ATTACHMENT F: CATEGORY 4 MACROINVERTIBRATE SAMPLE PROCESSING AND IDENTIFICATION

DRAFT STANDARD OPERATING PROCEDURES (SOP)

"Laboratory Processing of ESRP-GLE Macroinvertebrate Samples collected in St. Louis Bay/Howards Bay in the St. Louis River Freshwater Estuary"

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1.0 SCOPE AND APPLICATION

1.1 Scope

The standard operating procedures described in this document are to be used for sample sorting and taxonomic identification of macroinvertebrate samples collected in St. Louis Bay/Howards Bay of the St. Louis River freshwater estuary for the Ecosystem Services Research Program for Great Lakes Embayments (ESRP-GLE).

A recent intensive survey of multiple habitats across the entire St. Louis River estuary found about 240 macroinvertebrate taxa (A. Trebitz, personal communication). This SOP is for a survey with a much more restricted spatial extent (i.e., St Louis Bay between Grassy Point and the Blatnik [US 53] Bridge). Therefore, somewhat fewer than 240 total taxa are expected to be collected.

1.2 Application

The procedures in this SOP apply to all macroinvertebrate samples collected using methods described in ESRP-GLE Field Sample Plans. Samples are standard PONAR samples that have been field elutriated (500 um mesh). Sample processing and taxonomic identification procedures apply to preserved samples delivered in labeled jars to the macroinvertebrate processing Contract Laboratory.

2.0 SUMMARY OF METHOD

After formalin-preserved ESRP-GLE macroinvertebrate samples are received by the Contract Laboratory, they are washed to remove formalin. Fine sediment and organic matter should be minimal in the samples due to previous field-elutriation. Samples are then subsampled using a gridded tray or similar device. Subsamples (e.g., grid contents) are removed and examined under magnification and invertebrates are removed until at least 400 organisms are obtained. The sorted organisms are identified to a specified level, and preserved. After all identifications have been confirmed through QC procedures, organisms are pooled into a small number of higher-level taxa, dried, and weighed. Specific quality assurance practices apply to sorting and identification steps of the SOP.

2.1 Definitions

PONAR sample: An ESRP-GLE macroinvertebrate sample created by elutriating the contents of two standard PONAR dredge samples (total area = 1064 cm^2). The sample is preserved in 10% buffered formalin in one or more 500 mL jars.

Distinct taxon: a unique taxon in a sample. Distinct taxa can occur at any taxonomic level (e.g., genus, family, order). Distinct taxa are assigned a name based on the lowest feasible taxonomic level. Two specimens may be the same taxonomic level and have the same name, but they are distinct because they are not the same species and both count towards sample richness. Distinct taxa may be identified on the forms and in files by a numeric qualifier: Ephemeridae 1 (distinct = yes), Ephemeridae 2 (distinct = yes), Ephemeridae (distinct = no). Species are distinct taxa by default. Taxonomy Bench forms and electronic files will contain a field for identifying distinct taxa.

External sample label: label affixed to the outside of each sample jar. Information on the label includes Sample ID, collection date, jar number, and the total number of jars. The label shall remain on the jars, which are also used to hold the unsorted sample residue.

Internal sample label: Duplicate label placed inside each sample jar with the same information as the external sample label.

Incidental taxa: non-benthic taxa (non-benthic adults, terrestrials) in a sample that are removed from the subsample during sorting but which are not identified (unless it is not clear if they are aquatic or not) and do not count toward the sorting enumeration target. Zooplankton may be ignored. Zebra/quagga mussels (*Dreissena)* should be sorted from the samples, but should not count toward the sorting enumeration target. Non-aquatic adult specimens of aquatic taxa may be identified to confirm the likely presence of the taxa as larvae in the samples.

Lowest feasible taxonomic level: the lowest taxonomic level to which a specimen can be identified. The lowest feasible taxonomic level maybe limited by the availability of taxonomic keys and the maturity and condition of the specimens. A taxa-specific listing of taxonomic level of identification required for ESRP-GLE is provided (Table 1).

Macroinvertebrate sample tracking form: a paper copy of the form used to track the transfer of samples from EPA to the Contract Laboratory. The Contract Laboratory will design and provide this form.

Macroinvertebrate sorting bench form: a paper copy of the form used by the contract laboratory that includes sample metadata from the sample label, all the information needed to calculate the percent of the sample sorted, and the number of organisms sorted from the sample. The Contract Laboratory will design and provide this form. There is one sorting bench form per sample, although additional forms may be used as continuation forms if additional space is needed

for a sample. Guidance on what this form should include can be derived from the required contents of the Macroinvertebrate taxonomy report (Section 6.1).

Macroinvertebrate taxonomy bench form: a paper copy of the form used by the contract laboratory that includes sample metadata from the sample labels and a taxonomic name and count for all the specimens sorted from the subsample. There is one taxonomy bench form (multi-page) per sample. The Contract Laboratory will design and provide this form. Guidance on what this form should include can be derived from the required contents of the Macroinvertebrate taxonomy report (Section 6.2).

Macroinvertebrate sample information report: a spreadsheet with information from the macroinvertebrate sorting bench forms. The file will contain all the data for all the sites.

Macroinvertebrate taxonomy report: a spreadsheet with information from the macroinvertebrate taxonomy bench forms. The file will contain all the data for all the sites.

Percent of the sample sorted: the percent of the total sample that is subsampled in a gridded tray and from which the organisms were removed (sorted) and identified. For example, if 5 of 30 grids of a sample were sorted, the percent of sample sorted would be (5/30) x 100 = 16.7%.

Percent sorting efficiency (PSE): = (number of organisms found by original sorter/ (number found by original sorter + number found by QC sorter)) * 100.

Percent taxonomic agreement (PTA): = (number of agreeing identifications found by QC taxonomist/ (number of identifications made my original taxonomist))*100.

Reference collection: specimens of each distinct taxon identified from ESRP-GLE samples should be retained in a voucher collection. Specimens should be properly labeled and preserved in vials with ethanol or on slides and a catalog of the collection maintained. Taxonomic references used and experts consulted should be listed.

Sample pre-processing: processing steps for the samples prior to subsampling, sorting, or identification. Pre-processing includes washing the formalin out of the sample, removing large organic and inorganic debris by hand and removing fines <500 μm by sieving. In most samples, field elutriation will have eliminated most debris and fines.

Sample sorting: separation of macroinvertebrates from organic debris into labeled vials.

Sorted sample residue: organic debris remaining from the portion of the sample that is sorted under magnification. The sorted residue is placed in a new container with 70% ethanol and labeled appropriately. The sorted residue is the portion of the sample that is "re-sorted" to determine the PSE.

Sorting enumeration target: the target number of organisms removed from the sample during sorting. For ESRP-GLE, the sorting enumeration target is 400 organisms. Zebra mussels (*Dreissena*) do not count toward the sorting enumeration target. In all cases, partial grids should be sorted.

Subsampling: random selection of grids from a gridded subsampler that are to be sorted under magnification.

Taxonomic identification: assignment of a taxon name to all the specimens sorted from subsamples excluding incidental taxa.

Taxa-specific biomass: dry weight of organisms pooled across taxa into higher-level groups

Unsorted sample residue: organic debris remaining from the portion of the sample that is not sorted. The unsorted sample residue is placed in the original containers with the original label with either ethanol or recycled formalin and labeled appropriately.

2.2 Health and Safety Warning

1. Samples should be washed, sorted, and identified in areas with adequate ventilation.

2. Formalin-preserved samples should be stored in an area with adequate ventilation.

3. Preparation of specimens on microscope slides with mounting media should be preformed with adequate ventilation.

2.3 Cautions

1. Damaged and early instar larval specimens may be impossible to identify to the desired taxonomic level.

2. Identification keys do not exist for all groups that allow identification to the lowest taxonomic level.

3. QC checks must be performed by qualified personnel.

4. Appropriate personal safety protection should be used (gloves, lab coat, eye protection).

3.0 APPARATUS AND MATERIALS (partial list – may be modified by contract laboratory)

500-µm sieves
Small and large Caton gridded subsamplers or comparable device
Delimiter for removing grid contents from subsampler
Vials for sorted specimens and voucher collection
Vial labels
Stereo dissecting microscope with fiber-optic light source
Compound microscope
Forceps
Sorting pans or dishes
Ethanol (70%) for preservation of specimens and sorted sample residue
Slide preparation supplies
Safety equipment
Tally counters
Bench forms
Personal safety protection

4.0 PROCEDURES

4.1 Sample tracking

1. One or more **sample tracking forms** will be filled out which lists all the samples and their condition in the samples received by the contract laboratory. The EPA and Contract Laboratory representatives both sign the completed form(s). The form is copied and the Contract Laboratory representative gets a copy.

2. Jars containing **sorted sample residue**, **unsorted sample residue**, and vials containing the sorted and identified specimens may be transferred back to MED for storage. A macroinvertebrate tracking form is also used to track this transfer.

4.2 Sample Processing

1. In an area with adequate ventilation, the contents of all the jars for a sample are poured into one or more 12" (recommended) 500-µm sieve for cleaning. Waste formalin should be retained and disposed of properly. It may also be recycled and used to preserve the unsorted sample residue. Place the original internal sample label in the jars holding the unsorted sample residue.

3. The sieve contents are gently washed with water to remove the formalin from the sample and to further remove fines <500 µm from the sample. Do not use a strong flow to wash the samples because soft-bodied organisms will be damaged. Large inorganic and organic particles (gravel, macrophytes, wood) should be washed in the sieve and returned to the original sample jar(s).

4.3 Subsampling

1. Record the label information on a **macroinvertebrate sorting bench form** including the site ID, sample ID, collection date, number of jars, and the name of the sorter.

2. Transfer the sample from the sieve onto the gridded screen of the subsampler(s). Do not overload the subsamplers. Add enough water to distribute the sample across the grid. Use a spoon to distribute the sample, jiggle the subsampler to level, and evenly distribute the sample across the grid. Use either all small or all large subsamplers for a sample (do not mix sizes).

3. Lift the screen out of the tray, pour excess water from the tray, and replace the screen. Leave enough water in the tray to keep the screen contents moist.

4. Record the number of grids sorted to reach the **sorting enumeration target** of 400 organisms (excluding *Dreissena*) and the total number of grid squares (30 in a standard Caton subsampler).

5. Use a delimiter with the same dimensions of a grid and remove the selected grid contents with a spatula, spoon, scoop, forceps, or dropper (whichever is most effective). Place the contents of each grid contents into a watch glass or dish that can be examined under a dissecting scope. If an organism straddles a grid line, it belongs to the grid that contains its head or most of its body if no head is apparent (e.g., worms).

6. The sorted sample residue should be placed in a new container with 70% ethanol and labeled appropriately.

4.4 Sample sorting

1. Place each dish containing all or part of a subsample (contents of a grid) under a dissection scope and examine the contents for benthic aquatic organisms at ≥ 10X magnification. Organisms picked out should be preserved in vials filled with 70% ethanol labeled with the sample ID.

2. Record enumeration data for each subsample on the macroinvertebrate sorting bench form.

3. If the sample contains <400 organisms (excluding *Dreissena*), the entire sample should be picked.

4. Non-aquatic adult and terrestrial insects should be sorted from the sample and preserved, but they do not count toward the sorting enumeration target of 400 organisms and are not included in taxa-specific biomass. Ignore zooplankton.

5. *Dreissena* mussels should be sorted from samples (or subsamples, if necessary) and counted, but they do not count toward the sorting enumeration target of 400 organisms.

6. Once the enumeration target is reached, record the total number of grids and number of random grids sorted so that the percent of sample sorted can be calculated.

   An 8-hour limit is imposed on total picking time. Stop at the grid that is finished at the 8-hour mark, regardless of whether the 400-count target has been reached. Record on the bench sheet that picking was halted due to time limit. If the target can be reached in a short amount of additional time, go on and pick another grid.

7. Do not sort partial grids. Once a grid has been started, it should be finished even if the sorting enumeration target of 400 organisms will be exceeded.

8. The sorted sample residue (examined grid contents) should either be re-sorted by a QC sorter (see Section 5.2) or placed in a new container(s) filled with 70% ethanol and labeled appropriately.

9. The unsorted sample residue should be placed in the original container(s) filled with 70% ethanol or recycled formalin.

4.5 Taxonomic identification

1. Transfer the applicable information from the **macroinvertebrate sorting bench form** to the **macroinvertebrate taxonomy bench form**.

2. Transfer vial contents to watch glasses or dishes filled with water plus a few drops of dish detergent. Identify all the specimens to the lowest feasible taxonomic level using the most current taxonomic reference material. Attempt to achieve at least the taxonomic level specified in Table 1.

3. Sort specimens during identification so that the number or specimens of each life stage (larvae, pupae, adult) of each taxon can be counted. Record the count for each stage for each taxon and whether or not the specimens constitute a distinct taxon.

4 If one life stage of an organism can be identified to a lower taxonomic stage than another, only the most specific taxon will be identified as distinct.

5. Damaged organisms should be counted only if they can be identified. Mollusk shells and insect cases should be counted only if occupied.

6. Do not identify or count incidental taxa (terrestrials, non-aquatic adults). Do count and identify zebra/quagga mussels.

7. Prepare slide mounts, as necessary, for small dipteran larvae and pupae, worms, water mites (mount ventral side up), and any other specimens too small to identify with a dissecting microscope. Multiple specimens can be mounted on a slide. Label slides with at least the ESRP-GLE sample ID.

   Taxonomists with extensive experience identifying Oligochaeta and Chironomidae will follow the identification procedures for those groups as written in EPA's GLNPO SOP LG407, Standard Operating Procedure for Benthic Invertebrate Laboratory Analysis. These procedures describe identifications of certain Oligochaeta and Chironomidae taxa using a dissecting microscope.

8. Identified organisms should be placed in labeled (Sample ID, taxa, or taxon) vials such that re-identification of the sample for QC purposes is facilitated. Every taxon need not be placed in its own vial, but this will facilitate taxon-specific biomass determinations later.

9. Uncertain taxa should be sent to a consulting taxonomist for confirmation.

10. As appropriate, extract specimens from the sample as type specimens for the reference collection. When a sample is chosen as the source for a specimen in the collection, all specimens of that taxon in that sample should be placed in the reference collection. The reference collection should be augmented with

additional specimens (1 vial/source sample) throughout the project.

11. Record the extraction of reference specimens from the sample in a column on the macroinvertebrate taxonomy bench form (see section 6.1).

4.6 Taxon-specific biomass

1. After all the taxonomic identifications of all samples have been confirmed as described in Section 5.3; voucher specimens extracted, the taxonomic identifications rectified as needed for internal consistency, and entered into a spreadsheet, **a preliminary draft of the data will be transmitted to EPA**. On instructions from EPA, the contractor will pool the organisms into a small number of taxa groups for biomass determination, including important local taxa and composite higher-level taxa.

2. Tentative biomass groups (to be confirmed after examining the preliminary data) may include:

   Annelida, Chironomidae, other Diptera, *Hexagenia*, other Ephemeroptera, other Insecta, Crustacea (excluding crayfish), Crayfish, *Dreissena*, other, Mollusca, other organisms

3. Organisms in confirmed taxon-specific biomass groups should be composited into vials or weigh boats, dried in a drying oven at 55°C for at least 48h, allowed to cool in a desiccator for 24h, and weighed to the nearest 0.1 mg on an analytical balance.

4. Record data on the **macroinvertebrate taxonomy bench form** (or a specific form at the discretion of the contract laboratory).

5. Note any voucher specimens that were removed from the samples prior to weighing.

5.0 QUALITY CONTROL AND QUALITY ASSURANCE PRACTICES

5.2 Sorting Check

1. Each sorter should be given samples in batches of 10.

2. One randomly selected sample from each batch of sorted samples should be checked for missed organisms in the sorted residue by an experienced "QC"sorter. The percent sorting efficiency (PSE) should be calculated and recorded on a bench form.

3. If the PSE is <90% for the sample re-sorted by the QC sorter, 2 more samples

are re-sorted from the batch. If the mean PSE for all 3 samples is ≥90%, the batch of 10 samples is considered to meet the performance objective for sorting. (Additional organisms found in samples that pass the sorting check test do not need to identified and should be returned to the sorted sample residue.)

4. If the mean PSE for all 3 samples is ≤ 90%, the remaining 7 samples in the batch should be re-sorted. Additional organisms found in samples should be identified and added to the total for samples in the batch.

5. A record of sorting checks will be maintained and should accompany the deliverable.

5.3 Taxonomic identification

1. The contract laboratory QC officer will randomly assign 10 percent of the samples as QA samples to be re-identified by an in-house or external QC taxonomist.

3. The performance objective for taxonomic identification is a percent taxonomic agreement (PTA) of > 90%.

4. The QC officer will compare the original and QA bench forms for re-identified samples to calculate PTA.

5. If PTA is <90%. The specific mismatches should be examined in more detail. EPA samples containing problematic taxa will be reexamined and taxonomic identification errors corrected.

6.0 DATA REPORTING AND RECORDS MANAGEMENT

6.1 Macroinvertebrate sample information report

1. The Contract Laboratory will provide an electronic spreadsheet (*.xls) with selected information from the macroinvertebrate sorting bench form. The file will contain all the data for all submitted samples.

2. The required data fields (columns) include

   Sample ID: Sample IDs will be in the form “SLB10-3-19-005_B” or HB10-1-1-005_B. The first string “SLB10” indicates the project/year; the second string “3” indicates the EPA focus area at the site; the third string “19” indicates the EPA location identifier; the fourth string “005” indicates the sample depth; the last string “B” indicates that the sample is a benthos sample”

Sample collection data: date from jar labels.

Lab sample ID number: unique code that may be assigned to samples by Contract Laboratory.

Number of jars containing the sample: data from sample labels.

Date samples received from EPA: self explanatory.

Date sample sorted: self explanatory.

Sorter: self explanatory.

Percent of sample sorted: calculated from the macroinvertebrate sorting bench form for the sample.

QC information: PSE if applicable

3. After the file has been error-checked by the Contract Laboratory, the reports should be transmitted to the WAM for approval.

6.2 Macroinvertebrate taxonomy report

1. The Contract Laboratory will provide an electronic spreadsheet (*.xls) with selected information from the macroinvertebrate taxonomy bench forms. The file will contain data for all submitted samples.

2. The required data fields include

Sample ID: date from jar labels.

Sample re-identified for QA: indicates (Yes or No) if the data are from a sample re-identified by a QC taxonomist.

Sample collection date: date from jar labels.

Lab sample ID number: unique code that may be assigned to sample by Contract Laboratory.

Date identified: self explanatory.

Taxonomist: self explanatory.

Taxon IT IS: ITIS number assigned to each taxon identified in each sample

Taxon name: data from macroinvertebrate taxonomy bench forms.

Number of individuals: data from macroinvertebrate taxonomy bench forms.

Number of larvae: data from macroinvertebrate taxonomy bench forms.

Number of pupae: data from macroinvertebrate taxonomy bench forms.

Number of adults/others: data from macroinvertebrate taxonomy bench forms.

Proportion of sample sorted: data from macroinvertebrate sorting bench forms, convert percentage to proportion.

Multiplication factor: (1/proportion sorted) used to convert subsample abundance to whole sample abundance.

Whole sample abundance: self-explanatory.

Taxonomic phylum: self-explanatory.

Taxonomic class: self-explanatory.

Taxonomic order: self-explanatory.

Taxonomic family: self-explanatory.

Taxonomic subfamily: Applies primarily to chironomids that cannot be identified to genus.

Taxonomic tribe: Applies primarily to chironomids that cannot be identified to genus.

Taxonomic genus: self-explanatory.

Taxonomic species: self-explanatory.

Distinct taxa: data (Yes or No) from macroinvertebrate taxonomy bench forms.

Dry weight of each taxon-specific biomass group: this will require a

number of fields equal to the number of groups. This information should be provided in a separate spreadsheet or a different worksheet of the taxonomy report file.

Data flags: data qualifiers defined by the contract lab. Examples may include immature specimens, damaged specimens, decomposed specimens, non-aquatic taxa.

Lab comments: data from macroinvertebrate taxonomy bench forms. This field includes special circumstances and data about transfer of specimens to the reference collection.

3. After the file has been error-checked by the Contract Laboratory, draft final reports should be transmitted to the WAM for approval.

6.3 QA/QC Reporting

1. The Contract Laboratory will submit an annual report to the WAM summarizing achievement of the performance objectives for sorting and taxonomic identification.

2. The data for re-identified samples that fail the QA test (PTA <`90%) should be used to correct the Macroinvertebrate Taxonomy Report for that sample (not entered as a separate sample). The specific changes should be documented on the bench sheets. Re-identified and corrected samples should be identified in the report. Samples that pass the PTA QA test do not need to be corrected (except in the case of systematic problems identified by re-identifications that should be corrected for all samples.

6.4 Reference collection

1. The Contract Laboratory will maintain a reference collection for the submitted samples

Table 1. Taxonomic level of effort. Consult EPA WAM for taxa not included in this table.

Taxon	Level of identification
Phylum Annelida	
Class Branchiobdellida	Class
Class Hirundinea	Species
Class Oligochaeta	Genus
Class Polychaeta	Species
Phylum Arthropoda	
Class Arachnoidea (Acarina)	Family
Class Arachnida	Family
Class Insecta	
Coleoptera	Genus unless monotypic
Diptera	Genus unless otherwise specified below; pupae to family
Ceratopogonidae	Family[1]
Chironomidae	Genus[2]; pupae to tribe/subfamily
Dolichopodidae	Family
Dryomyziidae	Family
Phoridae	Family
Sciomyzidae	Family
Scathophagidae	Family
Syrphidae	Family
Ephemeroptera	Genus unless monotypic
Ephemeridae	Species
Hemiptera	Genus unless monotypic
Lepidoptera	Genus unless monotypic
Megaloptera	Genus unless monotypic
Odonata	Genus unless monotypic
Plecoptera	Genus unless monotypic
Trichoptera	Genus unless monotypic; pupae to family
Class Malacostraca	
Amphipoda	Species
Decapoda	Species
Isopoda	Genus
Mysidacea	Species
Class Ostracoda	Class
Phylum Cnidaria	Phylum (except *Hydra*)
Phylum Mollusca	
Class Bivalvia	Genus[3]
Class Gastropoda	Genus
Family Hydrobiidae	Family (except *Potamopyrgus antipodarum*)
Phylum Nematoda	Phylum
Phylum Nemertea	Phylum
Phylum Platyhelminthes	Class (except *Polycelis coronata*)
Terrestrial arthropods and plankton	Ignore

[1] Identify *Dasyhelea*, *Bezzia/Palpomyia*, *Atrichopogon*, *Probezzia*, *Forcipomyia* to genus.

[2]Some taxa are identified as composite genera due to difficulty in separating them at genus. These include *Conchepelopia/Helopelopia*, Thienemannimyia group (other than *Conchepelopia/Helopelopia*), some *Microchironomus* and *Cryptotendipes* as *Microchironomus/Cryptotendipes* (those not fitting description in key), and *Cricotopus/Orthocladius*.

[3]*Sphaerium/Musculium* may be identified as a composite genera.