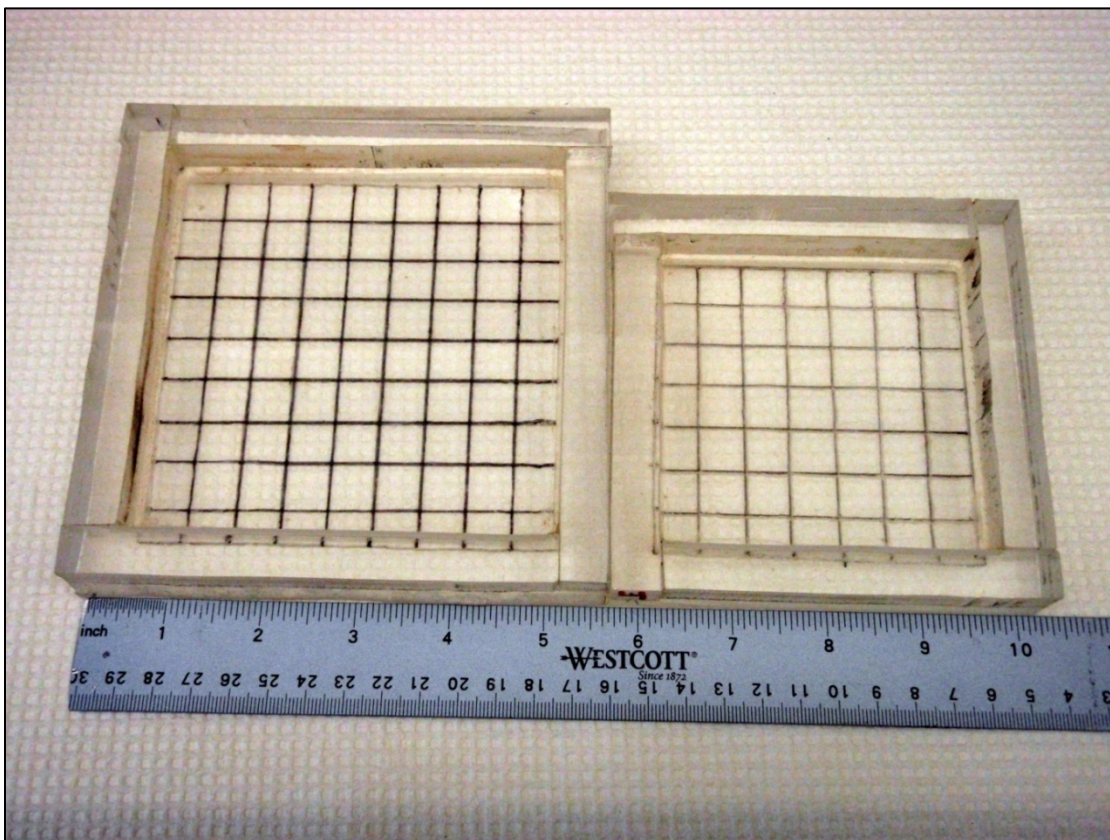


Figure 3. 81-grid (left) and 49-grid (right) estimation trays that are used to estimate the mean number of benthic macroinvertebrates in stage-1 sampling grids.

2.3. Sorting Organisms

Contents of each randomly chosen stage-1 or stage-2 grid (see section 2.6.2. Determining the Specific Processing Strategy) are sorted separately using 10X magnification. All identifiable organisms are sorted but mollusk shells and caddisfly cases are only sorted if the animals are present in the shells. Only a portion of colonial organisms, such as Bryozoans or Porifera, is sorted to document their presence in the sample. Vertebrates, exuviae, invertebrate eggs, microcrustaceans, subaquatic (e.g., collembolans) and terrestrial organisms are not sorted; similarly, insect pupae are not sorted because their abundance could be disproportionately great and not representative (i.e., drifting from upstream of sampling area during emergence).

Once sorting has begun, the grid is sorted to completion even if numeric or time criteria are exceeded. Organisms are enumerated as they are removed from each grid and pre-sorted into categories (Table 2). Organisms are placed in polyseal-capped vials containing 70% ethanol. The sort-time criteria, excluding time required to prepare the sample and estimate grid densities, are eight hours for a 300 organism fixed-count target.

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Table 2. Taxonomic categories used for presorting benthic macroinvertebrates into vials as they are removed from subsampling grids.

Taxonomic categories	
Gastropoda (snails)	Plecoptera (stoneflies)
Bivalvia (clams)	Heteroptera (true bugs)
Oligochaeta (segmented worms)	Megaloptera (dobsonflies/fishflies/alderflies)
Hirudinea (leeches)	Trichoptera (Caddisflies)
Hydrachnidia (water mites)	Lepidoptera (moths)
Decapoda (crayfish/shrimp)	Coleoptera (beetles)
Amphipoda/Isopoda (scuds/sow bugs)	Diptera (true flies)
Ephemeroptera (mayflies)	Chironomidae (midges)
Odonata (damselflies/dragonflies)	Others (nematodes/flatworms)

2.4. Sorting Large-Rare Organisms

Large-rare taxa (e.g. crayfish, hellgrammites, stoneflies) are often present at such low densities that it is unlikely that they are encountered in random subsamples. These organisms are often long-lived and ecologically important taxa that should be included in monitoring programs; consequently, the quantitative sample-processing method includes these large-rare taxa by visually sorting them for 15 minutes from the unsorted portion of the sample. If inorganic debris is separated from the sample, this debris is also sorted for large-rare organisms.

2.5. Interferences

Inorganic debris in the sample matrix interferes with obtaining a uniform distribution of the sample matrix in the subsampling frame. Substantial amounts of inorganic debris are separated from the sample matrix by elutriation before the organic portion of the sample is distributed in the subsampling frame. Large detritus is removed, rinsed, inspected for attached organisms, and discarded. Samples with substantial amounts of filamentous algae are distributed as evenly as possible. Algae (rarely encountered in ERMN samples) are cut with scissors to aid removal of randomly selected grids from matrices that contain filamentous algae.

A large sample matrix can reduce subsampling and sorting efficiency. The volume of most samples can be sufficiently field-processed to reduce the collected volume to < 750 mL. Although very rare, laboratory splitting might be necessary if the total sample volume exceeds 750 mL.

2.6. Procedure

2.6.1. Estimating mean organism abundances in the 5.1 by 5.1 cm stage-1 grids using estimation trays.

The mean number of organisms in a 1.3 by 1.3 cm estimation tray grid (B) is first determined by averaging the number of organisms in each of three randomly chosen estimation tray grids (A_i):

$$B = \frac{1}{3} \sum_{i=1}^3 A_i$$

The estimated number of organisms in each stage-1 grid (C_i) is subsequently determined from each of five estimation trays as: $C_i = e \times B$, where $e = 49$, if the 8.9 x 8.9 cm estimation tray is used or $e = 81$, if the 11.4 x 11.4 cm estimation tray is used. The mean number of organisms per stage-1 grid (D) is then calculated as follows:

$$D = \frac{1}{5} \sum_{i=1}^5 C_i$$

The value of D is used to determine an appropriate subsampling strategy.

2.6.2. Determining the specific processing strategy

The fixed-count and time criteria for quantitative sample processing can be achieved in different ways. For example, the criteria can be achieved by processing different numbers of stage-1 grids (one stage subsampling) and by subsequent subsampling of a subset of the stage-1 grids (two stage subsampling). The number of combinations that could be used is large, thus it would be possible to apply substantially different processing procedures to samples with similar numbers of organisms. A more standard approach for determining when and how a sample should be subsampled is important; consequently, sample processing procedures were developed on the basis of the average density per grid in the stage-1 subsampling frame (Table 3). The sample processing procedures are used so that no fewer than three randomly selected subsample grids are sorted. No fewer than three grids are sorted because the distribution of organisms within a subsampling frame may be clumped (Moulton et al., 2000). The following process also strives to achieve total sorted organism counts only slightly in excess of the target.

The procedure for processing a sample to a 300-organism fixed count begins by estimating the average number of organisms per stage-1 grid (D); from that estimate, the appropriate subsampling frame size can be chosen (e.g., if two-stage subsampling is necessary; Table 3).

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Table 3. Subsampling frame selection based on estimated organism density in samples.

Estimated organism density per grid in the stage-1 subsampling frame (D_n)	Subsampling frame sizes (in number of 5.1 by 5.1 cm grids)					
	Number of stage-1 grids to sort			Number of stage-2 grids to sort		
	12	24	42	12	24	42
$D_{300} \leq 120$ $D_{100} \leq 120$	3-12	3-24	3-42			
$120 < D_{300} \leq 216$ $40 < D_{100} \leq 72$	Two stage subsampling using FIVE stage-1 grids			4-6		
$216 < D_{300} \leq 432$ $72 < D_{100} \leq 144$					4-7	
$432 < D_{300} \leq 1,008$ $144 < D_{100} \leq 336$						3-6
$1,008 < D_{300} \leq 1,260$ $336 < D_{100} \leq 420$	Two stage subsampling using FOUR stage-1 grids					3-4
$1,260 < D_{300} \leq 1,680$ $420 < D_{100} \leq 560$	Two stage subsampling using THREE stage-1 grids					
$D_{300} > 1,680$ $D_{100} > 560$	Additional subsampling is necessary					

1. If $D \leq 120$, then two-stage subsampling is not necessary (see Table 3). The amount of one-stage subsampling is determined by estimating the total number of stage-1 grids (rounded to the nearest integer ≥ 3) needed to reach the fixed count target (E). E is determined as:

$$E = \frac{300}{D}$$

The entire sample is sorted (≤ 8 hr) if E is greater than or equal to the number of grids in the stage-1 subsampling frame; otherwise, E randomly selected stage-1 grids are sorted. Processing begins with the five originally chosen stage-1 grids used to determine D . If fewer than five grids are needed to reach 300 BMI, then the first three or four stage-1 grids chosen are sorted. If more than five grids are needed, then additional stage-1 grids are chosen at random from the stage-1 subsampling frame are sorted until 300 organisms are removed.

2. If $D > 120$, two-stage subsampling is necessary (see Table 3). Two-stage subsampling involves: 1) randomly selecting three to five stage-1 grids, 2) uniformly redistributing material from these stage-1 grids onto a stage-2 subsampling frame, and 3) then randomly selecting a subset of grids (stage-2 grids) to sort from the stage-2 subsampling frame.
3. A series of calculations are made to determine: 1) the number of stage-1 grids that are combined and placed in the stage-2 subsampling frame, 2) the size of the

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stage-2 subsampling frame, and 3) the estimated number of stage-2 grids that are combined to obtain the stage-2 subsample are all based on a series of calculations.

The number of organisms in an aggregation of three, four, or five stage-1 grids (G) is determined as:

$$G_i = i \times D$$

Where $i = 3, 4$, or 5 stage-1 grids; D = the average number of organisms per stage-1 grid.

The estimated number of stage-2 grids (H_k) to be sorted to reach the fixed-count target is then determined for the available stage-2 subsampling frames as follows:

$$H_k = \frac{300}{G_i / k}$$

Where $k = 12, 24$, or 42 (i.e., the stage-2 subsampling frame size).

Whenever possible, G_5 should be used to calculate H_k . Values of H_k are always rounded up to the nearest integer and should be greater than or equal to 3 and less than or equal to 7; however, some stage-1 subsampling frames may have too high a density (D) to achieve an H_k greater than or equal to 3 and less than or equal to 7 when using G_5 . In these cases, G_4 followed by G_3 should be used to calculate H_k .

When multiple H_k 's are valid for a given G_i , then the estimated number of organisms that would be sorted from stage-2 grids (I_k) may be calculated to aid in choosing k as follows:

$$I_k = H_k \times \frac{G_i}{k}$$

Where $3 \leq H_k \leq 7$.

The value of I_k can be compared to the fixed-count target and used to select the most appropriate combination of i (the number of stage-1 grids combined and placed in the stage-2 subsampling frame) and k (the stage-2 subsampling frame size). Whether or not I_k is used to select the most appropriate subsampling strategy, the i randomly selected stage-1 grids are recombined and uniformly distributed on the appropriately sized stage-2 subsampling frame (k).

These calculations consider the original organism density, the size of the stage-2 subsampling frame, the fixed-count target, and the estimated number of organisms in the final (stage-1 and stage-2) subsample. This procedure can produce a fixed-count subsample slightly in excess of 300 organisms from a sample containing <

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70,560 organisms. If the estimated number of organisms contained in the sample exceeds 70,560, the sample must be processed differently.

2.6.3. Determination of the Laboratory Correction Factor

If a sample is subsampled in the laboratory, a laboratory subsampling correction factor is calculated (Table 4). The laboratory correction factor is recorded on (1) the Subsampling and Preliminary Enumeration Worksheet and (2) the Identification and Enumeration Bench Data Sheet as $a:b$, where a is the combined numerator and b is the combined denominator.

Table 4. Formulas for calculating the laboratory subsampling correction factor based on 1- or 2-stage subsampling.

	1-Stage subsampling	2-Stage subsampling ¹
Laboratory subsampling correction factor (L)	$L = \frac{W}{X}$	$L = \frac{W}{X} \times \frac{Y}{Z}$

2. *Determination of field-correction factor.* – If the collected sample was subsampled in the field, the abundance of each taxon is corrected for field subsampling by applying a field-correction factor (F) as calculated below:

$$F = \frac{V_{\text{collected}}}{V_{\text{submitted}}}$$

where $V_{\text{collected}}$ = total volume of sample collected in the field and $V_{\text{submitted}}$ = total volume of sample submitted for processing.

¹ When 2-stage subsampling, X will typically be 5

3.0. Quality Control of Sample Processing

3.1. Sorting effectiveness

The primary purpose of re-sorting is to detect and then correct sorting error. For example, re-sorting can (1) discover a subsample grid that was inadvertently missed during the initial sorting effort or (2) to sort taxa that are systematically overlooked. Sorting effectiveness is determined by re-sorting the sorted sample remnant.

To detect sorting errors, the remnant of samples are periodically re-sorted at 10 X magnification by the Aquatic Ecologist for at least 10 % of the time that the sample was originally sorted. All organisms recovered are added to the original sort vials and become a permanent part of the sample. The total number of organisms obtained during the re-sorting period is recorded on the estimation worksheet, and sorting effectiveness, expressed as a percentage (E_s) is calculated as follows:

$$E_s = 100 \cdot \frac{S}{R + S'}$$

Where R = the total organisms obtained during the re-sort of the grid remnants, and S = the total organisms originally obtained from the sorting grids. It is expected that ≥ 80 % of the organisms be removed during the original sort.

New taxonomists should be evaluated by using a more stringent sorting effectiveness procedure. Sorting effectiveness checks are performed on all grids as they are sorted for at least the first five samples processed by a new taxonomist and no time limit is imposed. The purpose of this procedure is to ensure that sorting standards and operational issues are understood before new taxonomists begin to process samples on their own. After achieving the sorting standards (typically after processing five samples), new taxonomists are evaluated by using the normal sorting effectiveness procedures.

3.2. Documentation

After a sample has been sorted (usually during the identification stage), the Aquatic Ecologist confirms the recorded accuracy of the subsampling strategy and the resulting correction factors. This task is performed by initialing the appropriate space on the Subsampling and Preliminary Enumeration Worksheet.

4.0. Benthic Macroinvertebrate Identification and Enumeration

4.1. Overview

The ERMN uses BMI identification and enumeration methods that are slightly modified from those developed by Moulton et al. (2000). Taxonomic identification of BMIs requires experienced personnel that have broad knowledge of taxonomic principles and BMI groups – the Aquatic Ecologist is responsible for accurate identification of BMIs. Dichotomous keys are routinely used to identify BMI which provide a formal, repeatable, and stepwise method for arriving at a name for BMI based on their morphological and meristic characters. It is desirable to identify BMI to the lowest level of taxonomic classification (e.g., Order, Family, Genus) possible because ecological characteristics and responses to water-quality conditions are more specific at lower taxonomic levels (e.g., Genus) than at higher levels (e.g., Family).

4.2. Interferences

Most dichotomous keys for BMI are constructed on the basis of morphological characters that are found in mature (i.e., late instar) larvae. Many BMI collected in samples are too immature or damaged during collection, transport, and laboratory processing to be identified to the target (i.e., Genus) level of identification. If the characters required to identify BMI are missing or obscured, the identification is terminated at a higher taxonomic level than desired (e.g., Family or Order); consequently, higher level determinations are justified on the bench data sheet (Appendix B) to facilitate the interpretation of taxonomic data used for analyses. Standardized supporting notes are used to justify BMI where the target taxonomic level is not achieved or to convey additional information about BMI identifications (Table 5). Although identifications to target levels are not always possible, BMI taxonomists often have the expertise have access to a verified reference collection, and can often identify specimens to lower taxonomic levels.

Table 5. Standardized notes that 1) reflect when organism(s) are retained or 2) justify why benthic macroinvertebrate identifications to the target taxonomic level were not achieved.

Note	Definition
imm.	'Immature' - usually used because identification to targeted level was not possible because the organism(s) is/are too immature
dam.	'Damaged' - usually used because identification to targeted level was not possible because the organism(s) is/are too damaged
indet.	'Indeterminate' – includes all synonyms thereof and means that identification to targeted level not supported for recently molted organisms, mature and intact organisms because of undocumented variation or indistinct characters, required case is missing/damaged, or required habitat/ecological information is missing/unavailable
Ref.	'Reference' denotes that an organism(s) is/are placed in a reference collection
Sex	...Unidentifiable due to lack of required sexual characters

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4.3. Taxonomic Literature

Because taxonomy is a dynamic process (e.g., species are continually being described), previous designations are often revised which requires the construction of new identification keys and re-examination of the validity of some taxa. Consequently, taxonomic identifications are checked against the most current and widely accepted list of names for a particular group to ensure their validity and use.

The BMI taxonomic literature is diverse and widely scattered but Merritt and Cummins (2008) is the primary dichotomous key used by the ERMN. Because that key does not include non-insect BMI (e.g., crustaceans and gastropods), the ERMN relies on several other keys for non-insects and certain insect taxa (Table 6). Peer reviewed publications and organizations (e.g., The Society for Freshwater Science) are annually consulted via the internet to ensure that the ERMN stays current with changes to BMI taxonomy.

Table 6. Primary dichotomous keys used by the Eastern Rivers and Mountains Network to identify benthic macroinvertebrates.

Group	Key
Insects	Merritt and Cummins (2008)
Crustaceans	Rogers and Hill (2008)
Gastropods	Peckarsky et al. (1990)
Baetidae, Capniidae, Leuctridae, Simuliidae	Pfeiffer et al. (2008)
Cambaridae	multiple

4.4. Reference and Voucher Collections

The ERMN does not have resources to curate a museum of all ERMN BMI collections; consequently, the ERMN maintains 1) a physical BMI voucher collection in the laboratory and 2) digital reference photographs that are stored on the ERMN server. The voucher collection serves primarily as a Quality Assurance measure and is maintained as evidence for the ERMN's ability to accurately identify BMI taxa throughout the network.

Preference for selecting voucher specimens is given to organisms that are mature, intact, and when possible, are available in a series (i.e., several specimens of a taxon in a single sample). Specimens may be selected despite their condition if they represent the only verifiable record of a particular taxon. As additional taxa are documented from ERMN network streams, representative voucher specimen(s) are stored in 70% ethanol. When high quality voucher specimens (e.g., mature and undamaged larvae) are available, one set of specimen(s) for each taxon will be accessioned into the NPS museum system with catalog numbers associated with the ERMN. Specimens to be entered into the NPS museum will be verified by a second expert taxonomist before they are accessioned.

In addition to the physical voucher collection, digital photographs are taken of each 'new' taxon to the ERMN master taxa list. The digital images are intended primarily as a reference collection that is easily and safely distributed when colleagues have questions or interest regarding BMI found in the ERMN. The digital collection is continually updated as better (i.e., more mature or intact) specimens are available.

4.5. Taxonomic Specialists

Taxonomic specialists outside the ERMN are consulted with problematic taxonomic issues or to confirm identifications. Specialists have a demonstrated record (e.g., peer reviewed publication) in their area of taxonomic interest and should particularly be consulted if there is need for verification of threatened and endangered species.

4.6. Taxonomic Procedures

The ERMN follows guidelines that are based on those recommended by the USGS (Moulton et al., 2000) and USEPA (Barbour et al., 1999). In general, mollusks, crustaceans, and insects are identified to the Genus level whereas aquatic worms are identified to the Class level (i.e., Oligochaeta) when possible (Table 7). Other BMI groups (e.g., flatworms and nematodes) are typically identified at higher taxonomic levels (e.g., Phylum). This target level of identification allows the ERMN to use more detailed analyses than would be possible at coarser taxonomic levels (Family); moreover, it allows considerably more samples to be identified than at a species-level effort.

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Table 7. Levels of benthic macroinvertebrate taxonomic identification used by the Eastern Rivers and Mountains Network.

Taxon	Level of Identification	Taxon	Level of Identification
Porifera	Family	Petaluridae	Genus
Cnidaria	Genus	Plecoptera	
Platyhelminthes	Class	Capniidae	Genus
Nemertea	Genus	Chloroperlidae	Genus
Nematomorpha	Phylum	Leuctridae	Genus
Bryozoa	Phylum	Nemouridae	Genus
Gastropoda	Genus	Peltoperlidae	Genus
Bivalvia	Genus	Perlidae	Genus
Polychaeta	Family	Perlodidae	Genus
Aphanoneura	Family	Pteronarcidae	Genus
Oligochaeta	Class	Taeniopterygidae	Genus
Hirudinea	Family	Heteroptera	
Hydrachnidia	Order	Belostomatidae	Genus
Amphipoda	Genus	Corixidae	Genus
Isopoda	Genus	Gelastocoridae	Genus
Decapoda	Genus	Gerridae	Genus
Collembola	Order	Hebridae	Genus
Ephemeroptera		Hydrometridae	Genus
Ameletidae	Genus	Macroveliidae	Genus
Baetidae	Genus	Mesoveliidae	Genus
Baetiscidae	Genus	Naucoridae	Genus
Caenidae	Genus	Nepidae	Genus
Ephemeridae	Genus	Notonectidae	Genus
Ephemerellidae	Genus	Ochteridae	Genus
Heptageniidae	Genus	Pleidae	Genus
Isonychiidae	Genus	Saldidae	Genus
Leptophlebiidae	Genus	Veliidae	Genus
Potamanthidae	Genus	Megaloptera	
Siphonuridae	Genus	Corydalidae	Genus
Odonata		Sialidae	Genus
Calopterygidae	Genus	Neuroptera	
Coenagrionidae	Genus	Sisyridae	Genus
Lestidae	Genus	Trichoptera	
Protoneuridae	Genus	Apataniidae	Genus
Aeshnidae	Genus	Halplidae	Genus
Cordulegastridae	Genus	Beraeidae	Genus
Corduliidae	Genus	Heteroceridae	Family
Gomphidae	Genus	Histeridae	Family

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Taxon	Level of Identification	Taxon	Level of Identification
Libellulidae	Genus	Brachycentridae	Genus
Macromiidae	Genus	Calamoceratidae	Genus
Trichoptera - continued		Lamprolidae	Family
Dipsudopsidae	Genus	Limnichidae	Genus
Glossosomatidae	Genus	Lutrochidae	Genus
Goeridae	Genus	Melyridae	Family
Helicopsychidae	Genus	Microsporidae	Genus
Hydropsychidae	Genus	Ptilidae	Family
Hydroptilidae	Genus	Psephenidae	Genus
Lepidostomatidae	Genus	Ptilodactylidae	Genus
Leptoceridae	Genus	Salpingidae	Family
Limnephilidae	Genus	Scirtidae	Family
Molannidae	Genus	Staphylinidae	Family
Odontoceridae	Genus	Tenebrionidae	Family
Philopotamidae	Genus	Diptera	
Phryganeidae	Genus	Athericidae	Genus
Polycentropodidae	Genus	Blephariceridae	Genus
Psychomyiidae	Genus	Canacidae	Genus
Rhyacophilidae	Genus	Ceratopogonidae	Genus
Uenoidae	Genus	Chaoboridae	Genus
Lepidoptera		Chironomidae	Family
Arctiidae	Genus	Corethrellidae	Genus
Cosmopterigidae	Genus	Culicidae	Genus
Nepticulidae	Genus	Deuterophlebiidae	Genus
Noctuidae	Genus	Dixidae	Genus
Pyrilidae	Genus	Dolichopodidae	Family
Tortricidae	Genus	Dryomyzidae	Genus
Coleoptera		Empididae	Genus
Amphizoidae	Genus	Ephydriidae	Family
Anthicidae	Family	Muscidae	Family
Carabidae	Family	Nymphomyiidae	Genus
Chrysomelidae	Family	Pelecorhynchidae	Genus
Curculionidae	Family	Phoridae	Family
Dryopidae	Genus	Psychodidae	Genus
Dytiscidae	Genus	Ptychopteridae	Genus
Elmidae	Genus	Sarcophagidae	Family
Epimetopidae	Genus	Tabanidae	Genus
Georyssidae	Genus	Tanyderidae	Family
Gyrinidae	Genus	Thaumaleidae	Family
Helophoridae	Genus	Tipulidae	Genus

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Taxon	Level of Identification	Taxon	Level of Identification
Hydraenidae	Genus		
Hydrochidae	Genus		
Hydrophilidae	Genus		
Hydrosaphidae	Genus		

4.7. Recording Results

As BMIs are identified, each taxon (with its abundance) is recorded on the bench data sheet (Appendix B) along with its life stage [L = larva(e), P = pupa(e), A = adult(s)] and supporting taxonomic note(s) when applicable. Species-level identifications are recorded for monotypic genera and each taxon is placed in a 4-6 dram vials(s) containing 70% ethanol with an identification label. Vials are inventoried against recorded data on the bench data sheet to check for unrecorded names and to ensure that each name listed is represented by at least one organism.

All complete and fragmented BMIs are enumerated if at least the head is present. Organisms without heads or with incomplete heads are not enumerated. Although mollusks are frequently identified to Genus or Species by using shell characteristics, the organism must be present for the individual to be identified and enumerated.

5.0. Quality Control

5.1. Verification of Taxonomic Identifications

The ERMN will randomly select 10 identified and enumerated samples (i.e., all taxa collected from a site) upon completion of sampling both panels (every two years) for verification by an external, certified BMI taxonomy laboratory. If discrepancies occur between the ERMN and contracted laboratory, they will be corrected by consulting the appropriate taxonomic literature and/or training.

5.2. Review of benthic macroinvertebrate data

The taxa chosen for taxonomic verification are also re-enumerated by the contracted taxonomic laboratory to determine the accuracy of the original count. As general guidance, differences in enumeration for each BMI taxon are maintained within the enumeration limits specified in Table 8. Enumeration differences that result from changes in the level of identification following taxonomic verification are not assessed as enumeration errors.

Table 8. Performance limits used to evaluate the enumeration of benthic macroinvertebrates [+ , plus; ± , plus or minus; %, percent].

Actual count for a given taxon in the sample		Acceptable Deviation from the recorded value
Lower limit	Upper limit	
1	5	±0
6	15	±1 organism
16	35	±2 organisms
36	55	±3 organisms
56	85	±4 organisms
86+		±5% rounded up

6.0. Literature Cited

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Appendix A. Quantitative BMI Sample Processing – Subsampling and Preliminary Enumeration Worksheet

Park Code:			Reach Name:			Collection Date:							
						Subsampling Date:							
Stage-1 subsampling frame			Stage-2 subsampling frame			Estimation tray		Correction factor ($W \times Y$)/($X \times Z$)					
Total grids in stage-1 subsampling frame (W)			Total grids in stage-2 subsampling frame (Y)			Total cells in estimation tray (e):							
Total stage-1 grids used (X)			Total stage-2 grids used (Z)			Total cells counted from tray:							
Stage-1 subsampling frame grid density estimation													
Subsampling Frame coordinates			Estimation Tray Coordinates/Counts (A_i)					Total A_i (A_1) + (A_2) + (A_3)	B $A_i / 3$	C_i ($B \times e$)	Time		
Grid #	Row (R)	Column (C)	R/C	(A_1)	R/C	(A_2)	R/C	(A_3)					
1													
2													
3													
4													
5													
									$\Sigma =$	G =			
									$\Sigma/5 =$	D=G/5=			
Preliminary counts from individual sorted stage-1 or stage-2 grids													
Grid #	Row	Column	Count	Time	Grid #	Row	Column	Count	Time	Stage-1 (if $D \leq 120$)		Stage-2 (if $D > 120$)	
1					14								
2					15					E=300/D=		G=i×D=	
3					16								
4					17							H=300/	
5					18							(G/k)=	
6					19								
7					20								
8					21								
9					22								
10					23								
11					24								
12					25								
13					26					Total organisms = counted		Time to process	

B = Avg. organisms/estimation grid

C_i = Est. organisms/stage 1 grid

D = (sum of C_i)/5

E is calculated if $D \leq 120$. $E = 300/D$

G = the sum of C_i ; it is the number of organisms in an aggregation of three, four, or five stage-1 grids. i.e., $G_i = i \times D$; where $i = 3, 4$, or 5 stage 1 grids.

H_k is the estimated number of stage-2 grids to be sorted. $H_k = 300/(G/k)$; where $k = 12, 24$, or 42 (i.e., the stage-2 subsampling frame size)

$I = 3, 4$, or 5 stage-1 grids (for ERMN purposes this number should always be 5)

K = 12, 24, or 42 (i.e., the stage-2 subsampling frame size)

Sample ID: _____ Collection date: _____ Field split: _____
Sort by: _____ Date: ____/____/____ Prep time: _____ hr(s) Sort time: _____ hr(s)
ID's by: _____ Date: ____/____/____ Time: _____ hr(s)

LS = life stage. All vials and taxa accounted for _____ (initials) Data entry (initials) _____
 Entry date____/____/____
 Continue on back: YES / NO (circle one) Page_____ of _____

[illegible]

ID, identification; hr(s), hour(s); Prep, preparation; LS = life stage.

All vials and taxa accounted for _____ (initials) Data entry (initials) _____ Entry date _____/_____/_____

 Page _____ of _____