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Purpose

This technical standard operating procedure (TSOP) describes the method for processing, sorting, and preparing macroinvertebrate samples for taxonomic identification in a consistent manner to yield high quality, reproducible, and reportable data for making aquatic life use assessments. Macroinvertebrate samples processed using this TSOP include those collected using the Indiana Department of Environmental Management (IDEM) Multihabitat (MHAB; IDEM 2023a), Hester-Dendy (H-D; IDEM 2023b), and Riffle Kick (KICK; IDEM 1992) methods as well as the United States Environmental Protection Agency (U.S. EPA) National Aquatic Resource Survey (NARS) methodology for sampling benthic macroinvertebrates in lakes (U.S. EPA 2022) and wadeable streams and rivers (U.S. EPA 2017).

Scope

This TSOP applies to agency staff located in the Office of Water Quality (OWQ), Watershed Assessment and Planning Branch (WAPB) responsible for processing and identifying macroinvertebrate community samples collected from rivers, streams, and lakes as part of IDEM's ambient water quality monitoring program. This TSOP covers the processes of sub-sampling and methods for the enumeration and identification of macroinvertebrates.

Authorizing Signatures

I approve and authorize this technical standard operating procedure (TSOP):

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Stacey Sobat, Chief Probabilistic Monitoring Section, Office of Water Quality

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Kristen Arnold, Branch Chief WAPB, Office of Water Quality

This TSOP is consistent with agency requirements.

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Quality Assurance Staff Indiana Department of Environmental Management

<u>6/6/2023</u> Date

6/6/2023

Date

6/7/2023

Date

6/13/2023

Date

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1.0. Overview Flowchart

2.0. Procedure

2.1. Procedural Flowchart

Step 1-5 procedural flowcharts are shown in Section 2.2, followed by the step-by-step descriptions.

2.2. Procedural Steps

Step 1. Select and prepare an unprocessed sample from storage.



- A. Select an unprocessed macroinvertebrate sample container from the flammable cabinets in the IDEM macroinvertebrate laboratory (Room 125) or IDEM macroinvertebrate sample storage room (Room 122). If the unprocessed macroinvertebrate sample consists of more than one container (i.e., the label contains "1 of 2" or "2 of 2") locate both sample containers before proceeding.
- B. Complete the following columns of the IDEM OWQ Biological Studies Laboratory Chain of Custody form (Appendix 1) to sign out the sample containers selected in Step 1.A.:
 - 1. Sample Type: Enter a brief description of the project the sample was collected under (e.g., "2021 Probabilistic").
 - 2. Macro number: Identification number for macroinvertebrate community samples consisting of the last two digits of the year the sample was collected, two digits for the month the sample was collected, two digits for the day the sample was collected, one digit for the crew chief's assigned logbook identification number, and two digits for the chronological order in which the sample was collected that day (YYMMDD###). For example, the third sample collected on 7/30/2021 using logbook #7 would have Macro# 210730703.
 - 3. AIMS sample number: AA/AB/AC number assigned to each individual watershed sampling event conducted by

IDEM OWQ field crews and used to identify the sampling event in the AIMSII database.

- 4. The volume and number of sample containers removed for processing.
- Removed from Storage for Processing: The date (MM/DD/YYYY) and time (24 hr.) the sample was removed.
- 6. Processing Room number: The room number for the laboratory in which the sample will be processed.
- 7. Initials: The initials of the sample processor.
- C. Print two blank copies of the "Macroinvertebrate lab datasheet" (Appendix 2), one copy of the "Macroinvertebrate labels" (Appendix 3) associated with the sample that will be processed, and one blank copy of the "Macroinvertebrate slide record" (Appendix 4). The "Macroinvertebrate labels" form should be printed on "Rite in the Rain ©" or other waterproof archival paper using a laser printer and then paper clipped to the "Macroinvertebrate lab datasheet" and "Macroinvertebrate slide record" forms. Instructions for locating these forms can be found beginning on page 45 of the AIMSII User Guide (IDEM 2023).
- D. If the unprocessed macroinvertebrate sample type is:
 - 1. H-D, KICK, Transect Jab (TJ) or Transect Kick (TK) go to Step 2.A.
 - National Lake Assessment (NLA) See steps 1-9 of Section 5.5.2 Subsampling and 1-10 of Section 5.5.3 Sorting of EPA 2022 for instructions on processing these samples, then go to Step 2.K.
 - 3. MHAB go to Step 3



Step 2. Subsample unprocessed samples

- A. Place a stainless-steel sieve with 500µm mesh openings into an empty processing tray (or plastic storage container, such as the ones used while field subsampling) slightly larger than the sieve. Empty the contents of the sample container(s) into the sieve and use tap water to rinse any remaining contents from the sample container into the sieve.
 - Locate and remove the sample label(s) that was placed into the sample container(s) when the sample was collected and check that the Macro #, IDEM Sample # and any other identifying information matches the information on the sample container lid, the "Macro Count Labels", and the "MHAB Report" or the "KickHD Report". If any of the sample numbers do not match, check the original copies of the field data sheet (Appendix 5) and QHEI form (Appendix 6), and the macroinvertebrate field logbook entry (Appendix 7) to resolve the discrepancy. Retain the sample label(s).
- B. If the macroinvertebrate type is:
 - 1. H-D go to Step 2.C.
 - 2. KICK, Transect Jab (TJ), or Transect Kick (TK) go to Step 2.D.
- C. Select an individual plate from the disassembled H-D sampler components in the sieve and use a glass microscope slide (or other flat-edged utensil) to scrape any macroinvertebrates or debris from all faces and edges of the plate into the sieve. Use the squeeze bottle filled with 80% isopropyl alcohol to rinse any scraped slides. Place the scraped H-D plates into a large tray to dry. H-D plates which are broken, showing signs of wear, or were deployed in areas of known sediment contamination should be discarded.
- D. Remove the sieve from the processing tray and rinse under a gentle stream of tap water until the rinse water flows clearly. Empty the contents of the sieve into a 30-square wire mesh Caton tray placed into its associated plastic tray for subsampling. Use a squeeze bottle filled with tap water to rinse any remaining material from the sieve into the Caton tray. Pour the preservative from the processing tray back into the original sample container(s), then rinse and dry the processing tray. Add tap water to the plastic subsampling tray until there is about 1 cm of water covering the bottom of the tray (about 750ml) and

then use forceps or gentle agitation to spread the contents relatively evenly throughout the tray. The water level in the Caton tray should be just enough to keep the sample wet but not so much that material begins floating. If the tray does not appear level, increase the height of the lowest corner or edge.

- E. If the macroinvertebrate sample type is:
 - 1. H-D, KICK, or Transect Kick (TK) go to Step 2.F.
 - 2. Transect Jab (TJ) go to Step 2.N.
- F. Use a random number generator (i.e., <u>www.random.org</u>) to select a square. Place the subsampling device over the borders of the selected square. Use a pipette, forceps, spade tip forceps or other tools (e.g., a spoon or a putty knife) to remove as much of the material from within the randomly selected square as possible and place this material into the bottom half of a glass petri dish. Add tap water to the petri dish until all the material is submerged. For organisms and other material that are on the borders of the square, if over half of it is within the square, then remove it as well.
- G. Place the petri dish underneath a dissecting microscope. Use forceps to remove all organisms from the petri dish and place them into a 250ml Nalgene jar filled halfway with 80% isopropyl alcohol. Use a tally counter to count each individual specimen that is removed. Record the square the subsample was taken from, and the number of organisms removed from it; also record the current total number of removed organisms. Continue counting and removing organisms even if the target number of organisms has been reached (e.g., pick the last selected square to completion). Once all apparent organisms have been removed, empty the remaining contents of the petri dish into a temporary sample residual container. Follow these rules about deciding if a specimen should be counted:
 - Exuvia, empty mollusk shells, terrestrial organisms, vertebrates, and organisms without heads are not counted. Currently, all organisms that live within the water column (e.g., the Hemiptera families Corixidae or Nepiidae) or on the water surface (e.g., the Hemiptera families Geriidae or Veliidae; Collembola) are counted.
 - 2. Microcrustaceans such as Cladocera, Copepoda, or Ostracoda are not counted; all crayfish, freshwater shrimp, Amphipoda, and aquatic Isopoda are counted.

- H. Has the target number of organisms (100 \pm 10% for KICK or H-D; 300 + up to 10% for Transect Kick; or other goal specified in project workplan) been met? (Note: if 25% of the squares in the subsampling tray have been removed and fewer than 25% of the required number of organisms have been counted, the remainder of the tray can be consolidated, and all specimens removed.)
 - 1. Yes go to Step 2.I.
 - 2. No return to Step 2.F.
- I. Is this sample a Percent Sorting Efficiency (PSE) sample (i.e., first five samples and every 10th sample thereafter for each individual staff member)?
 - 1. Yes go to Step 2.J.
 - 2. No go to Step 2.K.
- J. A second staff person will empty the contents of the temporary sample residual container into a petri dish and remove any additional specimens that are encountered. These specimens are counted separately with a tally counter and then added to the same container as the subsample (note: these specimens are only counted and not identified at this time). The number of missed organisms is then entered into the "Residuals" section of the "MHAB Report" or "KickHD Report" lab bench sheet along with the initials of the second staff person and date the residuals were re-picked. After the residual organisms have been picked, the remaining material may be poured through a sieve and disposed.
- K. Is this sample a lab duplicate sample (i.e., every 10th sample processed by a lab staff during a given year)?
 - 1. Yes go to Step 2.L.
 - 2. No go to Step 2.M.
- L. If enough sample material remains for a second pick, return to Step 2.H, and complete a second pick of the appropriate subsample size of organisms following Steps 2.H–2.J. If the Normal of this sample is a PSE sample, then the residuals of the lab duplicate sample do not require a residual pick. Specimens removed as a lab duplicate are stored in a separate sample jar. If there is not enough sample material remaining after the initial pick, then treat the next viable sample as the lab duplicate instead.

- M. Remove a sample label from the "Macro Sample Labels" sheet and write the initials of the sample processor and the date the sample processing was completed on the back of the label. A lab duplicate sample has ".5" added to the end of the IDEM Sample # and Macro # on the label. Insert the new label and the original label into the 250ml Nalgene jar. Add additional 80% isopropyl alcohol until the jar is 75% full.
- N. Will this sample have a timed pick component?
 - 1. Yes go to Step 2.O.
 - 2. No go to Step 2.Q.
- O. Use a pair of forceps to remove specimens from the sample tray for the specified length of time (5 minutes, 10 minutes, 15 minutes). All removed specimens are placed into a separate 250ml Nalgene jar from the random subsample. Specimens are picked on a single organism basis with the goals of obtaining the greatest diversity and number of specimens within the given picking time.
- P. Remove a sample label from the "Macro Sample Labels" sheet and write the initials of the sample processor and the date the sample processing was completed on the back of the label. Insert the new label and the original label into the 250ml Nalgene jar. Depending on the length of time that the sample was picked, add ".05", ".10" or ".15" to the end of the IDEM Sample # and Macro #. Add additional 80% isopropyl alcohol until the jar is 75% full.
- Q. Return the picked subsample to the flammable storage cabinet used for storing samples. Complete the following columns of the IDEM OWQ Biological Studies Laboratory Chain of Custody form (Appendix 1) to sign in the subsample containers (Note: this step is only completed if identification of the subsample will not begin immediately after the sample is picked. Otherwise, proceed directly to Step 2.R and then Step 3.C):
 - Placed in sorage after processing: The date (MM/DD/YYYY) and time (24 hr.) the sample was returned to storage.
 - 2. Storage room number: The room in which the sample will be stored.
 - 3. Initials: The initials of the sample processor.
 - 4. Number of Nalgene voucher jars: The number of 250ml jars containing the subsample.

R. Pour the remaining sample material through a sieve to remove excess water. Transfer the remaining sample material to the original sample jar(s) containing the original sample preservative and insert a new sample label that indicates that this is a sample remainder. Return this remainder container to the flammable storage cabinet. This container should be maintained until the subsample(s) taken from it have been identified and verified. If no new or rare taxa were encountered, the remaining sample can be drained of its preservative and disposed of into a trash can in a well-ventilated room.

Step 3. Remove macroinvertebrates from subsample.



- A. Select a processed macroinvertebrate sample container from the flammable cabinets in the IDEM macroinvertebrate laboratory (Room 125) or IDEM macroinvertebrate storage room (Room 122). If the macroinvertebrate sample consists of more than one container (i.e., the label contains "1 of 2" or "2 of 2") locate both sample containers before proceeding.
- B. Complete the following columns of the IDEM OWQ Biological Studies Laboratory Chain of Custody form (Appendix 1) to sign out the sample container selected in Step 3.A.

- 1. Sample type: Enter "M" for macroinvertebrate sample type and a brief description of the project (e.g., "2021 Probabilistic").
- Macro number: Identification number for macroinvertebrate community samples consisting of the last two digits of the current year, two digits for the current month, two digits for the current day, one digit for the crew chief's assigned logbook identification number, and two digits for the chronological order in which the sample was collected that day (YYMMDD###). For example, the third sample collected on 7/30/2021 using logbook #7 would have Macro# 210730703.
- 3. AIMS sample number: AA/AB/AC number assigned to each individual watershed sampling event conducted by IDEM OWQ field crews and used to identify the sampling event in the AIMSII database.
- 4. The volume and number of sample containers removed for processing.
- 5. Removed from Storage for Processing: The date (MM/DD/YYYY) and time (24 hr.) the sample was removed.
- 6. Processing Room number: The Room # for the laboratory in which the sample will be processed.
- 7. Initials: The initials of the sample processor.
- C. Locate the laboratory bench sheet and sample labels that were printed in Step 1.C. If these sheets are not available, follow the instructions in Step 1.C to create new bench sheets (i.e., an unprocessed MHAB sample).
- D. Agitate the contents of the subsample container, decant excess isopropanol through a sieve into a waste container, and slowly pour the remaining contents into a petri dish (or more than one petri dish if necessary). Rinse sieve and subsample container into the petri dish with 80% isopropanol to ensure all organisms have been collected. Remove the sample label from the petri dish and confirm that the sample identification numbers match those that are on the sample jar label. If the contents of the petri dish are cloudy due to sediment suspended in the alcohol, allow the sediments to settle to the bottom of the dish before proceeding.

- E. Use fine tipped forceps to remove macroinvertebrates from the petri dish and place them into a series of watch glasses or spot plate wells filled with 80% isopropanol. Removed macroinvertebrates can be organized into groups or orders (i.e., a watch glass containing Ephemeroptera, Plecoptera and Trichoptera; another watch glass containing Coleoptera and Hemiptera) to facilitate identification.
- F. Is this sample a PSE MHAB sample?
 - 1. Yes follow the instructions in Step 2.J for completing a residual pick then go to Step 4
 - 2. No go to Step 4.
- Step 4. Identify and enumerate macroinvertebrate samples.



- A. Select a watch glass or spot plate well containing a group of taxonomically similar macroinvertebrates. Identify these specimens to the lowest practical taxonomic level using recommended taxonomic references listed in Appendix 8. (Note: oligochaetes and chironomids are not identified at this time. Also, identifications for the KICK and H-D sample types are generally taken to family level). If a specimen requires a slide mount of specific body parts (i.e., legs or mouthparts) see the instructions for making slides in Step 4.F.
- B. All specimens that are identified to the same taxonomic identification are placed into a glass shell vial (typically, 1 dram but 2-dram, 4-dram and 1-ounce olive jars may also be used if there are numerous or large specimens). A single label is cut out from the Macroinvertebrate Label sheet page (Appendix 3) and the identification of the specimens, initials of the identifier and date the specimens were identified is written on the label. If a slide was used in the identification, enter the slide number and position of the slide-mounted part in the notes onto the label. The label is inserted into the vial with the identification visible to the outside of the vial and the vial is then filled with 80% isopropyl alcohol from a squeeze bottle. A piece of cotton is rolled into a ball, soaked in 80% isopropyl alcohol, and inserted into the end of vial (the cotton ball should stay in place when the vial is inverted but not be so large as to crush any specimens in the vial). Also, place the specimen associated with that slide mount into a smaller vial, which is then placed into the larger vial. Include a small label listing the slide number associated with that specimen in the smaller vial.
- C. Enter the taxonomic identification of the specimens into the uppermost blank line of the macroinvertebrate bench sheet. Include the number of specimens of those taxa and any pertinent notes regarding the identification (i.e., number of larvae or juveniles and adults, number of male or female specimens; if a slide was used in the identification, enter the slide number and position of the slide mounted part in the notes).
- D. Have all specimens that do not require slide mounting been identified?
 - 1. Yes go to Step 4.E.
 - 2. No return to Step 4.A.

- E. Do you have taxa which require slide mounting for proper identification (i.e., chironomids, oligochaetes, or elmid beetles)?
 - 1. Yes go to Step 4.F.
 - 2. No go to Step 5.
- F. Locate a glass slide. On the frosted or white end of the slide write the Sampling Event #, Macro #, stream name and slide number. The slide number consists of the first initial of the identifier, two digits for the year the sample was collected and then three digits for the order in which the slide was made. While wearing gloves and using a fume hood or fume extractors turned to the highest setting, place a drop of mounting media (PVA or CMCP mixture) onto the left area of the slide.
- G. Mounting taxa:
 - 1. Mounting body parts of large taxa: For any taxa that requires examining specific body parts under a compound microscope (e.g., Baetid mouthparts or Elmid genitals), mount the body parts from at least one specimen per 10 individuals. Once you have dissected the necessary body parts from a specimen, pick them up with forceps, dab them against a piece of Kimwipe to remove excess alcohol, and place into the mounting media. Lower a 12mm diameter round glass cover slip onto the body parts. If possible, all pieces from a single specimen should be mounted under a single cover slip. Repeat this process for each necessary specimen. In the slide record sheet (Appendix 4), enter the slide number, ascending numbers for the number of cover slips that were placed on the slide, and what body parts were mounted. These slides may be identified immediately and then placed within the fume hood to dry.
 - 2. Mounting chironomid larvae: Select up to six chironomid specimens that appear to be the same size, starting with the largest specimens and moving towards smaller specimens. Picking them up with forceps, dab them against a piece of Kimwipe to remove excess alcohol, and place into the mounting media. With very large specimens you may only be able to place three specimens into one drop of mounting media. Using forceps and a probe, arrange the specimens so that they have the same orientation with the head pointed towards the bottom edge

of the slide and the curvature of the body pointed towards the left side of the slide. If the specimens are very large, the head may need to be removed and placed near the body with the ventral side pointing up. Lower a 12mm x 12 mm glass cover slip onto the specimens and gently press or slide the slip so that the heads are pointed ventral side up and the mandibles spread outwards. Repeat the process for a second drop of mounting media placed on the right side of the slide. In the slide record sheet (Appendix 4) enter the slide number and ascending numbers for the number of chironomid larvae that were placed on the slide.

- 3. Mounting chironomid pupae: Chironomid pupae are placed into the mounting media and the abdomen is separated from the head and thorax using a pin or forceps. The head should be mounted pointing towards the bottom of the slide and the abdomen should be mounted with the dorsal side pointed up. In the slide record sheet (Appendix 4) enter the slide number and ascending numbers for the number of chironomid pupae that were placed on the slide.
- 4. Mounting oligochaete specimens: Oligochaete specimens are placed in a row with the heads (if determinable) pointing down. Place a cover slip on top. In the slide record sheet (Appendix 4) enter the slide number and ascending numbers for the number of oligochaetes that were placed on the slide.
- H. Once a slide has been filled with specimens, it should be placed within the fume hood to dry. CMCP mounting resin, slides, and other equipment necessary to be stored in the fume hood should be placed as far from hood vents as possible. Slides should be allowed to dry for at least two weeks to ensure optimal conditions for taxa identification. While drying, slides should be checked regularly for the formation of air bubbles. If air bubbles are present, they should be filled in with additional mounting medium.
- Once slides have dried sufficiently, select a slide. Identify these specimens to the lowest practical taxonomic level using recommended taxonomic references, specified in Appendix 8. Once you identify a mounted specimen, write the taxa name

with the specimen's corresponding number on the slide record sheet.

J. Once all slide-mounted taxa have been identified, enter the taxonomic identification of the specimens into the uppermost blank line of the macroinvertebrate bench sheet. Include the number of specimens of that taxon and any pertinent notes regarding the identification (i.e., number of larva or pupa).

Step 5. Store completed samples.



- A. Once all specimens have been identified or slide-mounted, place all the individual specimen vials into a larger 16-ounce glass olive jar (use two jars if one is not large enough). Place vials into the jars with the cotton stuffed end pointing up and pour enough 80% isopropyl alcohol into jars to cover all the specimen vials. Place the original sample label into the jar or into the "1 of 2" jar if more than one storage jar is being used. Create a second sample label for the "2 of 2" jar. If the label tape from the original sample is still available place it onto the side of the "1 of 1" jar. Write in permanent marker on top of both jars the AIMS sample number, Macro number, stream name, and "1 of 2" or "2 of 2" if necessary. If the sample is part of a "Normal-Duplicate" sample pair, place a colored sticker on top of the jar if it is the "Normal" sample.
- B. Place the sample jar back into storage. Fill out the following sections of the COC form (Appendix 1): Returned to storage, date and time, room number and initials. Update any progress charts, noting completion of Stage 1 for the sample.
- C. Once slides have been identified, place them within a slide box for storage. The outside of the slide box should be labeled with the identifier's name, sampling year, and type of samples the

slides are associated with (i.e., MHAB, KICK, H-D, Transect Jab, Transect Kick). Record the slide's AIMS sample number and slide number in the box's slide index.

D. Once samples have been placed into storage, they will be kept for a minimum of 10 years, to allow time for any required further review of the samples. After 10 years, all samples from a given sampling year will be removed from storage. Any specimens that may be a new state distribution record or a new species that could be added to the macroinvertebrate reference collection will be sent to an independent expert for verification and then added to the reference collection for indefinite storage. All other specimens will be offered to researchers or organizations that may have an interest in the collection. If specimens are unable to be dispersed to others, they will be disposed.

2.3. Related Technical Issues

- A. Health and Safety Warnings
 - 1. Ventilation systems
 - a. Fume Hood
 - *i.* Turn on fume hood before opening.
 - *ii.* Proper user height for fume hood is 6-8 inches.
 - *iii.* Limit storage of chemicals, slides, etc. to necessary amounts to ensure adequate air flow. Store items away from vents.
 - b. Extraction arms
 - *i.* Use extraction arms on the highest setting.
 - *ii.* Face of extraction arm should be placed as close to working area as possible, ideally 2-3 inches.
 - 2. Before using isopropyl alcohol, review the Safety Data Sheet (SDS) stored in the macroinvertebrate laboratory in the IDEM Shadeland office and be sure to wear required PPE.
 - a. Isopropyl alcohol poses the following health hazards:
 - i. H225 Flammable liquid
 - ii. H319 Causes serious eye irritation.
 - iii. H336 May cause drowsiness or dizziness.
 - iv. Harmful to aquatic life
 - b. Required PPE:
 - i. None, but gloves and eyewear are available.
 - c. Proper spill clean-up:

- i. Notify Chemical Hygiene Officer (CHO) of spill for assistance in cleanup and waste disposal.
- ii. Less than 100 ml: Wipe spill with paper towel and dispose of in well-ventilated trash receptacle.
- iii. More than 100 ml: Locate spill clean-up station and use chemical absorbers to absorb spill. Place soiled chemical absorbing pads into air-tight plastic bag provided in spill clean-up station. Store bag in flammable cabinet. Coordinate with CHO for proper disposal of waste.
- 3. Before using CMCP, review the SDS stored in the macroinvertebrate laboratory in the IDEM Shadeland office and be sure to wear required PPE.
 - a. CMCP poses the following health hazards:
 - i. H331 Toxic if inhaled.
 - ii. H371 May cause damage to organs.
 - iii. H314 Causes severe skin burns and eye damage.
 - iv. H302 Harmful if swallowed.
 - b. Required PPE:
 - i. Chemical resistant latex or nitrile gloves
 - ii. Adequate ventilation (extraction arms set to the highest setting or fume hood)
 - c. Proper spill clean-up:
 - i. Notify CHO of spill for assistance in cleanup and waste disposal.
 - ii. Less than 10 mL: Wearing gloves, wipe spill with paper towel and dispose of in well-ventilated trash receptacle.
 - iii. More than 10 mL: Increase airflow into room by opening doors, windows, turning on fume hood, etc. Wearing gloves, locate spill clean-up station and use chemical absorbers to absorb spill. Place soiled chemical absorbing pads into air-tight plastic bag provided in spill clean-up station. Coordinate with CHO for proper disposal of waste.
- B. Cautions
 - 1. Ensure that in-process picking samples are kept moist with sufficient water and covered when not actively being picked.
 - Ensure that samples that are in watch glasses or spot plate wells have sufficient alcohol to prevent specimens from becoming dry. Stack watch glasses and cover (i.e., with a 500ml Nalgene wide mouth jar) if they will not be processed in one day. If they cannot be

identified within several days, they should be emptied into a container and re-sorted when possible.

- 3. Make sure that slides have enough mounting media and add more if needed to prevent the development of "air fingers" or bubbles.
- 4. Follow manufacturer's instructions for microscope cleaning and maintenance.
- C. Interferences
 - 1. Improper storage of specimens at any point during the process may result in specimens drying out, which makes them fragile and often degrades identifying characteristics. This may result in incorrect or insufficiently specific identifications.
- D. Calibration
 - The ocular micrometer on the Leica DM750 microscope was calibrated with a stage micrometer to ensure that measurements are accurate. The calibration was done before the ocular reticle was used for measurements. It is unknown whether the stage micrometer is traceable to a NIST standard.
- E. Troubleshooting
 - 1. Not applicable

3.0. Roles

- 3.1. Responsibilities
 - A. IDEM biologist
 - 1. Processes macroinvertebrate samples
 - 2. Identifies and enumerates macroinvertebrate samples.
 - 3. Processes appropriate taxa to be made into permanent mounts.
 - 4. Records taxonomic information in AIMSII
 - 5. Exercises proper laboratory safety read SDS sheets, use ventilation systems when required, and use appropriate PPE.
 - B. Chemical hygiene officer
 - 1. Conduct weekly safety inspections in macroinvertebrate lab and storage rooms, including eyewash fountains, safety showers, and fire extinguishers.
 - 2. Coordinate the proper disposal of hazardous chemical waste from the macroinvertebrate lab and storage rooms annually.
 - Conduct annual safety training on macroinvertebrate lab equipment and procedures. This includes reading and interpreting a Material SDS, using Personal Protective Equipment (PPE) when handling

hazardous chemicals, operation of safety equipment (i.e., hoods, eyewash fountains, safety showers, fire extinguishers), and proper labeling and storage of hazardous chemicals.

- 4. Help coordinate the annual maintenance and inspection of ventilation systems. This includes inspection of motors, fans, pulleys, and belts, as well as routine lubrication.
- 5. Physical copies of the Material SDS's in each lab and storage room must be maintained and updated annually or whenever a chemical is added. This includes the MSDS On-line application.
- 6. Respond to emergency spills/leaks in macroinvertebrate lab and storage rooms (coordinating appropriate cleanup and disposal, notification of Agency Health and Safety Director, completion of spill forms, recommendations for future spill prevention).
- C. Quality control taxonomist
 - 1. Perform taxonomic verification and enumeration on internal quality control samples.
- D. Lab custodian
 - 1. Maintains historic macroinvertebrate samples for at least 10 years after identification.
 - 2. Works with potential interested parties to disburse historic samples after a 10-year holding period.
 - 3. Properly disposes of any samples that cannot be disbursed when historic samples are unable to be stored.
 - 4. Maintain macroinvertebrate reference collection, including getting potential reference specimens verified by an outside taxonomist.
- 3.2. Training requirements
 - A. Macroinvertebrate identification
 - 1. IDEM biologist
 - 2. Quality control taxonomist
 - B. Slide mounting
 - 1. IDEM biologist
 - C. Macroinvertebrate sample receiving and check in/out of the laboratory.
 - 1. IDEM biologist
 - 2. Lab custodian
 - D. Handling and disposal procedures of laboratory chemicals
 - 1. IDEM biologist
 - 2. Chemical hygiene officer
 - 3. Lab custodian

4.0. Required Forms, Equipment, or Software List

4.1. Forms

- A. IDEM OWQ Biological Studies Laboratory Chain of Custody
- B. Macroinvertebrate lab datasheet
- C. Macroinvertebrate slide record sheet

4.2. Equipment

- A. Straight Forceps (4A and finer tipped)
- B. Bent-tipped forceps
- C. Lead pencil
- D. Archival ink pen
- E. Rite-in-the-Rain paper (or other waterproof archival paper)
- F. 80% Isopropyl alcohol
- G. 500ml squeeze bottles
- H. Fine tip permanent marker
- I. Watch glasses
- J. Ceramic spot plates
- K. White labeling tape
- L. 12 mm x 12 mm square glass coverslips
- M. 12 mm round glass coverslips
- N. Fume hood
- O. Nederman fume extractor arms
- P. Glass microscope slides with a frosted edge for writing
- Q. Scissors
- R. Kim-Wipes cleaning tissues
- S. Lens paper
- T. Microscope slide boxes
- U. Glass cleaner
- V. Chemical resistant gloves (latex or nitrile)
- W. 500 µm sieve (U.S. Standard No. 35 sieve)
- X. Immersion oil for microscopy
- Y. Nikon MZ800N Dissecting Scope or similar
- Z. Leica DM750 Compound Scope or similar with 10x 1000x magnification and Phase Contrast capabilities
- AA. Attachable compound scope camera
- BB. CMPC mounting media
- CC. Acid cabinet
- DD. Glass Petri dish
- EE. Stiff paint brush

- FF. Taxonomic identification keys for macroinvertebrates
- GG. Cotton swabs
- 4.3. Software
 - A. AIMSII database
 - B. SPOT Pro 5.6 imaging software

5.0. Records Management

- 5.1. IDEM OWQ Biological Studies Laboratory Chain of Custody original hard copy is retained until it is scanned and uploaded to AIMSII database or the Virtual File Cabinet (VFC), after which it will be recycled.
- 5.2. Taxonomist writes identification and enumeration information on the Macroinvertebrate Bench Sheet which is entered into AIMSII and then undergoes two rounds of quality control for data entry errors. The original hard copy is retained until it is scanned and uploaded to AIMSII database or VFC, after which it will be recycled.
- 5.3. AIMS Database can be found at: <u>https://aims.idem.in.gov/AIMS/Pages/Login/</u>

6.0. Definitions

- 6.1. "AIMS sample number" A number assigned to each individual watershed sampling event conducted by Indiana Department of Environmental Management (IDEM) field crews. The number is used to identify the sampling event in the Assessment Information Management System database (AIMSII database).
- 6.2. "Ambient" Surrounding environmental conditions.
- 6.3. "Agency staff" Any employee or representative of IDEM including regular employees, temporary employees, contractors, and interns.
- 6.4. "Assessment Information Management System database (AIMSII database)" IDEM database containing information related to water chemistry, aquatic habitat, macroinvertebrate and fish communities, fish tissue analyses, sediments, algae, and E. coli bacteria data generation through WAPB, External Data Framework, and NPS sampling events.
- 6.5. "Chain of custody (COC)" The records documenting the possession of samples from the time obtained until disposed or shipped off-site.
- 6.6. "Crew chief" The agency staff person who leads a field crew when conducting field sampling activities.

- 6.7. "Field crew" The team of agency staff who conducts field sampling activities. Field crews must contain at least one full-time agency staff member from the Probabilistic or Targeted Monitoring Sections in the crew chief position and one or more full-time IDEM staff or Governor's Summer Interns or compensated interns.
- 6.8. "Indiana Department of Environmental Management (IDEM)" An agency of Indiana State Government whose mission is to implement federal and state regulations to protect human health and the environment while allowing the environmentally sound operations of industrial, agricultural, commercial, and government activities vital to a prosperous economy.
- 6.9. "Macroinvertebrate" Aquatic animals which lack a backbone, are visible without a microscope, and spend some period of their lives in or around water.
- 6.10. "Macroinvertebrate Sample Number" An identification number used to identify a macroinvertebrate sample. The macroinvertebrate sample number is constructed by combining the last two digits of the year, the two digit designation for the month, the two digit designation for the day, the number of the Field Notebook used by the crew leader to record the sampling event, and a two digit number indicating the order in which the current sample was collected on that day. For example, the fourth sample collected by the field crew using Field Notebook #3 on July 15, 2018 would read: MHAB 180715304 ("18" =2018, "07" = July, "15" = 15th day of the month, "3" = Field Notebook #3, "04" = fourth sampled collected that day). The macroinvertebrate sample number does not take precedence over the AIMS sample Number.
- 6.11. "Office of Water Quality (OWQ)" The Office of Water Quality within the IDEM.
- 6.12. Quality assurance (QA)" An integrated system of management activities involving planning, implementation, documentation, assessment, reporting, and quality improvement to ensure that a process, item, or service is of the type and quality needed and expected by the client.
- 6.13. "Quality control (QC)" The overall system of technical activities that measures the attributes and performance of a process, item, or service against defined standards to verify that they meet the stated requirements established by the customer; operational techniques and activities that are used to fulfill requirements for quality. In other words, QC involves measuring the "thing produced" against a standard to ensure it is a quality product that meets the identified need.

- 6.14. "Safety data sheet (SDS)" A sheet containing data regarding the properties of a particular substance or product. It is information intended to provide workers and emergency staff with procedures for handling or working with a particular substance or product in a safe manner.
- 6.15. "Technical standard operating procedure (TSOP)" A standard operating procedure that involves environmental data generation, manipulation, or compilation of an analytical process.
- 6.16. "Virtual File Cabinet (VFC)" The agency's electronic digital image document repository system, that stores, files, indexes, redacts, reassembles, and securely accesses electronic documents of all types both received and created by the various program areas within the agency.

7.0. Quality Assurance and Quality Control

- 7.1. Quality control (QC) of macroinvertebrate projects will be documented by QC checks of both field and laboratory data. Duplicate macroinvertebrate field samples will be collected at ten percent of the sites sampled for each sampling project to evaluate sampling methodology precision. The collection and identification of these duplicate samples will be equally distributed between current IDEM macroinvertebrate biologists, preferably with the same biologist collecting and identifying both the normal and its duplicate sample. After the normal samples are processed and identified in the lab, all specimens are sent for verification by non-IDEM macroinvertebrate taxonomists at an external laboratory. Based on the external verification results, Percent Difference in Enumeration (PDE) will be calculated comparing the difference in total number of specimens counted and Percent Taxonomic Difference (PTD) will be calculated based on the number of differing identifications between IDEM and external taxonomists.
- 7.2. Macroinvertebrate samples will also undergo internal QC along with external verification. Each IDEM biologists' first five samples identified in the lab, and every tenth sample afterwards, will have its residuals checked (as in Step 2.I above) to calculate PSE. Then, after the sample is identified, a quality control taxonomist (usually the Macroinvertebrate Program Manager or most experienced taxonomist in the lab) will verify the sample and calculate PDE and PTD for that sample.

8.0. References

8.1. Agency Policies

IDEM. 2008. <u>Personal Protective Equipment Policy</u>, A-059-OEA-08-P-R0, May 1 2008. Indiana Department of Environmental Management, Office of External Affairs, Indianapolis, Indiana.

IDEM. 2010. <u>Health and Safety Training Policy</u>, A-030-OEA-10-P-R2, revised October, 1 2010. Indiana Department of Environmental Management, Office of External Affairs, Indianapolis, Indiana.

IDEM. 2019. <u>IDEM Hazard Communication (HazCom) Plan</u>. Indiana Department of Environmental Management, Office of Program Support, Indianapolis, Indiana.

IDEM. 2021. Office of Water Quality Watershed Assessment and Planning Branch Laboratory Safety Plan. Indiana Department of Environmental Management, Office of Program Support, Indianapolis, Indiana.

8.2. Agency Standard Operating Procedures

IDEM 1992. Standard Operating Procedures for Conducting Rapid Assessment of Ambient Water Quality Using Benthic Macroinvertebrate. Biological Studies Section, Surveillance and Standards Branch, Office of Water Management, Indiana Department of Environmental Management, Indianapolis, Indiana. IDEM/OWM/WQSSB/BSS-SOP, December 1992, revision number 1.

IDEM 2020. <u>Quality Assurance Program Plan (QAPP) for Biological</u> <u>Community and Habitat Measurements</u>. B-003-OWQ-WAP-XXX-20-Q-R0. Indiana Department of Environmental Management, Office of Water Quality, Watershed Assessment and Planning Branch, Indianapolis, Indiana.

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8.3. Other Guidance

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Klemm, D.J., P.A. Lewis, F. Fulk and J.M. Lazorchak. 1990. <u>Macroinvertebrate Field and Laboratory Methods for Evaluating the</u> <u>Biological Integrity of Surface Waters</u>. EPA/600/4-90/030. Environmental Monitoring Systems Laboratory, Monitoring Systems and Quality Assurance, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes.
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Pyron, M., J. Beugly, E. Martin, and M. Spielman. 2008. Conservation of the Freshwater Gastropods of Indiana: Historic and Current Distributions. American Malacological Bulletin 26: 137-151.

U.S. EPA. 2017. <u>National Rivers and Streams Assessment 2018/19</u>: Field <u>Operations Manual – Wadeable</u>. EPA 841-B-17-003a. U.S. EPA, Office of Water, Washington, DC.

U.S. EPA. 2022. <u>National Lakes Assessment 2022. Field Operations</u> <u>Manual. Version 1.2</u>. EPA 841-B-16-011. U.S. EPA, Washington, DC.

U.S. EPA. 1999. Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. <u>Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition</u>. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

Date:____

9.0. Appendices

Appendix 1: IDEM OWQ Biological Studies Laboratory Chain of Custody

Indiana Department of Environmental Management OWQ Chain of Custody Form	Project:
	OWQ Sample Set or Trip #:

I Certify that the sample(s) listed below was/were collected by me, or in my presence.

Signature:									Se	ction:				
Sample Media (🗆	Water, 🗖 Alga	ae,□ Fisl	h, 🗆 Ma	icro, 🗆 🤇	Cyanoba	acteria/I	Microcy	stin, 🗆	Sedimer	nt)				
Lab Assigned	IDEM	iple rpe		M.	mI. M.	al II	120 ml P (Bact)) ml ene	ml ene	ml Ss	Date and Ti	me Collected		ne check
Number / Event ID	Control Number	Sample Type	ID	1000 ml P.N.M.	1000 ml G.N.M.	40 ml Vial	120 P (B	2000 ml Nalgene	250 ml Nalgene	125 ml Glass	Date	Time		er bottle eresent
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													+	
P = Plastic	G = Glass	N.I	M. = Na	rrow Mo	outh	Bact =	Bacter	iologica	l Only		L Should samples	s be iced?	Y	N
M = MS/MSD	B = Blank	D	= Dupli	cate		R = R	evisit							

<u>Carriers</u>

I certify that I have received the above sample(s).					
Signature	Date	Time	Seals	Intact	Comments
Relinquished By:			v	N	
Received By:			'		
Relinquished By:			v	N	
Received By:				N	
Relinquished By:			v	N	
Received By:			T	N	
IDEM Storage Room #					

Lab Custodian

I certify that I have received the above sample(s), which has/have been recorded in the official record book. The same sample(s) will be in the custody of competent laboratory personnel at all times, or locked in a secured area.

Signature:____

Date:_____ Time:____

Lab:_____

Address:_____

Revision Date: 4/27/2016

Appendix 2: Macroinvertebrate Bench Sheet

OWQ/WAPB Macroinvertebrate Community Assessment MHAB Bench Sheet

Sample #	Macro Eve	nt#	Macro S	ample Type		Stream Name	/Location		C	oanty
L 100-Organi im Sub Sample										
	Sample	#of	🗹 Sab	Sample Comp. Sort Date #	eted 🛛	🗆 Phase	1 Completed ID Date		Phase 2	Completed
15-minute Sub Sample 100-Organi im (Lab Dup)	Date	Vials	Sorter	Sort Date #	S <i>qn ares</i>	klentiker	ID Date	kde	ntifier	ID Date
100-Organi illi (Lab Edp)										
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Appendix 3: Example of macroinvertebrate label sheet

Labels For: MHAB AB51654; Macro# 220927601; 15-Min

WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-08-0002: East Fork White River
IN County-Jackson 27-September-2022	IN County-Jackson 27-September-2022	IN County-Jackson 27-Beptember-2022	IN County: Jackson 27-September-2022	IN County-Jackson 27-September-2022
MCO	MCO	MCO	MCO	MCO
CR 375 West	CR 375 West	CR 375 West	CR 375 West	CR 375 West
MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MH/AB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601
Lathude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376
15-Min Preserved in 80% Isopropanol	15-Min Preserved in 80% Isopropanol	15-Min Preserved in 80% Isopropanol	15-Min Preserved in 80% Isopropanol	15-Min Preserved in 80% Isopropanol
WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River
IN County:Jackson 27-September-2022	IN County:Jackson 27-September-2022	IN County:Jackson 27-September-2022	IN County:Jackson 27-September-2022	IN County-Jackson 27-September-2022
MCO	MCO	MCO	MCO	MCO
CR 375 West	CR 375 West	CR 375 West	CR 375 West	CR 375 West
MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601
Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376
15-Min Preserved in 80% toopropanol	15-Min Preserved in 80% Isopropanol			
WEU-06-0002: East Fork White River	WEU-08-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River
IN County-Jackson 27-September-2022	IN County:Jackson 27-September-2022	IN County: Jackson 27-September-2022	IN County: Jackson 27-September-2022	IN County-Jackson 27-September-2022
MCO	MCO	MCO	MCO	MCO
CR 375 West	CR 375 West	CR 375 West	CR 375 West	CR 375 West
MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601
Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376
15-Min Preserved in 80% isopropanol	15-Min Preserved in 80% Isopropanol	15-Min Preserved in 80% isopropanol	15-Min Preserved in 80% Isopropanol	15-Min Preserved in 80% Isopropanol
WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River
IN County:Jackson 27-September-2022	IN County-Jackson 27-September-2022	IN County:Jackson 27-September-2022	IN County: Jackson 27-September-2022	IN County-Jackson 27-September-2022
MCO	MCO	MCO	MCO	MCO
CR 375 West	CR 375 West	CR 375 West	CR 375 West	CR 375 West
MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601
Latitude 38.85297 Longitude -96.11376	Latitude 38.85297 Longibude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376
15-Mn Preserved in 80% isopropanol	15-Min Preserved in 80% Isopropanol	15-Min Preserved in 80% Isopropanol	15-Min Preserved in 80% Isopropanol	15-Min Preserved in 80% Isopropanol
WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River
IN County:Jackson 27-September-2022	IN County-Jackson 27-September-2022	IN County:Jackson 27-September-2022	IN County-Jackson 27-September-2022	IN County-Jackson 27-September-2022
MCO	MCO	MCO	MCO	MCO
CR 375 West	CR 375 West	CR 375 West	CR 375 West	CR 375 West
MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601
Latitude 38.85297 Longitude -96.11376	Latitude 38.85297 Longibude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376
15-Min Preserved in 80% isopropanol	15-Min Preserved in 80% Isopropanol	15-Min Preserved in 80% Isopropanol	15-Min Preserved in 80% isopropanol	15-Min Preserved in 80% Isopropanol
WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River	WEU-06-0002: East Fork White River
IN County-Jackson 27-September-2022	IN County:Jackson 27-September-2022	IN County:Jackson 27-September-2022	IN County-Jackson 27-September-2022	IN County-Jackson 27-September-202
MCO	MCO	MCO	MCO	MCO
CR 375 West	CR 375 West	CR 375 West	CR 375 West	CR 375 West
MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601	MHAB AB51654; Macro# 220927601
Latitude 38.85297 Longitude -06.11376	Latitude 38.85297 Longibude -86.11376	Latitude 38.85297 Longitude -86.11376	Latitude 38.5297 Longitude -86.11376	Latitude 38.85297 Longitude -86.11376
15-Min Preserved in 80% isopropanol	15-Min Preserved in 80% Isopropanol	15-Min Preserved in 80% isopropanol	15-Min Preserved in 80% Isopropanol	15-Min Preserved in 80% Isopropano

Appendix 4: Macroinvertebrate slide record

AB#:	Macro#:	Stream:		ld:	of
Slide#	Identification		Slide#	Identification	
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Appendix 5: Example of a completed macroinvertebrate field data sheet

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Site D	Descript	ion: C	R 300 No	rth														
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	EDROCK[5] ER OF BEST T		stural substrates; igno r more [2]	re sludge from p		USTRINE[0] VE[-1]	MODERATE [-1]
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of margir	nal quality; 2-Mo	derate amount	s, but not of highe	est quality or	in small amounts of I	highest quality;	AMOUNT
					ders in deep or fast or deep, well-define		Check ONE (Or 2 & average) EXTENSIVE > 75% [11]
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DOVE	RHANGING VEC	ETATION [1]	Q ROOTWAL	DS[1]	AQUATICMACRO	PHYTES[1]	NEARLY ABSENT < 5%[1
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Appendix 6: Example of a completed macroinvertebrate QHEI form


220712601	Clear Spring Creek @ CR 200N, Jackson Co
AB51669	12, July 2022 DTB MGMLL
INRB22-025	ImRiffle, 50m Miller, collivers tokend
MHAM	NORMAL
220712602 AB51690 INRB20-025 MHAB	Clear Spring Creek @ CR 200 N, Jackson Co DTB IM RIFFLEKICK, SOMMHAB MERO DUPLICATE completed SOM upstream of Normal

Appendix 7: Example of a macroinvertebrate field logbook entry

Appendix 9: List of macroinvertebrate taxonomic references and lowest practical taxa identification levels

This appendix is based on previously compiled lists of Indiana's benthic macroinvertebrate taxa (Pyron et al. 2008; Hellenthal et al. 2005) and should not be considered an exhaustive list of potential taxa in the state.

Phylum	Class	Order	Family	Genus	Recommended level of ID	Notes	Recommended Keys
Platyhelmenthes					Platyhelmenthes		n/a
Annelida	Clitellata	Tubificida	Naididae (includes "Tubificidae")		Species	If genetalia is required for identification, leave at lowest possible level (including subfamily + chaetae type, for Naidinae and Tubificinae)	Kathman & Brinkhurst 1999
		Branchiobdella	Branchiobdellidae		Family		
			Erpobdellidae		Family		
	Hirudinida		Glossiphonidae		Family		Thorp & Covich 2016
		Hirudinida	Macrobdelliade		Family	Helobdella stagnalis can potentially be IDed to species	
			Lymanaeidae	Fossaria <i>Lymnaea stagnalis</i> Stagnicola Pseudosuccinea	Genus		
			Physidae	Physa Physella	Genus		Thorp & Covich
Mollusca	Gastropoda Basommator	Basommatomorpha	Planorbidae	Ferrissia Laevapex fuscus Gyralus Helisoma anceps Planorbella Planorbula armigera Promenetus exacuous	Genus	Formerly Ancylidae	2016; Pekarski 1990; FWGNA Ohio 2020; Jokinen 1992; Branson 1987
			Bithyniidae	Bithynia tentaculata	Species		
			Hydrobiidae		Family		

		Basommatomorpha	Pleuroceridae	Elimia livescens Leptoxis praerosa Lithasia obovata Pleurocera	Genus	Thorp & Covich 2016; Pekarski		
	Gastropoda		Pomatiopsidae	Pomatiopsis cincinnatiensis	Genus	1990; FWGNA Ohio 2020;		
Mollusca		Heterostropha	Valvatidae	Valvata	Genus	Jokinen 1992;		
		Architaenioglossa	Viviparidae	Bellamya <i>Campeloma decisum</i> Viviparus	Genus	Branson 1987;		
			Corbiculidae	Corbicula fluminea		Thorp & Covich		
	Bivalvia	Veneroida	Pisidiiade		Genus	2016; Pekarski 1990		
		Unionida	Unionidae		DO NOT COLLECT	1990		
Nematoda					Phylum	Thorp & Covich 2016		
Nematomorpha	Gordioida		Gordiidae Chordodidae	Gordius Chordodes Gordionus Neochordodes Paragordius Pseudochordodes	Species	Poinar & Chandler 2004; Schmidt- Rhaesa et al. 2003		
	Arachnida	"Acari"			"Acari"	Thorp & Covich 2016; Pekarski 1990; Smit 2020		
				Amphipoda	Gammaridae	Gammarus Echinogammarus ischnus	Genus	Thorp & Covich
			Crangonictidae	Crangonyx		2016; Pekarski		
			Hyallelidae	Hyallela		1990		
Arthropoda		Isopoda	Asellidae	Caecidota	Genus			
	Malacostraca	1300004	Ascillac	Lirceus	Genus			
				Cambarus	Form I male: Species;			
		Decapoda	Cambaridae	Faxonius	Form II male: Species, if possible; Female: If	Taylor, Schuster,		
			Cambaridae —	Orconectes	feasible, group with males; otherwise,	& Wylie 2015		
				Procambarus	leave at family			

			Entomobryidae				
			, Hypogastruidae				
			Isotomidae				
			Onychiuridae		F 11		Merritt, Cummins
	Collembola	Collembola	Paronellidae		Family		& Berg 2019
			Poduridae				
			Sminthuridae				
			Tomoceridae				
			Ameletidae	Ameletus	Species		
				Acentrella			1
				Acerpenna			
				Baetis	Currier		
				Callibaetis	Species		
				Camelobaetidius waltzi			
				Centroptilum	Genus	Identification will likely	
				Cloeon dipterum	Genus	require mounting of mouthparts, proleg, and/or gills	
			Baetidae	Diphetor hageni			
				Heterocloeon			
				Paracloeodes	Species		
Arthropoda		Insecta Ephemeroptera		Plauditus			
				Procloeon			
				Procideon			
				usa			Klubertanz 2016; Morse et al. 2017
	Insecta			Pseudocloeon			
			Baetiscidae	Baetisca	Species		WOISE et al. 201
			Dubtibuludo	Brachycercus	Species		1
				brachycorodo		<i>Caenis latipennis</i> can	
			Caenidae		"Caenis diminuta	potentially be IDed to	
				Caenis	Group" or "Caenis	species out of "C.	
					hilaris Group"	diminuta group"	
				Attenella attenuata			
				Dannella lita			
			Ephemerellidae	Drunella walkeri	Species		
			cplienterentude	Ephemerella	Species		
				Eurylophella			
				Serratella			
				Ephemera	Genus		
			Ephemeridae	Hexagenia			
				Pentagenia vittigera	Species		

	1	1		A nonconstruction		I	1
				Anepeorus simplex	Curacian		
				Epeorus	Species		
				Heptagenia			
				Leucrocuta	Genus		4
						Identification will	
				Maccaffertium		require mounting	
			Heptageniiidae			maxilla	
				Nixe	Species		1
			Raptoheptagenia				
				cruentata			
				Rhithrogena manifesta			
				Stenacron	Genus		
				Stenomena femoratum	Species		Klubertanz 2016;
		Ephemeroptera	Isonychiidae	Isonychia	Genus		Morse et al. 2017
			Leptohyphidae	Tricorythodes	Genus		Worse et al. 2017
				Choroterpes basalis			1
			Leptophlebiidae	Leptophlebia	Species		
		ecta		Paraleptophlebia			-
			Metropodidae	Siphloplecton			
Arthropoda	Insecta			interlineatum	Species		
Antinopoda	msecta		Oligoneuriidae	Homoeoneuria	- .		
				ammophila	Species		
			Delawite and in a	Ephoron	Co i		
			Polymitarcydiae	Tortopus primus	Species		
			Potamanthidae	Anthopotamus	Species		1
			Pseudironidae	Pseudiron centralis	Species		1
			Siphlonuridae	Siphlonurus	Species		1
				Calopteryx	- ·		
			Calopterygidae	Hetaerina	Species		
				Archilestes grandis			
			Lestidae	Lestes	Species		
				Amphiagrion saucium			
				Argia		Leave at genus if	Merritt, Cummins,
		Odonata		-		necessary gills are	& Berg 2019;
			Coenagrion resolutum		missing	Westfall & May	
			Coenagrionidae	Chromagrion conditum	Species	missing	2006
			Enallagma				
				Ischnura			
				Nehalennia			
			1	ivenaiennia		1	1

				Aeshna Anax Basiaaeschna janta Boyeria Gomphaeschna furcillata Epiaeshna heros Nasiaeshna pentacantha	Species		
			Corduliidae	Cordulia shurtleffi Dorocordulia libera Epicordulia princeps Helocordulia uhleri Neurocordulia Somatochlora Tetragoneuria	Species		
			Corduligastridae	Cordulegaster Arigomphus	Species		{
Arthropoda	Insecta	Odonata	Gomphidae	Angomphus Dromogomphus Erpetogomphus designatus Gomphus Hagenius brevistylus Ophiogomphus Progomphus obscurus Stylogomphus albistylus Stylurus	Species	lf identifiable in the field, do not collect	Merritt, Cummins, & Berg 2019; Tennessen 2019
			Libellulidae	Celithemis Erythemis simpliciollis Erythrodiplax Ladona Leucorrhinia Libellula Nannothemis bella Pachydiplax longipennis Pantala Perithemis Plathemis lydia	Species		

			Libellulidae	Sympetrum Tramea	Species		Merritt, Cummins,
		Odonata	Macromiidae	Didymops transversa	Species		& Berg 2019;
			Wacronnidae	Macromia sp.	Genus		Tennessen 2019
			Petaluridae	Tachopteryx thoreyi	Species		
				Allocapnia			
			Capniidae	Nemocapnia carolina			
				Paracapnia angulata			
				Alloperla			
			Chloroperlidae	Haploperla brevis			
				Sweltsa naica			
				Leuctra			
			Leuctridae	Paraleuctra sara			
				Zealeuctra			
				Amphinemura			
		Insecta Plecoptera		Nemoura trispinosa			
			Nemouridae	Prostoia			
Arthropoda	Insecta			Shipsa rotunda			
				Soyedina vallicularia			
				Acroneuria	Species		Morse et al. 2017
				Agnetina	Species		Morse et al. 2017
			Perlidae	Attaneuria ruralis			
			Periluae	Eccoptura xanthenes			
				Paragnetina Perlesta			
				Perlinella			
				Clioperla clio			
				Cultus decisus			
				Diploperla robusta			
			Perlodidae	Helopicus nalatus			
			Hydroperla				
			Isogenoides varians				
			Isoperla				
			Pteronarcyidae	Pteronarcys	コート		
			Taeniopteryigidae	Strophopteryx fasciata			
				Taeniopteryx			

					Species	Nymphs left at family or genus (if able to ID to genus)	To genus: Epler 2006; To species: see below
			Belastomatidae	Belastoma Lethocerus	Species		Epler 2006; Hilsenhoff 1984
				Corisella Hesperocorixa Palmacorixa			Hilsenhoff 1984
			Ramphocorixa acuminata			Epler 2006	
			Corixidae	Sigara	Species	Only males can be positively IDed to species; females can be grouped with like males	Hilsenhoff 1984
			Gelastocoridae	Trichocorixa	Engaige		Epler 2006
			Gelastocoridae	Gelastocoris oculatus	Species		Epler 2006
Arthropoda	Insecta	Insecta Hemiptera		Aquarius Gerris Limnoporus			Epler 2006 Taylor 1996
			Gerridae	Metrobates hesperius Neogerris hesione Rheumatobates	Species		Epler 2006
				Trepobates			Hilsenhoff 1984
				Hebrus			Hilsenhoff 1984
			Hebridae	Merragata	Species		Epler 2006
			Hydrometridae	Hydrometra martini	Species		Epler 2006
			Mesoveliidae	Mesovelia mulsanti	Species		Epler 2006
			Naucoridae	Pelocoris femortus	Species		Epler 2006
				Nepa apiculata			Epler 2006
			Nepidae	Ranatra	Species		Epler 2006 + Hilsenhoff 1984
			Notonectidae	Buenoa	Species		Epler 2006; Hilsenhoff 1984
				Notonecta			Hilsenhoff 1984
			Ochteridae	Ochterus banksi	Species		Epler 2006
			Pleidae	Neoplea striola	Species		Epler 2006
			Saldidae	b di anguna li a	Family		Epler 2006
			Veliidae	Microvelia Rhagovelia	Species		Epler 2006 Hilsenhoff 1984

			Brachycentridae Dipseudopsidae Glossosomatinae Goeridae Helicopsychidae	Brachycentrus Micrasema Phylocentropus Glossosoma Agapetus Protoptila Goera stylata Helicopsyche borealis	Species Species Species Species Species	Leave pupa at family (unless monotypic/easily identifiable, i.e. Helicopsyche)	
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Cheumatopsyche Diplectrona <i>Homoplectra doringa</i> Hydropsyche Potamyia flava	Genus Species	"Hydropsyche betteni group," "Hydropsyche bidens group", "Hydropsyche rossi/simulans" or "Hydropsyche morosa (or checkerboard) Group"	Morse et al. 2017; Wiggins 1996
			Hydroptilidae	Agraylea multipuncata Dibusa angata Hydroptila Neotrichia minutisimella Ochrotrichia Orthotrichia Oxyethira Stactobiella delira	Genus	Only take to genus if identifiably Hydroptila or final instar, leave at family otherwise	
			Lepidostomatidae Leptoceridae	Lepidostoma sommermanae Ceraclea Leptocerus americanus Mystacides Nectopsyche Oecetis Setodes oligius Triaenodes	Species Species		

			Limnephilidae	Anabolia Frenesia missa Hydatophylax argus Ironoquia Limnephilus Platycentropus radiatus Pseudostenophylax uniformis Pycnopsyche	Species Genus		
		Molannidae	Molanna	Species			
			Philopotamidae	Chimarra Dolophilodea distinctus Wormaldia moesta	Species		
Arthropoda	Insecta	Trichoptera	Phryganeidae	Agrypnia vestita Banksiola crotchi Fabria inornata Oligostomis ocelligera Phryganea Ptilostomis	Species		Morse et al. 2017; Wiggins 1996
			Polycentropodidae	Cernotina spicata Cyrnellus fraternus Neureclipsis crepuscularis Nyctiophylax Polycentropus	Species	"lacertina species group"	
			Psychomiidae	Lype diversa Psychomyia flavida	Species		
			Rhyacophilidae	Rhyacophila	Species]
			Uenoidae	Neophylax	Species		
		Lepidoptera	Crambidae	No list available for Indiana under current systematics	Genus until we can find a species key	Make sure it is a truly aquatic taxa	Merritt & Cummins 3E (to ensure aquatic family); Merritt, Cummins, & Berg 2019 (for genus)

		Megaloptera	Corydalidae Sialidae	Chauliodes Corydalus cornutus Neohermes concolor Nigronia Sialis Climacia areolaris	Species Genus		Merritt, Cummins, & Berg 2019; Rasmussen & Pescador 2002
		Neuroptera	Sisyridae	Sisyra	Species		
						Larva left at genus	Merritt, Cummins, & Berg 2019 (Genus only)
			Artematopodidae	Eurypogon harrisi	Species		
			Chrysomelidae		Family		
Arthropoda In	Insecta		Curculionidae	Bagous Brachybamus electus Euhrychiopsis lecontei Lissorhopterus Listronotus Notiodes Onychylis nigriostris Stenopelmus rufinasus Tanysphyrus lemnae	Species	Make sure it is an aquatic taxa; Do not count larvae	Bayer & Brockkmann 1975
		Coleoptera	Dryopidae	Helichus Pelonomus obscurus	Species		Ciegler 2003
		Coleoptera		Acilius Agabus	Species Genus		
			Dytiscidae	Bidessonotus inconspicuous Celina Colymbetes sculptilis Copelatus Coptotomus Cybister fimbriolatus Desmopachria convexa Dytiscus Graphoderus Hydaticus Hydroporus Hydrovatus Hygrotus	Species		Larson 2001 (only after IDed to genus)

Processing and Identification of Macroinvertebrate Samples

		Dytiscidae	Ilybius Laccophilus Laccornis Liodessus Matus <i>Neoscutopterus</i> <i>angustus</i> Rhantus <i>Stictotarsus</i> <i>griseostriatus</i> Thermonectus Uvarus	Species		Larson 2001 (only after IDed to genus)	
			Ancyronyx variegatus Dubiraphia		Male genetailia required for ID; Females can be grouped with males if thought to be same speices	Hilsenhoff & Schmude 1992	
Arthropoda	Insecta	Coleoptera		Macronychus glabratus	Species		
Antinopoda	mseeta		Elmidae	Microcylloepus pusillus			
				Optioservus		Male genetailia may be required for ID	White 1978
				Oulimnius latiusculus		Genus not separated in Hilsenhoff & Schmude 1992	Merrit, Cummins, & Berg 2019; Ciegler 2003
			Stenelmis		Male genetailia generally required for ID; Females can be grouped with males if thought to be same speices	Unlabeled dissertation <i>Stenelmis</i> key?	
		Gyrinide	Dineutus Gyrinus	Genus		Merritt, Cummins,	
		- / · · · · · · -	Gyretes compressus	Species		& Berg 2019	
		Haliplidae	Haliplus Peltodytes	Species		Gunderson & Otremba 1998	
			Helophoridae	Helophorus	Species		Hilsenhoff 1995a

		Lanternrius Lapsus tristus Neoheterocerus Tropicus pusillus Gymnochthebius nitidus Hydraena	Heteroceridae	Heterocerus Lanternrius <i>Lapsus tristus</i> Neoheterocerus	Species	Ciegler 2003 (check species descriptions for correct genus names; key was developed using old classification)
			Species	Epler 2010. Thomas 2001. (Limnebus). Beetles of the Northeast. Downie & Arnett. 1996 (All)		
		Hydrochidae	Hydrochus	Species	Hilsenhoff 1995a	
Arthropoda	Insecta	Coleoptera	Hydrophilidae	HydrochusSpeciesAnacaena limbatumBerosusChaetarthriaCrenitisCymbiodytaDerallus altusEnochrusHelochares maculicollisHydrobiusHydrocharaHydrochusHydrophilusLaccobiusParacymusPhaenonotumexstriatumSperchopsis tesselatus	Merrit, Cummins, & Berg 2019 (larvae and adult genus); Archangelsky 1997 (larvae); Hilsenhoff 1995a and 1995b (species). Hydrophilidae Regional Key. Testa & Lago 1994 (Phaenontem extriatum genus and species key).	
			Limnichidae	Eulimnichus Limnichites <i>Limnichoderus ovtus</i>	Genus	Arnett et al. 2002

			Lutrochidae	Lutrochus laticeps	Species		Merritt, Cummins, & Berg 2019
			Noteridae	Hydrocanthus iricolor Suphisellus	Species		Ciegler 2003
			Psephenidae	Psephenus herricki	Species		Merritt, Cummins,
			rsephenidae	Ectopria	Genus		& Berg 2019
		Coleoptera	ra Ptiliidae Actidium Genus Ptiliodactylidae Paralichus trivittus Species	Genus		Merrit, Cummins & Berg 2019	
				Species		Merrit, Cummins, & Berg 2019	
				Ptilodactyla			Ciegler 2003
	Insecta		Scirtidae		Family		Merrit, Cummins & Berg 2019
Arthropoda		Diptera Athericidae Atherix variegata Ge Atrichopogon "Bezzia group"				Leave larvae at genus level except for Chironomidae; Leave pupa at family except for Chironomidae and pupariums ("Muscomorpha")	
			Genus		Merritt, Cummins,		
				Atrichopogon			& Berg 2019
				"Bezzia group"			
			Ceratopogonidae	"Ceratopogon group"	Genus or genus group		

Arthropoda	Insecta	Diptera	Chironomidae (Orthocladiinae) Chironominae (Podonominae)	Acricotopus Brillia flavifrons Crdiocladius Chasmatonotus atripes Corynoneura Cricotopus Epoicocladius flavens Eukiefferiella brevinervis Heterotrissocladius changi Hydrobaenus pilipes Metriocnemus Nanocladius Orthocladius Orthocladius Parakiefferiella subaterrima Parametriocnemus lundbeckii Paraphenocladius exagitans Psectrocladius vernalis Rheocricotopus Smittia Thienemanniella simils Xylotopus par Zalutschia lingulata	Larvae to species if key available. Pupa to genus	"Cricotopus/ Orthocladius" acceptable "Cricotopus/ Orthocladius" acceptable	Epler 2001 (larvae); Merritt, Cummins, & Berg 2019 (pupae)
			Chironominae (Prodiamesinae)	Parochlus kiefferi Monodiamesa tuberculata Prodiamesa olivacea			

				Ablabesmyia		"mallochi group",	
				Brundiniella eumorpha			
				Cantopelopia gesta			
		Conchapelopia		"Thienemannimyia group" for early instars			
				Djamabatista pulcher			
				Guttipelopia guttipennis			
			Chironominae	Hayesomyia senata Labrundia pilosella Larsia key available. Pupa to		Epler 2001 (larvae); Merritt,	
			(Tanypodinae)	Meropelopia	genus	"Thienemannimyia group" for early instars	Cummins, & Berg 2019 (pupae)
			Monopelopia tenuicalcar				
Arthropoda				Procladius Natarsia baltimoreus Psectrotanypus dyari Tanypus			
	Insecta	Diptera					
				Thienemannimyia senata		"Thienemannimyia group" for early instars	
				Zavrelimyia			
		Aedes					
				Anopheles			
				Coquillettidia perturbans			
			Culicidae	Culex Culiseta Orthopodomyia Psorophora Toxorynchites rutils septentrionalis	Genus		Merrit, Cummins, & Berg 2019
				Uranotaenia sapphirina			
				Wyeomyia smithii			

			Dixidae	Dixa recens Dixella	Genus				
			Dolichopodidae		Family	1			
						Empididae	Chelifera Chelipoda Hemerodromia	Genus	
			Ephydridae		Family	1			
Arthropoda	Insecta	Diptera	Limoniidae		Merrit, Cummins, & Berg 2019				
		Muscidae Family	Family						
			Pediciidae	Dicranota Pedicia	Genus				
			Psychodidae	Pericoma Philosepedon Psychoda Telmatoscopus Trichomyia nuda	epedon hoda Genus oscopus				
		Sarcoph	Sarcophagidae		Family				
			Scathophagidae		Family	1			
			Sciomyzidae		Family	1			
			Simuliidae	Cnephia Prosimulium Simulium	Genus				

			Stratiomyidae	Caloparyphus tetraspilus Euparyphus Hedriodiscus Hermetia illucens Hoplitimyia constans Labostigmina Nemotelus Odontomyia Oxycera Sargus Stratiomys	Genus		
			Syrphidae	idae Family		Merrit, Cummins, & Berg 2019	
Arthropoda	Insecta	Chrysops Diachlorus ferruge Goniops Tabanidae Hamatabanus Hybomitra	Diachlorus ferrugatus Goniops Hamatabanus Hybomitra Merycomyia Stonemyia	Genus			
			Tanyderidae	Protoplasa fitchii	Genus		J I
			Tipulidae	Brachyprema Liogioma Phalacrocera Tipula	Genus		