Illinois Environmental Protection Agency

Bureau of Water

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Standard Operating Procedure for Sample Processing for the Macroinvertebrate Index of Biotic Integrity (mIBI)

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1.0 Scope and Application

The instructions included in this Standard Operating Procedure (SOP) describe how to obtain a 300 ± 20% fixed-count macroinvertebrate subsample and how to enumerate and identify the individual members at the proper taxonomic resolution in order to properly compute and interpret the macroinvertebrate Index of Biotic Integrity (mIBI).

The SOP is part of a larger mIBI protocol that includes specific macroinvertebrate collection, identification, computation, and application instructions. To ensure a correct application of the mIBI, follow the protocols outlined in the module *"Calculation of the macroinvertebrate Index of Biotic Integrity (mIBI)"*. To ensure that samples are appropriate for the mIBI, follow the protocol described in *"Methods to Collect Aquatic Macroinvertebrates from Wadeable Streams for Biotic Integrity Assessments".*

The mIBI represents how macroinvertebrates respond to and integrate the chemical, physical and biological effects of human-caused impacts on streams and their watersheds when compared to a subset of least disturbed locations sampled during 2001.

2.0 Summary of Method(s)

- Collect samples according to the method outlined in "*Methods to Collect Aquatic Macroinvertebrates from Wadeable Streams for Biotic Integrity Assessments*".
- Rinse the sample with water to remove preservatives.
- Assign unique numbers to identify each of the thirty 6x6-cm grid cells in the screened subsample tray.
- Load the rinsed sample materials into the screened subsample tray.
- Evenly distribute the materials over the subsample tray.
- Submerge the loaded subsample tray in water to partially suspend the sample materials.
- Remove the subsample tray from the water to facilitate random distribution of the sample over the entire screen.
- Randomly select four cells from the gridded subsample tray.
- Place the contents of the selected cells into a shallow white pan. Partially fill the pan with liquid to facilitate macroinvertebrate sorting.
- Sort 300 ± 20% aquatic macroinvertebrates using a ring-light magnifier. Exclude macroinvertebrates (e.g., semi-aquatic taxa and others listed in, "*Illinois Environmental Protection Agency Tolerance List and Functional Feeding Group Classification*", in Appendix A.) from the 300 ± 20% count.

- If fewer than 300 ± 20% macroinvertebrate exist in the initial four cells, randomly select and sort additional sample material, one cell at a time, until the target number of organisms is obtained.

- If greater than 300 ± 20% macroinvertebrates exist in the initial four cells then reduce the amount of material and macroinvertebrates sorted. Place all of the materials from the initial four cells into a second gridded screen and conduct a "*Level-2"* subsample. Subsample the second gridded screen as before and obtain materials from the four ("*Level-2"*) cells. Repeat as necessary (e.g., "*Level-3"*, "*Level-4"* etc.) until the subsample contains 300 ± 20% macroinvertebrates.

- The subsample is complete.

- Identify macroinvertebrate with the taxonomic keys listed below in "*10.0 Macroinvertebrate Keys*".

- Refer to the module "*Calculation of the macroinvertebrate Index of Biotic Integrity (mIBI)"*, for information on the required levels of taxonomic resolution for specimen identification.

3.0 Interferences and Corrective Action

Proper application of the mIBI requires properly collected samples. To collect mIBI samples, follow the instructions outlined in the module "*Method to Collect Aquatic Macroinvertebrates from Wadeable Streams for Biotic Integrity Assessments*". In addition, a 300 ± 20% individual fixed-count subsample is required since this sample size was utilized to develop metric score expectations and mIBI interpretation criteria.

4.0 Safety

To reduce exposure to sample preservatives, protective eyewear and latex gloves should be worn. Process samples in a well ventilated area or under a fume hood. Depending on the preservative utilized, several rinsing and soaking steps may be needed to prepare the sample for processing.

5.0 Equipment and Supplies

Standardized gridded screen (595 micron screen, 30 cells, each 6 cm^2), shallow watertight tray for submerging the gridded screen, 6 cm scoop, 6 cm^2 metal dividing frame, forceps, scissors, white plastic or enamel pan for sorting, ring-light, specimen vials with caps or stoppers, sample labels, standard laboratory bench sheets, 95 percent ethanol for storage of specimens, sample log and tracking sheets.

6.0 General Guidelines (in part from Barbour et. al., 1999)

The procedures, outlined below, follow the methods established in the US EPA Rapid Bioassessment Protocol (Barbour et. al. 1999). These procedures use a fixed-count sub-sampling approach for sorting organisms from the materials included in the sample matrix. The reason for the subsample requirement is that the volume of material collected with the proportionally allocated multihabitat collection approach is typically impractical to process in its entirety. Sub-sampling reduces the effort required for the sorting and identification aspects of macroinvertebrate samples and provides a more

expedient expenditure of time (Barbour and Gerritsen 1996).

To facilitate processing and identification, the randomized 300 ± 20% fixed count subsample is sorted and preserved separately from the remaining sample. Document the level-of-effort, or proportion of sample processed by recording this information on the Laboratory Bench Sheet.

The instructions in this SOP describe two phases of macroinvertebrate sorting. The "*Phase-1"* sort is the source of all mIBI information. A "*Phase-1"* sort refers to the sub-sampling process utilized to randomly select 300 ± 20% macroinvertebrates from the sample. A *"Phase-1"* sort can progress in stages and each stage corresponds to a particular "*sub-sample level*". The level of sub-sampling needed to meet the 300 ± 20% target depends on the density of macroinvertebrates in the sample. "*Phase-2"* sort refers to a fifteen minute search conducted on all remaining unsorted material to check for obviously unique taxa not found during "*"Phase-1"* sub-sampling. "*Phase-2"* organisms are placed in a separate vial and are not included as part of the subsample. Exclude taxa obtained during the "*Phase-2"* sort from all mIBI computations. "*Phase-2"* taxa may be utilized, independent of the mIBI, as an aid when making water quality interpretations.

6.1 Specific Guidelines (in part from Barbour et. al., 1999)

- Record sample collection information on the benthic macroinvertebrate tracking sheets. These sheets are located in the sample log-book. Record sample label information plus the information from the Illinois EPA field assessment form on the benthic macroinvertebrate laboratory bench sheet. This information will include sample ID/station code, method of collection, allocation of jabs, number of containers per sample, initials of collector, start time, duration and sample collection date.

- Thoroughly rinse the sample in a No. 30 mesh (595-µm openings) sieve to remove preservative and fine sediment. Depending on the preservative utilized, this step may need to be initiated several days prior to sub-sampling and this step may need to be repeated daily. The log-book containing sample tracking sheets should be posted in the lab or sorting area to track this process. Large organic material (whole leaves, twigs, algal or macrophyte mats, etc.) not removed in the field should be rinsed, visually inspected, and discarded. Use large Rubbermaid type tubs to facilitate this activity. If the samples have been preserved in alcohol, it will be necessary to soak the sample contents in water for about 15 minutes to hydrate the benthic organisms, which will prevent them from floating on the water surface during sorting. If the sample is stored in more than one container, the contents of all containers for a given sample should be combined and homogenized at this time.

- Use Oregon Department of Environmental Quality standardized sub-sampling gear (Caton, 1991) to obtain 300 ± 20% randomly selected macroinvertebrates from the 20-jab sample. The subsample gear has two parts- the first part is a shallow watertight tray and the second part is a 600-micron screen. The screened tray is equally-divided into a grid of 30 clearly marked cells and each cell measures 6x6-cm. Place the screened tray into the watertight tray and spread the entire sample onto the screened grid. Pour enough water into the gear to immerse the sample. This activity will help suspend the sample and facilitate a more even distribution of sample materials over the screened grid. Then lift the screen out of the tray, which will cause the sample materials to settle evenly onto the screen. When samples are too large to be effectively-sorted in a single tray, place half of the homogenized sample in each of two gridded trays.

- Assign a unique number (e.g., 1-30) to each of the 30 cells in the grid of the screened tray. Randomly select four of the thirty gridded tray cells. Use a random number table or computerized random number generator to randomly select the four numbers. If two gridded trays are needed then eight cells should be selected- four from each of the two trays. The material contained in the four, randomly- selected, cells make the subsample.

- Beginning with the first-four randomly-selected cells (eight cells if two trays were used), remove all organisms and debris contained within the boundary of the pertinent cells and place these materials into a white pan to sort. When transferring organisms from the grid to the sorting pan, consider any organisms that straddle adjoining cells to be on the cell containing the head. In those instances where it may not be possible to determine the location of the head (worms for instance), the organism is considered to be in the cell containing most of its body. When a selected cell has organic debris lying over or entangled among the materials in an adjacent cells, it may be necessary to use a pair of scissors to cut materials along the gridline. Cutting will help prevent the unintended transfer of debris-entangled macroinvertebrates from one cell to another.

- After placing the randomly-selected materials into a shallow white pan, add a small amount of liquid to facilitate sorting. Use a ring light (approximately 1.75X) in the sorting process (this provides focused light on the sorting pan and helps standardize the visual acuity among sorters). Sort through the debris, and remove and count all the macroinvertebrates in the pan.

- If it becomes obvious, at any point during the sorting process, that the density of macroinvertebrates contained in the initial four-cells will exceed the subsample target (300 ± 20%), then suspend the sort. Exceeding the target number of organisms before all four cells are sorted essentially amounts to a "*false-start*". If a "*false-start"* occurs, place the just-sorted organisms back in with the unsorted portion of the initial four-cell subsample. Re-homogenize the material in the subsample. At this point, the composition of the re-homogenized material is as if the four cells were never sorted and were just now randomly selected and transferred from the subsample to the sorting tray. Since the "*false-start*" establishes that too many organisms exist in the re-homogenized material, these four cells must undergo a "*Level-2"* subsample before another sort is attempted. To perform the "*Level-2"* subsample, transfer the re-homogenized materials from the initial four cells into a second gridded tray. Distribute these materials evenly over the second tray and randomly select four new cells one at a time. The four newly-selected cells represent the "*Level-2"* subsample. Perform material dispersion and cell selection for the "*Level-2"* subsample in the same manner as for the initial four cells. It is important to note that *if* the target number of organisms is routinely exceeded in the first four cells, the sorter may elect to automatically perform the "*Level-2"* subsample rather than waste time due to "*false-start*" sorting.

- If the density of organisms is large enough that many more than the targeted number of organisms are contained in the four cells, then transfer the contents of the cells to a third gridded pan and continue as before. If the targeted subsample amount of 300 ± 20% organisms is reached, the subsample is finished. If less than the targeted 300 ± 20% organisms exist, continue randomly selecting and sorting cells, one-at-a-time, until the targeted subsample number is obtained. If picking through the entire next cell is likely to result in a subsample with greater than 300 ± 20% organisms, then subsample that single cell in the same manner as before. That is, spread the contents of the last cell into another gridded pan. Pick cells, one-at-a-time, until 300 ± 20% organisms are reached. The total number of cells for each subsample level should be noted on the laboratory bench sheet. Each cell selected for sorting, must be sorted in its entirety.

- Except for one situation, a 300 ± 20% organism fixed count subsample is required for all macroinvertebrate samples utilized for the mIBI. The exception occurs when the entire sample contains fewer than 240 total individuals. In this situation, the subsample will never reach the 300 ± 20% organism target. Use extreme caution when interpreting mIBI results based on fewer than 240 macroinvertebrates. A 300 ± 20% individuals fixed-count subsample is required since this sample size was utilized to develop metric score expectations and mIBI interpretation criteria

- Until the target number of organisms is obtained, be careful not to accidentally disturb the subsample tray between these activities (e.g., removing cell contents or while sorting) because this may cause a redistribution of specimens that could possibly change the probability of selection if additional cells must be sorted.

- It is important to note that organisms, which are damaged beyond recognition, should not be included in the subsample count. Likewise, exclude all Hemiptera and most semi-aquatic Coleoptera from the subsample count. Regardless of the number of semi-aquatic taxa omitted, the subsample procedure continues until the fixed-count target of 300 ± 20% organisms is attained. Refer to the tolerance assignments in Appendix A, to identify ineligible semi-aquatic taxa. The ineligible taxa have tolerance values of 99.9.

- Once this initial phase of sorting is completed and the target number of organisms is obtained, a fifteen minute "*Phase-2"* sort is conducted on all remaining unsorted material to check for obviously unique taxa not found while obtaining the require 300 ± 20% macroinvertebrates. "*Phase-2"* organisms will be placed in a separate vial and will not be included as part of the initial subsample. Also, exclude taxa obtained during the "*Phase-2"* sort from all mIBI computations. "*Phase-2"* taxa may be useful, independent of the m-IBI, as an aid when making site quality interpretations.

- Note the type of sample, sample ID/station code, sorter, date sorted and any comments regarding the sample (e.g., description of organic material in sample -- sand, fine organics), and sorting time on the bench sheet.

- For 10% of the samples, save the unsorted sample residue in a container labeled "sample residue"; this container should include the original sample label. Save the sorted debris in a separate container labeled "sorted residue" in addition to original label information. Length of storage and archival is to be determined.

- Place specimens sorted as the subsample **(**300 ± 20% organisms) into a glass vial, and preserve in 95 percent ethanol. Label the vial inside with the station code, stream name, collection date and sample ID/station code.

- Any entirely-sorted sample that falls below the designated subsample size of 300 ± 20% may provide spurious information. Use extreme caution when interpreting mIBI results based on fewer than 240 macroinvertebrates. A 300 ± 20% individuals fixed-count subsample is required since this sample size was utilized to develop metric score expectations and mIBI interpretation criteria

7.0 Quality Control (QC) for mIBI samples

- Ten percent of the sorted samples in each lot should be examined by laboratory QC personnel or a qualified co-worker. A lot is defined as a special study, basin study, entire index period, or individual sorter. The QC worker will examine the cells chosen and the tray used for sorting and will look for organisms missed by the sorter. Organisms found will be added to the sample vial. If the QC worker finds that less than 10% of the organisms remain in the sorting tray, the sample passes; if more than 10% are found, the sample fails. If 10 percent of the sample lot fails, another 10 percent is randomly selected until failure no longer occurs or the entire lot is checked. Sorters in-training will have their samples 100 percent checked until sorting efficiency reaches 90%. The results of the QC check are recorded on the Benthic Macroinvertebrate Laboratory Bench Sheet.

- After laboratory processing is complete for a given sample, all sieves, pans, trays, etc., that have come in contact with the sample will be rinsed thoroughly, examined carefully, and picked free of organisms or debris.

8.0 Organism Identification for mIBI Calculations

8.1 General Guidelines

- The taxonomic resolution of macroinvertebrate identification varies for mIBI calculations. In general, it is preferred to identify organisms to species level whenever practical. However, for mIBI calculations use generic resolution for insects and non-sphaerid bivalves, class resolution for leeches, worms and flatworms, and family resolution for crayfish, fingernail clams, and mussels. the taxonomic keys listed below for macroinvertebrate identification. Refer to "*Calculation of the macroinvertebrate Index of Biotic Integrity (mIBI)"* for specific information about the level of taxonomic resolution required for a given taxonomic group.

- When there is a question concerning the correct identification of a specimen, send it to other recognized authorities for confirmation.

- A voucher (reference) collection must be maintained to help resolve questions regarding the accuracy of taxonomic identifications. These specimens should be properly labeled, preserved and stored in the laboratory for future reference. Specimen labels should include the name(s) of the verifying person(s). Senior taxonomist must verify 10% of the sample identifications.

- Information on samples completed (through the taxonomy process) is recorded in the sample notebook or bench sheet to track the progress of each sample within the sample lot. The tracking of each sample will be updated as each step is completed (i.e., rinsing, sub-sampling, sorting and taxonomy).

8.1.1 Specific Guidelines for Organism Identification

- For identification and enumeration, examine organisms under a stereoscopic dissecting microscope. The microscope should have a magnification range of 7X to 120X. After they are identified and counted, all organisms are returned to a single sample vial containing 95% ethanol and the original field sample label, OR original field sample label information (see previous page, section 6.1. "For 10% of the samples, save the unsorted sample residue in a container labeled "sample residue"; this container should include the original sample label. Save the sorted debris in a separate container labeled "sorted residue" in addition to original label information."

- Some taxa require slide mounts for proper identification (e.g., chironomid larvae). Before mounting chironomids, the larvae are made more transparent (cleared) to facilitate the observation of internal structures utilized in taxonomic identification. The process of clearing and mounting the larvae may make certain characteristics difficult to see. Membranous structures such as the Lauterborn organs and the antennal blade become very faint after treatment in warm KOH. Overnight clearing in cool (room temperature) KOH is less damaging and will make structures more visible (Simpson and Bode, 1980). The anal and ventral tubules, preanal papillae, eyespots and body coloration should be observed under a dissecting microscope before the specimen is cleared. Another clearing/mounting agent is CMC-10 mounting media from Masters Group, Inc..

- After being picked from the sample, chironomid larvae should be sorted into groups based on location of eyespots, shape of head and possession of ventral tubules.

- Clear the larvae of soft tissue by placing them in cool 10% potassium hydroxide (KOH) overnight;

  Or

- Heat the KOH on a hot plate (low only) until the head capsule clears, approximately 20-30 minutes.

- Rinse out the KOH by soaking the larvae in distilled water approximately 10 minutes.

- Place the larvae through a graded alcohol series (50%, 70% and 95%) to remove all the water. Failure to remove the water may cause bubbles to appear. Minimum time per rinse is 10 minutes.

- Place approximately two drops of Canada balsam (Permount or other suitable mounting medium) on a microscope slide and arrange the midge with the ventral side up.

- Cover with a coverslip and press down on the head capsule to expose the mouthparts.

- Note: On large larvae the abdomen may prevent the depression of the head capsule. In such cases, mount the abdomen under a separate coverslip, but on the same slide.

9.0 Sample Integrity and Disposition

- To ensure the integrity of each sample, a label containing the information discussed in the subsection on labeling is included in each vial. In order to maintain chain-of-custody, samples are the responsibility of the collecting biologist. Keep samples in a secure location at all times (e.g. lock the vehicle whenever vacant). Final disposition of samples is also the responsibility of

the collecting biologist.

10.0 Macroinvertebrate Keys

Use any available literature as an aid to identify the organisms. However, take the final identification and recorded name from the following references.

PLATYHELMINTHES
TURBELLARIA: Family (Pennak, 1989 pgs 124-51)
ANNELIDA
OLIGOCHAETA: Class (Pennak, 1989 pg 290) / (Kathman and Brinkhurst, 1998)
HIRUDINEA: Family (Pennak, 1989 pgs 314-333)
ARTHROPODA
CRUSTACEA
ISOPODA: Genus (Pennak, 1989 pgs 462-73)
AMPHIPODA
Hyalellidae: species (Pennak, 1989 pgs. 474-88)
Gammaridae: Genus (Pennak, 1989 pgs. 474-88)
DECAPODA
Cambaridae: Genus/species (Page, 1985 INHS Bull. 33:4)
Palaemonidae: species (Page, 1985 INHS Bull. 33:4)
INSECTA
EPHEMEROPTERA
Siphlonuridae: Genus (Merritt and Cummins, 1996)
Isonychiidae: Genus (Merritt and Cummins, 1996)
Baetidae: Genus/species (Merritt and Cummins, 1996 / Morihara and McCafferty, 1979 TAES V.105 / Burks, 1953)
Heptageniidae: Genus/species (Merritt and Cummins, 1996 / Burks, 1953 / Lewis, 1974) / Bednarik and McCafferty, 1979)
Ephemerellidae: Genus (Merritt and Cummins, 1996)
Tricorythidae: Genus (Merritt and Cummins, 1996)
Caenidae: Genus (Merritt and Cummins, 1996)
Baetiscidae: Genus (Merritt and Cummins, 1996)
Leptophlebiidae: Genus (Merritt and Cummins, 1996)
Potamanthidae: Genus (Merritt and Cummins, 1996)
Emphemeridae: Genus (Merritt and Cummins, 1996)
Palingeniidae: Genus (Merritt and Cummins, 1996)
Polymitarcyidae: Genus (Merritt and Cummins, 1996)
ODONATA
ANISOPTERA
Cordulegasteridae: Genus (Merritt and Cummins, 1996 / Needham and Westfall, 1995 / Needham, Westfall and May, 2000).
Gomphidae: Genus (Merritt and Cummins, 1996 / Needham and Westfall, 1955 / Needham Westfall and May, 2000)
Aeshnidae (or Aeschnidae): Genus/species (Merritt and Cummins, 1996 / Needham and Westfall, 1955 / Needham, Westfall and May, 2000)
Corduliidae: Genus (Merritt and Cummins, 1996 / Needham and Westfall, 1955 / Needham, Westfall and May, 2000)
Libellulidae: Genus (Merritt and Cummins, 1996 / Needham and Westfall, 2000)

ZYGOPTERA

Calopterygidae: Genus (Merritt and Cummins, 1996 / Westfall and May, 1996)
Coenagrionidae: Genus (Merritt and Cummins, 1996 / Westfall and May, 1996)
Lestidae: Genus (Merritt and Cummins, 1996 / Westfall and May, 1996)

PLECOPTERA
Pteronarcyidae: Genus (Merritt and Cummins, 1996)
Taeniopterygidae: Genus (Merritt and Cummins, 1996)
Nemouridae: Genus (Merritt and Cummins, 1996)
Leuctridae: Genus (Merritt and Cummins, 1996)
Capniidae: Genus (Merritt and Cummins, 1996)
Perlidae: Genus (Merritt and Cummins, 1996)
Perlodidae: Genus (Merritt and Cummins, 1996)
Chloroperlidae: Genus (Merritt and Cummins, 1996)
HEMIPTERA
Gerridae: Genus (Merritt and Cummins, 1996)
Notonectidae: Genus (Merritt and Cummins, 1996)
Pleidae: Genus (Merritt and Cummins, 1996)
Belostomatidae: Genus (Merritt and Cummins, 1996)
Corixidae: Genus (Merritt and Cummins, 1996)
MEGALOPTERA
Sialidae: Genus (Merritt and Cummins, 1996)
Corydalidae: Genus/species (Merritt and Cummins, 1996)
NEUROPTERA
Sisyridae: Genus (Merritt and Cummins, 1996)
TRICHOPTERA
Hydropsychidae: Genus/species (Merritt and Cummins,1996 / Schmude and Hilsenhoff,1986)
Philopotamidae: Genus (Merritt and Cummins, 1996)
Polycentropodidae: Genus (Merritt and Cummins, 1996)
Psychomyiidae: Genus (Merritt and Cummins, 1996)
Glossosomatidae: Genus (Merritt and Cummins, 1996)
Hydroptilidae: Genus (Merritt and Cummins, 1996)
Rhyacophilidae: Genus (Merritt and Cummins, 1996)
Brachycentridae: Genus (Merritt and Cummins, 1996)
Lepidostomatidae: Genus (Merritt and Cummins, 1996)
Molannidae: Genus (Merritt and Cummins, 1996)
Phryganeidae: Genus (Merritt and Cummins, 1996)
Helicopsychidae: species (Merritt and Cummins, 1996)
Leptoceridae: Genus/species (Merritt and Cummins, 1996 / Glover and Floyd, 2004 / Floyd, 1995)
LEPIDOPTERA
Pyralidae: Genus (Merritt and Cummins, 1996)
COLEOPTERA
Gyrinidae: Genus (Merritt and Cummins, 1996)
Psephenidae (larvae only): Genus (Merritt and Cummins, 1996)
Scirtidae: Genus (Merritt and Cummins, 1996)
Haliplidae: Genus (Merritt and Cummins, 1996)
Hydrophilidae: Genus (Merritt and Cummins, 1996)

Dytiscidae: Genus (Merritt and Cummins, 1996)
Dryopidae: Genus (Merritt and Cummins, 1996)
Elmidae: Genus (Merritt and Cummins, 1996)
DIPTERA
Tipulidae: Genus (Merritt and Cummins, 1996)
Chaoboridae: Genus (Merritt and Cummins, 1996)
Culicidae: Genus (Merritt and Cummins, 1996)
Dixidae: Genus (Merritt and Cummins, 1996)
Psychodidae: Genus (Merritt and Cummins, 1996)
Simuliidae: Genus (Merritt and Cummins, 1996)
Chironomidae: Genus/species (Merritt and Cummins, 1996 / Epler, 1992)
Stratiomyidae: Genus (Merritt and Cummins, 1996)
Tabanidae: Genus (Merritt and Cummins, 1996)
Empididae: Genus (Merritt and Cummins, 1996)
Syrphidae: Genus (Merritt and Cummins, 1996)
Ephydridae: Genus (Merritt and Cummins, 1996)
Sciomyzidae: Genus (Merritt and Cummins, 1996)
Muscidae: Genus (Merritt and Cummins, 1996)
Athericidae: Genus (Merritt and Cummins, 1996)
MOLLUSCA
GASTROPODA: Genus (Pennak, 1989)
PELECYPODA
Corbiculidae: species (Pennak, 1989 / Cummings and Mayer, 1992)
Sphaeriidae: Genus (Pennak, 1989 / Cummings and Mayer, 1992)
Unionidae: Genus/species (Pennak, 1989 / Cummings and Mayer, 1992)

Note:
Class = key to the class level
Family = key to the family level
Genus = key to the genus level
Genus/species = contain genera with only one species for our area or genera which add large numbers and diversity to our samples at the species level. Current species level key are indicated for those genera.
species = only one species should occur for this group in our Illinois occurrence list.

11.0 Literature Cited

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