Alberta Biodiversity
Monitoring Institute

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Processing Aquatic Invertebrates

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Summary

This document describes the procedures, training, and resource materials used to process and identify aquatic invertebrates for the ABMI. A two-step process is implemented to identify aquatic invertebrates. First, field crews sort the specimens into coarse taxonomic groups. Technicians receive training so they can effectively separate aquatic invertebrates from debris and sediment, and sort the target specimens into coarse taxonomic groups. Training and quality control are conducted by a qualified lab supervisor. By having lab technicians remove the debris from the samples and sort the specimens into groups, less work is required by experts. Secondly, three taxonomic groups from rivers (*Ephemeroptera, Plecoptera, Trichoptera,)*, and eight taxonomic groups from wetlands (*Gastropoda, Ephemeroptera, Anisoptera, Zygoptera, Trichoptera, Hemiptera, Coleoptera, and Chironomidae)* are identified by taxonomic experts at the Royal Alberta Museum (RAM) to the lowest taxonomic level possible.

Data Management

Data Entry During Sorting

Lab technicians are responsible for filling information into the ABMI aquatic invertebrate on-line data portal (Appendix 1) while picking and sorting specimens. Data portal fields where no values are recorded are automatically assigned a "zero" value in the background database. If information from a sample or specimen cannot be described using existing categories in the data portal, then the lab technician selects the last (orange) line in the data portal to add the appropriate category from the drop down list.

Data Security and Back-up

Access to the on-line data portal requires password authentication by each user. Passwords and levels of access are controlled by the lab supervisor. The on-line data portal is the database interface only; the actual Oracle database is stored on a secure server at the ABMI Information Center located on the University of Alberta. This secure server is backed up at regular intervals on two additional off-site servers. Excel spread-sheet copies of the data are also backed up on RAM's external server which is in turn backed up at an additional off-site server.

Checking and Storing Data During Sorting

Data portal fields are checked for accuracy during the sorting process each time sample residue and picked organisms are checked as part of quality control. An Excel spread sheet is downloaded from the data portal by the lab supervisor once the sorting for an individual site has been completed (Appendix 2). The lab supervisor checks the data portal and Excel spread-sheet to ensure that all information is recorded accurately and that all data fields are filled in. The spread-sheet is then locked (using the protect worksheet function) and is stored on a secure computer. At the conclusion of the sorting session, the lab supervisor checks to ensure that spread-sheets for all ABMI sites are present. Excel spread-sheets act as a back-up in case of data portal failure.

Data Entry During Expert Identification

Taxonomic experts at RAM enter data directly into the aquatic invertebrate on-line data portal (Appendix 3). The taxonomic expert fills in the required information as specimens are identified. The total number of specimens identified for each sorted group is entered in the Advanced ID Count column. If more than one species is identified for a sorted group, the expert inserts a new row below the original row and records the total number of specimens identified as that species in the Advanced ID Count column. Automatic checks built into the data portal track species names and specimen counts to ensure accuracy (advanced ID counts total the original coarse sorting count for that group).

Transferring Data from the Taxonomic Expert to the ABMI Information Center

Once the advanced ID has been completed, the taxonomic expert notifies the ABMI Information Center that the data is ready for final review and release. The Information Center system analyst and information/data coordinator access the Oracle database directly and review the data for any discrepancies. All discrepancies are resolved prior to the data being released.

Specimen Management

To ensure that samples are not lost, all specimens received by the Sample Processing Center (RAM) are tracked using the Sample Tracking Log. All subsequent transfers of specimens, samples and data are recorded in the log.

Specimen Transfer from the Field to the Sample Processing Center

- At the end of each field shift, sample bottles containing aquatic invertebrates are packaged into coolers (to contain any possible leakage) by field crews, the sample information transferred from the field computer to a memory stick, and the coolers and memory stick delivered or shipped via courier to the Sample Processing Center (see ABMI Wetland Field Data Collection Protocols for more information).
- Samples are logged-in when they arrive at the Sample Processing Center. Samples are checked against the information contained on the memory stick to ensure that all sites have been received, and the correct number of sample bottles has been received from each site.
- The Sample Tracking Log includes information about the date the samples arrived, the location where the samples are stored, the ABMI sites where the samples were collected, the number of samples of each type from the site, and a detailed listing of the information about each sample.
- The ABMI lab coordinator ensures that all aquatic invertebrate sample bottles from each ABMI site are present in the storage facility and recorded in the sample tracking log.

Changing Preservative in Sample Bottles

- To maintain quality of the aquatic invertebrate specimens, preservative in the sample bottles is changed from formalin to ~80% ethanol between 4 and 14 days after the invertebrates are collected.
- Wearing gloves and safety glasses, remove the lid from the sample bottle, cover the opening with 500 µm mesh and secure it in place with a modified lid containing a large hole cut in the center.
- Decant the formalin into a temporary holding container (this is a precaution in case the lid falls off or does not seal properly) removing as much of the formalin as practical.
- Backwash the through the mesh with a small amount of 80% ethanol to dislodge any organisms that may be adhering to the mesh.
- Remove the lid and mesh, and carefully check that all organisms have been washed from the mesh back into the sample bottle. If there are organisms still adhering to the mesh, use 80% ethanol to carefully wash them back into the sample bottle.
- If the sample bottle is 1/3 or more full of organic debris and/or sediment, top up the bottle with 95% ethanol.
- If the sample bottle is less than 1/3 full of organic debris and/or sediment, top up the bottle with 80% ethanol.
- Tightly secure the original lid on the sample bottle, wipe down the outside of the bottle and apply ethanol workplace safety label.
- Pour the used formalin into the waste solution container, rinse the mesh and temporary holding container, and repeat with the next sample bottle.
- The lab coordinator ensures all samples are labeled properly. Each sample bottle should have been labeled by the field crews with: ABMI Site, Date, and Collector's Initials. In addition, there should have been a label (written on waterproof paper) with the same information inside each sample bottle. If any of this information is missing, the lab coordinator adds it.
- After the preservative in the aquatic invertebrate sample bottles has been changed from formalin to 80% ethanol, the change is recorded in the sample tracking log.

Specimen Transfer from the Sample Processing Center to the Sorting Facility

- Sample bottles containing aquatic invertebrates are transferred from the Sample Processing Center to the sorting location by the aquatic lab supervisor.
- The lab supervisor ensures that all sample bottles for each ABMI site are present at the sorting location, and records the new location, and the date of transfer in the sample tracking log.

Specimen Transfer from the Sorting Facility to the Sample Processing Center

- After aquatic invertebrates have been picked and sorted for all ABMI sites, the aquatic lab supervisor delivers the resulting samples to the Sample Processing Center. Four types of samples are transferred:
    1) Vials with sorted specimens,
    2) Sample bottles with sediment,
    3) Sample bottles with residue, and
    4) Sample bottles with sieved sample
- The ABMI lab coordinator ensures that all samples arrive, and then records the new location and date of transfer in the sample tracking log.

Specimen Transfer from the Sample Processing Center to the Taxonomic Expert

- Advanced ID is currently done by taxonomic experts at the Sample Processing Center (RAM). If external experts are used, the following procedures are used:
- Vials containing three taxonomic groups from rivers (*Ephemeroptera, Plecoptera, Trichoptera,)*, and eight taxonomic groups from wetlands (*Gastropoda, Ephemeroptera, Anisoptera, Zygoptera, Trichoptera, Hemiptera, Coleoptera, and Chironomidae)* are sent to an expert aquatic invertebrate taxonomist for identification to genus/species.
- Samples vials are packed within an inner carton, absorbent material, plastic liner, foam packing and an external cardboard box. Boxes are labelled and shipped by courier in accordance with current TDG regulations.
- The ABMI lab coordinator records the new location and the date of transfer in the sample tracking log.

Specimen Transfer from the Taxonomic Expert to the Sample Processing Center

- All specimens and materials received from the Sample Processing Center are returned after species have been identified.
- Samples are packed and shipped in the same manner as listed above.
- The ABMI lab coordinator checks to ensure that all samples have been returned and are properly labelled. Samples are organized and boxed for storage at the Sample Processing Center.
- The ABMI lab coordinator records the new location and the date of transfer in the sample tracking log.

Long-term Specimen Curation at the RAM

- All specimens and residual materials collected by the ABMI are gifted to, and where appropriate curated by, RAM.
- RAM retains all ABMI materials for 2 years. This includes specimens sorted to genus/species, specimens sorted to broad taxonomic groups, and residual material including non-sorted specimens and debris.
- After 2 years, reference specimens from each genus/species (or taxonomic group if the specimens were not identified to genus/species) and training specimens are retained by the RAM for use by the ABMI. All other ABMI specimens can be loaned, traded, distributed, or disposed as the RAM see fit.
- All specimen loans will be documented using procedures in the Royal Alberta Museum's Collections Management Policy (January, 2014).

Sample Processing

This protocol is designed to extract aquatic macro-invertebrates from the samples collected in the field, and determine the presence and abundance for these species.

Laboratory Equipment

Safety equipment (lab coat, nitrile gloves, safety goggles)
Dissecting microscope (10-40X) w/ cold (fiber optic) light source
Extraction equipment (forceps, eye dropper, bulb syringe, scoopula)
Sorting equipment (fine forceps, Petri dishes, 12-cell plates, tally counters)
ABMI Lab Protocols Manual
Marchant box
Scale (1 per lab)
Storage vials & stoppers
Ethanol & wash bottles
2.80 mm & 500 μm sieves
Air stones, tubing & pump
Coarse screen recovery box (modified litter pan)
4 L & 10 L buckets
Plastic pans & Ice cube trays
Administrative tools (pencils, scissors, vial labels etc.)
Lap top Computer with Microsoft Excel, and Firefox or Google Chrome web browser
Refrigerator (1 per five sorters)

Supervision of Aquatic Invertebrate Sorting

- A qualified lab supervisor oversees all stages of training, specimen picking and sorting by lab technicians.
- To be classified as a qualified lab supervisor, the person must have:
  1. More than 1 years' experience identifying aquatic invertebrates found in Alberta.
  2. Worked with the RAM appointed aquatic invertebrate expert for at least two days to ensure that the invertebrate sorting will be effective for the expert.
  3. Successfully completed an exam by identifying representative specimens from the coarse groups of aquatic invertebrates that are sorted for the ABMI (Appendix4). The exam consists of at least 100 specimens (with at least one specimen from each of the 33 taxonomic groups). More than 95% of the specimens on the test must be identified correctly.

Specimen Elutriation

- Ensure that all bottles collected at the ABMI site are present.
- Pour and rinse each sample bottle through a 500 μm sieve. Deposit the sieved material into a 10 L bucket and dump the used preservative into the waste solution container.
- When the last bottle from the site has been sieved, thoroughly rinse the sieve into the bucket to create a composite sample.
- For ABMI sites that contain large quantities of sediment, it is more efficient to process a few bottles at a time rather than trying to process them all at once.
- If all sample bottles from a site contain minimal vegetation or sediment, the samples can simply be rinsed through the 500 μm sieve without going through the complete elutriation process.
- Set up the elutriation components (see Figure 1 & 2).
- Run the elutriation process starting with a gentle flow, picking out and "washing" any coarse debris in the sorting/washing pan; save the coarse debris in a separate 4 L pail. Air stones are used in the 10 L bucket and/or the sorting/washing pan to help break up matted coarse debris and float organisms through the system. Run the elutriation process until the water is clear, or all of the coarse debris has been picked out & "washed".

- Periodically check the 500 μm sieve to make sure it does not become clogged and overflow in the sink. If the sieve is getting plugged, gently rinse as much fine material through the sieve as you can, and deposit the remaining material in the Marchant box before continuing with the elutriation process.
- Collect three 25 gram sub-samples from different locations throughout the sediment remaining in the 10 L bucket and/or coarse debris in the 4 L pail. If both types of "sediment" are present, select a total of three sub-samples based on the proportion of each type present.
- If >30 invertebrates are seen in the combined three sub-samples, repeat the above two steps.
- If <30 invertebrates are seen in the combined three sub-samples (and this has been verified by the lab supervisor), place all "sediment" into 1 L or 250 ml sample bottles (containing ~80% ethanol) and labelled with the RAM lot number, ABMI site number, number of bottles, and the word "sediment", before proceeding to the sorting phase.

Quality Control

- After lab technicians have completed elutriation for an ABMI site and checked the sediment for completeness, they submit the sediment sub-samples to the lab supervisor for inspection.
- The lab supervisor examines the "sediment" under a dissecting microscope (for exactly 2 minutes) and tallies the number of invertebrates seen.
- If the total number of invertebrates observed in the combined three sub-samples is >30 or more, lab technicians are instructed to repeat the elutriation process.

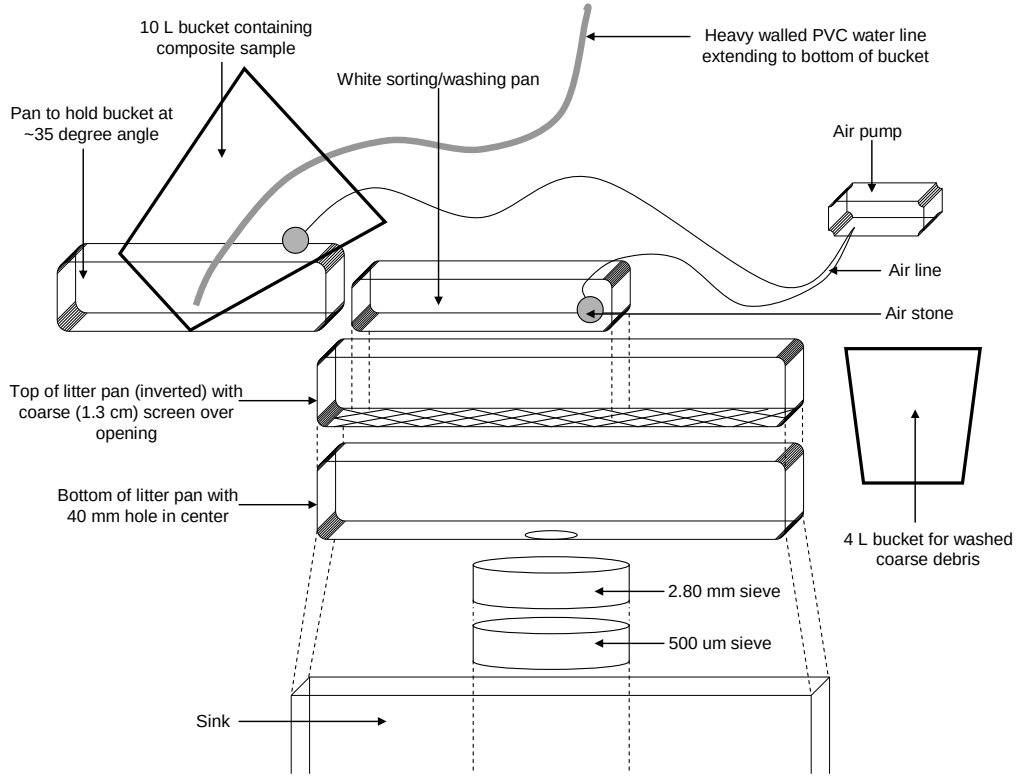

Figure 1: Schematic of elutriation components.

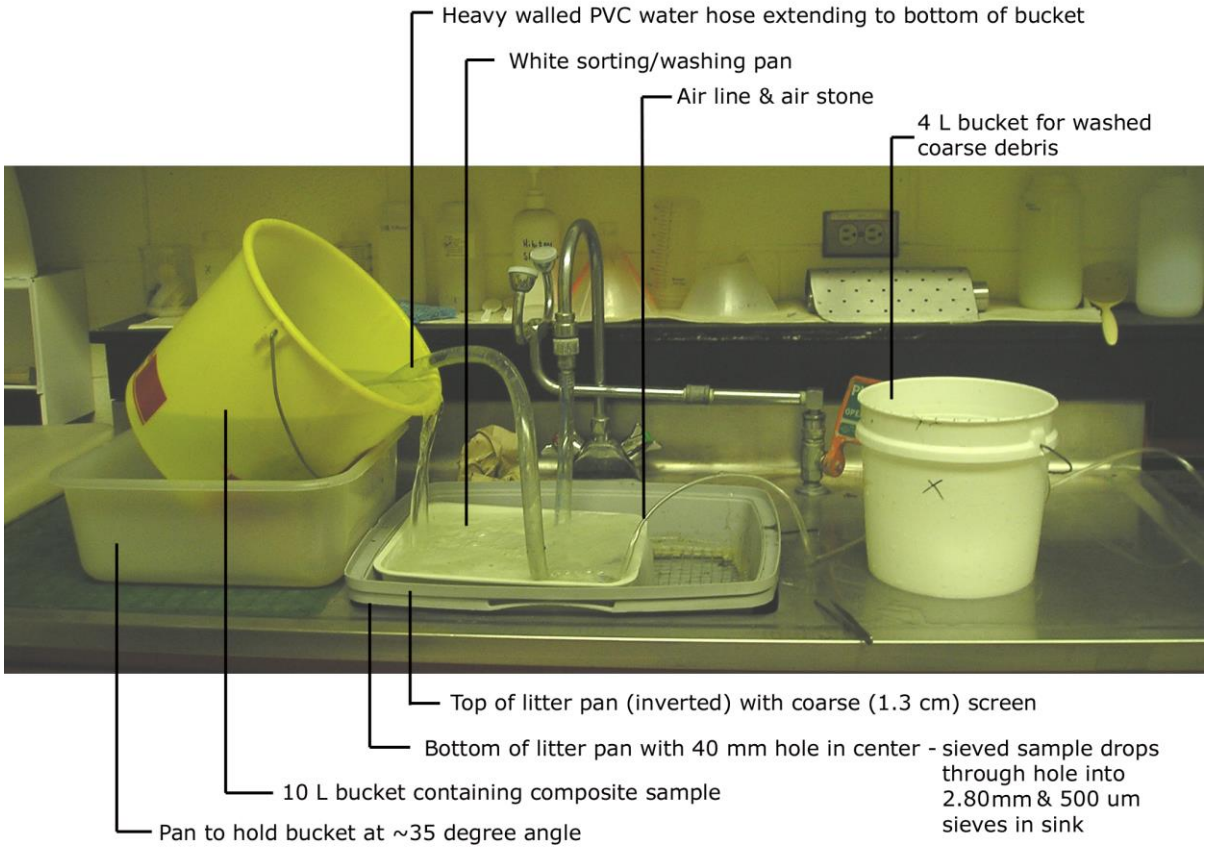

Figure 2: Photo of elutriation components.

Sorting to Taxonomic Groups

- Rinse the elutriated material from both the 2.80 mm and 500 µm sieves into a Marchant box and add enough water to just fill each of the 100 separate cells.
- Ensure that all sieves and elutriation components are completely clean before continuing with the sorting process.
- Clamp down the lid of the Marchant box, carefully invert the box, and gently swirl the water and elutriated material.
- Quickly and smoothly flip the box upright, and agitate gently to allow the material to settle into the cells.
- If the distribution of material in the Marchant box cells appears to be uneven, repeat the above two steps.
- Remove the lid and carefully inspect it to make sure that no organisms are adhering to the lid or caught in the seal. Rinse any trapped organisms into random cells of the Marchant box.
- Organisms that have not settled into a specific cell are considered to be in the cell over which the majority of the organism is located.
- Vegetation hanging over the wall of a cell is carefully cut along the cell wall so that the portions over each cell remain in the appropriate cell.
- Using the random number generated by the data portal select the corresponding cell in the Marchant box, and transfer the contents of that cell to a Petri dish. Be careful to ensure that all specimens are transferred from the cell to the Petri dish.
- Most aquatic invertebrate taxa encountered in the sample are classified as primary organisms (Table 1).
- Specimens from the groups *Porifera*, *Hydrozoa*, *Nematoda*, *Platyhelminthes*, *Cladocera*, *Ostracoda*, and *Copepoda* are classified as secondary organisms (Table 1). These taxa are not considered part of the macro-

invertebrate community and can be very prolific in some samples. If they were included as part of the 350 primary organisms few specimens from the target taxa would be sorted.

- The goal is to collect and sort at least 300 primary organisms that can be identified to genus/species, from each ABMI site. Since a portion of the sorted organisms may be immature or missing key features needed to identify them to genus/species, and these key features are not completely understood by sorting staff, a total of 350 undamaged primary specimens are sorted.

Table 1: Macro invertebrate taxa categorized based on whether they are primary or secondary organisms. Some primary organisms are sent to experts for advanced identification.

Primary Organisms	**Secondary Organisms**
Oligochaeta (aquatic worms)	Porifera (sponges)
Hirudinea (leeches)	Hydrozoa (hydras)
Gastropoda (snails & limpets)	Platyhelminthes (flatworms)
Bivalvia (clams)	Nematoda (roundworms)
Hydrachnida (aquatic mites)	Cladocera (water fleas)
Amphipoda (scuds)	Ostracoda (seed shrimp)
Isopoda (sow bugs)	Copepoda (copepods)
Decapoda (crayfish)	
Ephemeroptera (mayflies)	
Anisoptera (dragonflies)	
Zygoptera (damselflies)	
Plecoptera (stoneflies)	
Hemiptera (true bugs)	
Megaloptera (fishflies, alderflies)	
Lepidoptera (aquatic moths)	
Trichoptera (caddisflies)	
Coleoptera (beetle adult)	
Coleoptera (beetle larva)	
Chironomidae (midges)	
Ceratopogonidae (no-see-ums)	
Tabanidae (horse flies)	
Tipulidae (crane flies)	
Culicidae (mosquitoes)	
Chaoboridae (phantom midges)	
Simulidae (black flies)	
Other Diptera (true flies)	

- Using a 10-40x microscope with a cold (fiber-optic) light source, systematically sort through the contents of the Petri dish.
- Using tweezers transfer undamaged primary organisms into separate holding containers, sorting the specimens to the degree outlined in Appendix 4: Coarse Level Identification Guide for Aquatic Invertebrates.
- Secondary organisms are left in the residue and are not counted.
- Pupae, exuvia, terrestrial organisms, empty shells, and empty Trichoptera cases are left in the residue and are not counted.
- Damaged primary organisms are placed in a separate holding container and tallied at the completion of each cell. Record the number of damaged organisms in the data portal. Organisms are considered damaged if:
  - One or more major body regions (i.e. head, thorax or abdomen) are missing, or
  - >50% of a body region (excluding appendages) is mangled or broken off , or
  - >25% of the shell (if present) is broken.

- Once the contents of the Petri dish have been completely searched, tally the total number of undamaged primary organisms in each coarse group and record the number of individuals in the data portal.
- Submit the residue and identified organisms to the lab supervisor for verification by clicking on the "Cell Complete" button in the data portal. If QA is required, notify the lab supervisor. If no QA is required, continue with sorting.
- Transfer the sorted organisms into vials filled with 80% ethanol. Ensure that specimens are not "lost" during this transfer process.
- Transfer the remaining material in the petri dish to a separate holding container (all subsequent petri dish residues are combined in this same container and held until the completion of the site).
- If fewer than 350 undamaged primary organisms have been sorted, then select the next random cell from the Marchant box and process that cell. Continue this process until 350 undamaged primary organisms, or all 100 cells in the Marchant box, have been sorted.
- A minimum of 3 Marchant box cells must be sorted for each site even if 350 primary organisms are sorted within the first or second cell.
- If a total of 350 undamaged primary organisms are reached in the middle of sorting a cell, complete that cell before stopping.
- Note that some wetlands are dominated by secondary organisms; samples from these sites may contain less than 350 primary organisms even after sorting all 100 cells.
- If after sorting at least 3 cells it is apparent that there will be less than 350 primary organisms in the entire site, and the amount of sediment in the sample will allow it, the entire contents of the Marchant box can be transferred to a small plastic tray and the remainder of the site sorted in its entirety.
- Label the vials by inserting a waterproof label containing information on the coarse taxonomic group name, RAM lot number, ABMI site number, date collected, collector's name, and sorters' name.
- The remaining contents of each Petri dish are combined in a single a 250 ml bottle or 20 ml vial, preserved in ~80% ethanol, and labelled with the RAM lot number, ABMI site number and the word "Residue".

Unique/Mature Organism Search

- If at the conclusion of the sorting process there is still unsorted sample material in the Marchant box, conduct a unique/mature organism search on the remaining unsorted sample.
- Transfer the remaining contents of the Marchant Box to a shallow white plastic pan.
- Ensure the pan is located where there is good lighting.
- Set a timer for exactly 2 minutes – start the timer and begin visually searching the sample (i.e. without the use of a microscope) for any unique/mature primary organisms.
- Transfer any unique/mature primary organisms to a separate holding container – they do not need to be separated into coarse groups.
- Throughout the searching process the focus is on picking as many unique/mature primary organisms as possible within the time allotted, while adhering to the following criteria:
    - Pick only target taxa from the following groups: Gastropoda, Ephemeroptera, Anisoptera, Zygoptera, Plecoptera, Hemiptera, Trichoptera, Coleoptera (adults), and Coleoptera (larva).
    - Pick unique organisms that do not appear to be similar to other organisms that have already been sorted from the sample (look at both large and small organisms).
    - Pick mature organisms that appear to be similar to other organisms that have been sorted from the sample but have well developed wing pads, aquatic adult characters, and/or are much larger
    - Note: it is permissible to pick more than one example of each unique/mature organism, but do not become focused solely on that particular organism.
- If all of the unique/mature organisms appear to have been picked before the 2 minute time period has expired, continue to search the sample until the full 2 minutes has elapsed
- At the conclusion of the unique/mature organism search have the lab supervisor inspect the pan and picked material for quality control.
- Transfer all of the picked organisms to a single sample container filled with 80% ethanol. Label this container with the RAM lot number, ABMI site number and the words "UMOS Specimens".

- Once the ABMI site has been completed, the remainder of the unsorted sample is preserved in a 1 L or 250 ml bottle (containing ~80% ethanol) and labelled with the RAM lot number, ABMI site number and the words "Sieved Sample".
- Before proceeding to the next ABMI site, ensure that the Marchant box, lid, and large plastic pan have been completely cleaned.

Week 1: Training

Day 1: Goals and Expectations

- Lab technicians are familiar with the lab layout and equipment, and understand lab safety rules and protocols
- Lab technicians have a basic understanding of specimen extraction using the elutriation technique and are able to set up and run the process with minimal assistance.
- Lab technicians are familiar with the layout and content of the Appendix 4: Coarse Level Identification Guide for Aquatic Invertebrates, and have basic search images of the target organisms.
- By the end of day 1, each lab technician will have taken their first ABMI site to at least the end of the specimen extraction process.

Training:

Lab Safety

1. Review lab safety protocols

Specimen Extraction

1. Read Sample Processing (Pages 8-16 in this manual)
2. Watch a demonstration of the elutriation process
3. Run an elutriation on their first ABMI site

Aquatic Invertebrate Sorting and Identification

1. Read Appendix 4: Coarse Level Identification Guide For Aquatic Invertebrates

Quality Control

- The lab supervisor monitors the elutriation process as the technicians process their site.
- The lab supervisor inspects the sediment to ensure it meets quality control targets.
- The lab supervisor inspects Marchant box contents prior to cleaning up for the day.

Days 2-3: Goals and Expectations

- Lab technicians have a basic understanding of proper specimen picking and handling techniques, data recording and labelling methods, and the criteria for not counting organisms based on damage or level of development (i.e. pupae, exuvia etc.).
- Lab technicians know how to complete a UMOS search.
- By the end of day 3 each lab technician will have completed sorting their first ABMI site.

Training:

Aquatic Invertebrate Sorting and Identification

1. Watch presentation on identifying coarse taxonomic groups.
2. Watch demonstration on sorting techniques and data entry.
3. Watch demonstration on the UMOS search

Quality Control

- After picking and sorting specimens from a cell in the Marchant box, lab technicians submit the picked residue, and identified organisms from that cell to the lab supervisor for quality control.
- The first two cells from each site and the last cell are always inspected for quality control. Interim cells are randomly selected (1:3) for inspection by the data portal (note that if QA inspection is required the data portal will not allow the lab technicians to proceed to the next cell until the lab supervisor inspects and approves the data from that cell).
- The lab supervisor inspects the residue and organisms to evaluate whether ABMI accuracy targets have been achieved. These targets are:
    - at least 95% of the total primary organisms have been picked from each cell, and
    - at least 95% of the picked organisms have been correctly identified.

Days 4-5: Goals and Expectations

- Lab technicians are more familiar with specimen extraction using the elutriation technique and are able to set up and run the process unassisted.
- Lab technicians are more familiar with handling and picking aquatic invertebrates and data recording, to the extent that they are able to perform these tasks unassisted.
- Lab technicians are able to label bottles and vials in the required format.
- Through inspections by the lab supervisor, lab technicians will pick and sort aquatic invertebrates such that < 5% of the total primary organisms remains in the picked residue of each cell, and at least 95% of the picked organisms are correctly identified.
- By the end of day 5, each lab technician should have completed their second ABMI site.

Training:

Aquatic Invertebrate Sorting and Identification

1. Sort through the material from each cell (as describe above) in a systematic manner
2. Identify picked aquatic invertebrates using:
    - Appendix 4: Coarse Level Identification Guide For Aquatic Invertebrates
    - Collaboration with the lab supervisor and more knowledgeable colleagues
3. Submit picked residue and identified organisms to the lab supervisor for quality control prior to moving on to the next cell (see Quality Control Process)

Quality Control

- After picking and sorting specimens from of a cell in the Marchant box, lab technicians submit the picked residue, and identified organisms from that cell, to the lab supervisor for quality control.
- The first two cells from each site and the last cell are always inspected for quality control. Interim cells are randomly selected (1:3) for inspection by the data portal (note that if QA inspection is required the data portal will not allow the lab technicians to proceed to the next cell until the lab supervisor inspects and approves the data from that cell).
- The lab supervisor inspects the residue and organisms to evaluate whether ABMI accuracy targets have been achieved. These targets are:
    - at least 95% of the total primary organisms have been picked from each cell, and
    - at least 95% of the picked organisms have been correctly identified.
- If at any time a cell does not meet ABMI accuracy targets (i.e., field staff that have >5% error), then the cell in question, and all of cells they have completed subsequent to the previous successful inspection, are re-worked by the technician.

Week 2: Training

Goals and Expectations

- Sort through at least 3 more ABMI sites and identify aquatic invertebrates to the 33-group coarse level.
- Sort specimens such that <5% of the total primary organisms remain in the picked residue of each cell, with at least 95% accuracy of specimen identification.
- Submit picked residue and identified organisms to the lab supervisor for quality control prior to moving on to the next cell (see Quality Control Process).
- By the end of week 2, each ABMI field staff member should have completed the sorting and identification on at least 5 ABMI sites.

Quality Control

- After picking and sorting specimens from of a cell in the Marchant box, lab technicians submit the picked residue, and identified organisms from that cell, to the lab supervisor for quality control.
- The lab supervisor inspects the residue and organisms to evaluate whether ABMI accuracy targets have been achieved. These targets are:
    - at least 95% of the total primary organisms have been picked from each cell, and
    - at least 95% of the picked organisms have been correctly identified.
- The first two cells from each site and the last cell are always inspected for quality control. Interim cells are randomly selected (1:5) for inspection by the data portal (note that if QA inspection is required the data portal will not allow the lab technicians to proceed to the next cell until the lab supervisor inspects and approves the data from that cell).
- If at any time a cell does not meets ABMI accuracy targets (i.e., lab technicians that have >5% error), then the cell in question, and all of cells they have completed subsequent to the previous successful inspection, are re-worked by the technician.

Weeks 3 & 4:

Goals and Expectations

- Sort and identify aquatic invertebrates to the 33-group coarse level at a rate of 1 ABMI site/day.
- Sort specimens such that < 5% of the total primary organisms remain in the picked residue of each cell, with at least 95% accuracy of specimen identification.
- Submit picked residue and identified organisms to the lab supervisor for quality control prior to moving on to the next cell (see Quality Control Process).
- By the end of week 3, each lab technician should have completed the sorting and identification for at least 10 ABMI sites.
- By the end of week 4, each lab technician should have completed the sorting and identification on at least 15 ABMI sites.

Quality Control

- After picking and sorting specimens from of a cell in the Marchant box, lab technicians submit the picked residue, and identified organisms from that cell, to the lab supervisor for quality control.
- The lab supervisor inspects the residue and organisms to evaluate whether ABMI accuracy targets have been achieved. These targets are:
    - at least 95% of the total primary organisms have been picked from each cell, and
    - at least 95% of the picked organisms have been correctly identified.
- The first two cells from each site and the last cell are always inspected for quality control. Interim cells are randomly selected (1:10) for inspection by the data portal (note that if QA inspection is

required the data portal will not allow the lab technicians to proceed to the next cell until the lab supervisor inspects and approves the data from that cell).

- If at any time a cell does not meets ABMI accuracy targets (i.e., lab technicians that have >5% error), then the cell in question, and all of cells they have completed subsequent to the previous successful inspection, are re-worked by the technician.

Taxonomic Nomenclature

Taxonomy for insect arthropod coarse groups follows Merritt & Cummins (2008). Taxonomy for non-insect arthropod and non-arthropod coarse groups follows Thorp & Covich (2010).

Advanced Identification of Specimens

Three taxonomic groups from rivers (*Ephemeroptera, Plecoptera, Trichoptera,)*, and eight taxonomic groups from wetlands (*Gastropoda, Ephemeroptera, Anisoptera, Zygoptera, Trichoptera, Hemiptera, Coleoptera, and Chironomidae)* are sent to an expert aquatic invertebrate taxonomist for identification to the lowest taxonomic level possible.

Selecting the Expert

- The ABMI will select experts who are known specialists in the field of aquatic invertebrate taxonomy. To ensure the highest of standards, and to maintain ABMI's level of credibility, the ABMI will only select experts who can meet at least one of the following criteria:
  i. Expert is endorsed by the Royal Alberta Museum, or an associated museum (e.g.., Canadian Museum of Nature), as capable of identifying aquatic invertebrates with ≥95% accuracy.
  ii. Expert is endorsed by 2 members of the scientific community, recognized in the field of aquatic invertebrate taxonomy, as capable of identifying aquatic invertebrates with ≥95% accuracy.
- In addition, expert aquatic invertebrate taxonomists require level 2 (to genus) taxonomic certification by the Society for Freshwater Science (SFS – formerly NABS) for the taxonomic groups they will be identifying.

Identifying the Aquatic Invertebrate Specimens

- All specimens are to be identified to the lowest taxonomic level possible. Species names must be determined based on the Species References/Authorities listed below.
- RAM maintains the taxonomic keys, and if there is discrepancy between keys determines their order of precedence.
- If additional reference literature is needed to determine the species name, the expert will note this additional literature in the database.
- Specimens must be identified with ≥95% accuracy.
- Whenever possible, specimens are to be identified to species.
- Specimens from each sample vial are examined, identified, and the species name written directly on the back of the original label (or a separate slip of paper) along with the identification date and expert's initials, and inserted into the vial.
- If more than one taxonomic group is present in a sample vial, a new vial is created for each additional group and labeled with the ABMI site number, species name, identification date, and expert's initials.
- Isolate a voucher specimen for every unique species/taxon identified, and label the new vial as indicated above with the word "Voucher" on the label.
- Experts will also enter all required information into the ABMI on-line aquatic invertebrate data portal (Appendix 3).
- Specimens from the unique/mature organism search are maintained in separate vials and entered into the data portal using the UMOS category.

Identifying Chironomid Specimens

The identification of Chironomid specimens can be a time consuming process that involves additional steps to complete. To be fully confident in the identifications, all specimens need to be slide mounted; this involves clearing specimens, slide mounting, slide drying, and labeling/organizing of individual slide mounted specimens. In addition, the number of Chironomid specimens in each sample often outnumbers all other invertebrate specimens combined. Although the average Chironomid count for ABMI sites is around 100 specimens per site, many sites produce several hundred Chironomid specimens. For these reasons most monitoring programs do not identify Chironomids beyond the family level, if at all. To keep the identification of Chironomid specimens manageable the ABMI subsamples Chironomid specimens for any site where the sorting count exceeds 100.

Subsampling Chironomids

- Sites where the number of sorted Chironomid specimens exceeds 100 are subsampled using a ratio of the original number of Marchant box cells sorted so that the total number of specimens identified is as close to 100 as possible without going over [e.g. if 312 Chironomid specimens were sorted in 29 Marchant box cells we would subsample to 97 Chironomid specimens, and enter the subsampled Marchant box cell count as 9 (312/29x9 = 97)].
- Subsampling is done by placing all of the sorted Chironomids in a petri dish with a 98 cell numbered grid underneath. A random number table is used to select the first grid location and all of the Chironomid specimens that fall within this grid are removed for slide mounting. If all Chironomid specimens are removed from the first grid location, and the total specimens needed has not been reached, a second grid location is selected. The process continues until the desired number of subsampled specimens has been reached.
- Specimens destined for slide mounting are cleared in 85% lactic acid for 24 hours.
- Specimens are slide mounted directly from the lactic acid into PVLG mounting medium and dried for at least 6 weeks at 55-60°C.
    - During week 1 slides are checked daily for air being drawn under the cover slip as the mounting medium dries and shrinks. Aid bubbles are filled from the edge of the cover slip using a micropipette.
    - During week 2 slides are checked for air bubbles every second day.
    - At the end of the second week all slides are given a final ring coat of mounting medium.
    - During weeks 3-6 slides are periodically rotated in the drying oven to prevent hot spots causing discoloration.
- Dried slides from each site are placed in a slide box for storage until Advanced ID.

Advanced ID of Chironomids

- Chironomid specimens are first identified to the genus level using Merritt and Cummins (2008) and Epler (2001).
- Genera that do not match descriptions in either key are given a number designation for each distinct morphotype and the features that distinguish them are added to the keys. Unknown genera are recorded in the database using their number designations until such time as the actual identity can be worked out (e.g. Genus 1 RPH, Genus 2 RPH, Genus 3 RPH, etc.).
- A second round of ID is used to identify each genus from each site to species or morphospecies using recent primary literature and internally developed keys.
- Morphospecies that are thought to represent a species, or group of species, are given letter designations within each genus and the features that distinguish them are added to the keys. Morphospecies are recorded in the database using their letter designations until such time as the actual identity can be worked out (e.g. *Cricotopus sp.* E RPH, *Ablabesmyia sp.* E RPH, *Psectrocladius sp.* E RPH, etc.).
- A voucher of each species or morphotype is isolated from each site and maintained in the ABMI Chironomid voucher collection.

Verification Process

- Specimens that have been identified by experts will undergo a verification process by their peers to ensure accuracy.
- For each expert identifying ABMI aquatic invertebrates, 10% of the identified specimens (up to a maximum of 200) will be randomly selected for verification. Note that at least one randomly selected specimen from each species (or higher taxonomic group if the specimens are not identified to species) will be included.
- The ABMI lab coordinator will re-label each specimen with a reference number and send the specimens to a second expert that meets the above credibility criteria.
- The second expert will identify the specimens and record the species name beside the matching reference number on a provided data sheet.
- The second expert will ship the specimens back to the ABMI, and email the data sheet to the ABMI lab coordinator.
- The ABMI lab coordinator will compare the data between the two experts.
- Discrepancies are reviewed by both experts (plus additional experts if necessary) to determine the identification based on the most recent literature. If a discrepancy cannot be resolved, the specimen in question will be recorded in the database at the lowest taxonomic level that is agreed upon by the experts.
- If, after all discrepancies have been resolved, there is ≥5% error on the part of the initial taxonomic expert, then the genera/species with ≥5% mis-identifications are highlighted. All individuals the initial expert identified from the highlighted species are re-identified to confirm their identity.

Specimen Storage

- All specimens are stored in the vials for 2 years.
- After 2 years, all specimens are given to the Royal Alberta Museum.
- The ABMI will retain vouchers and enough reference specimens of each species plus additional specimens for training purposes.

Species References/Authorities

Taxonomy for Anisoptera follows Merritt and Cummins (2008), and Needham *et al.* (2000). Taxonomy for Chironomidae follows Merritt and Cummins (2008) and Epler (2001). Taxonomy for Coleoptera (Dytiscidae) follows Larson et al. (2000). Taxonomy of Coleoptera (*Gyrinus*) follows Oygur and Wolf (1991). Taxonomy for Coleoptera (Hydrophilidae) follows Smetana (1988). Taxonomy for Ephemeroptera (Caenis) follows Provonsha 1990. Taxonomy for all other Coleoptera and Ephemeroptera follows Merritt and Cummins (2008). Taxonomy for Gastropoda follows Clarke (1981) and Johnson *et al.* (2013) . Taxonomy for Plecoptera follows Stewart and Oswood (2006). Taxonomy for Trichoptera follows Wiggins (1998). Taxonomy for Zygoptera follows Westfall and May (2006).

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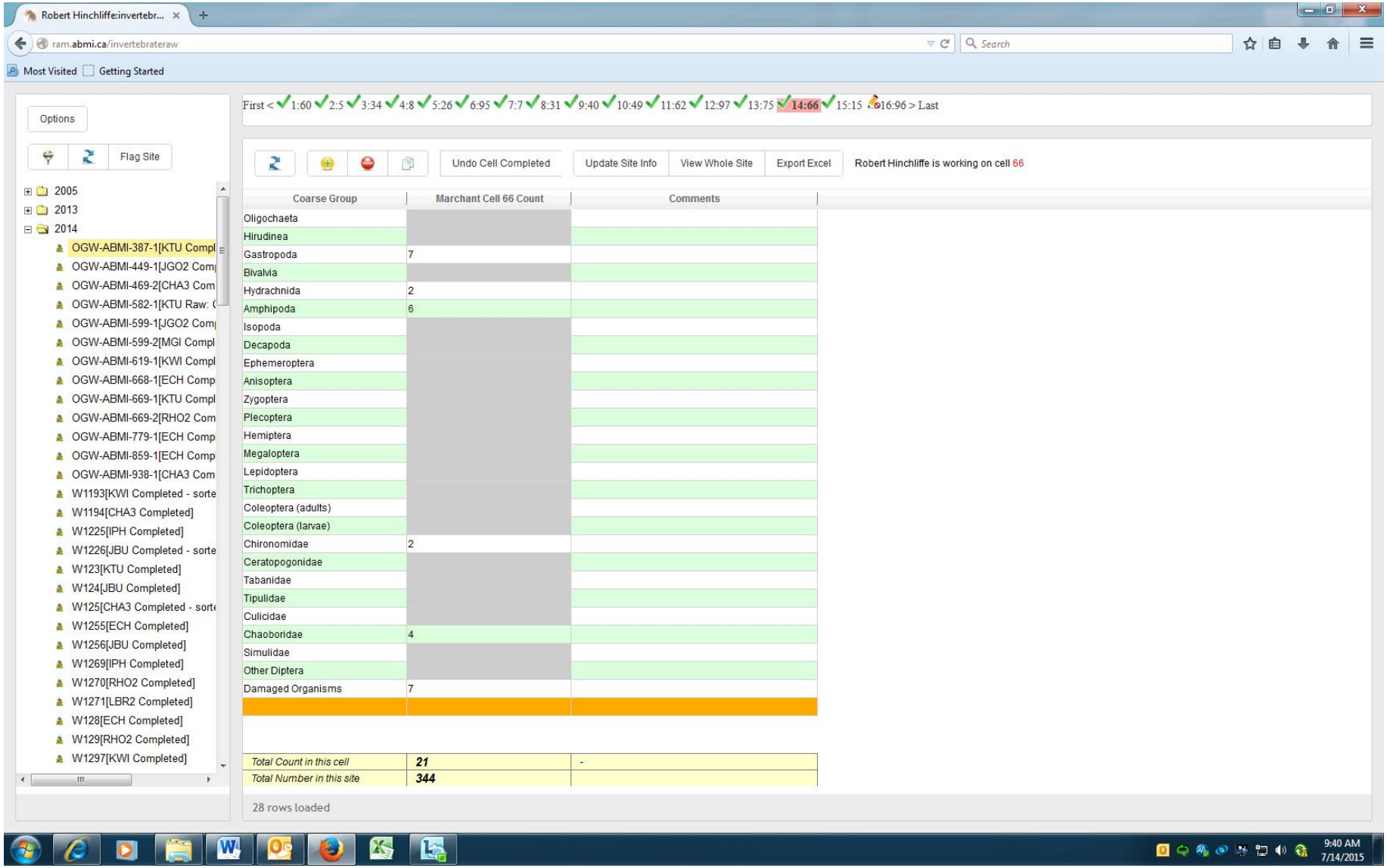
Appendix 1: On-line Data Portal Used By Field Staff During Picking And Sorting



Appendix 2: Aquatic Invertebrate Sorting Excel Spread-sheet

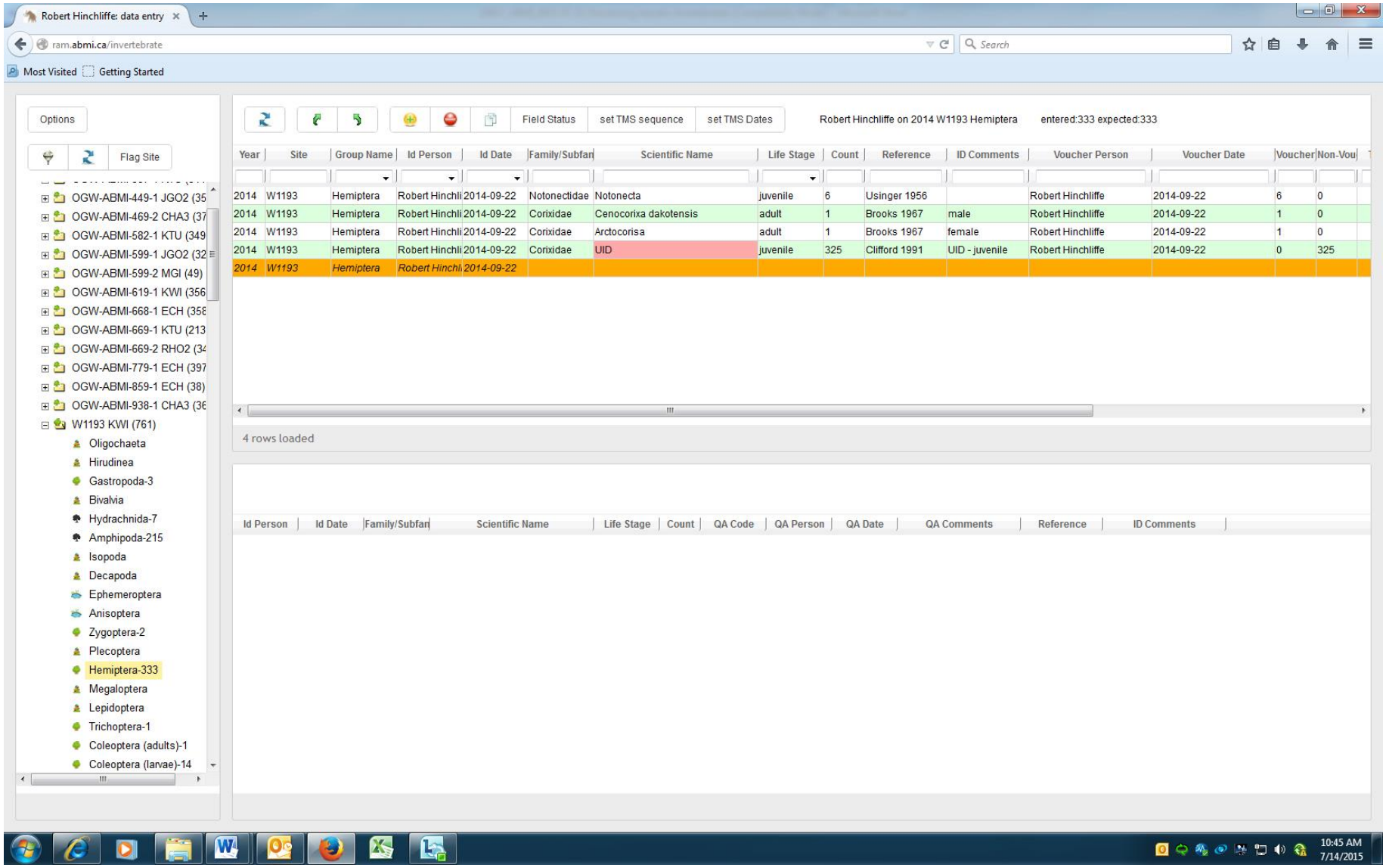
Alberta Biodiversity Monitoring Institute
Date Sorted:_____
Sorted by:_____

Aquatic Invertebrates
Sorting to Groups

ABMI Site #:_____
Year:_____

Marchant Cell Number																	
	Total/Group																
Oligochaeta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hirudinea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gastropoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bivalvia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrachnida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphipoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isopoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Decapoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ephemeroptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anisoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zygoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Plecoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemiptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Megaloptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lepidoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coleoptera (adults)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coleoptera (larvae)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabanidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tipulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Culicidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaoboridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other Diptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Damaged Organisms	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Primary Organisms	**0**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Marchant Cells Counted	0																
Unique/Mature Search																	

Appendix 3: Advanced ID Data Entry



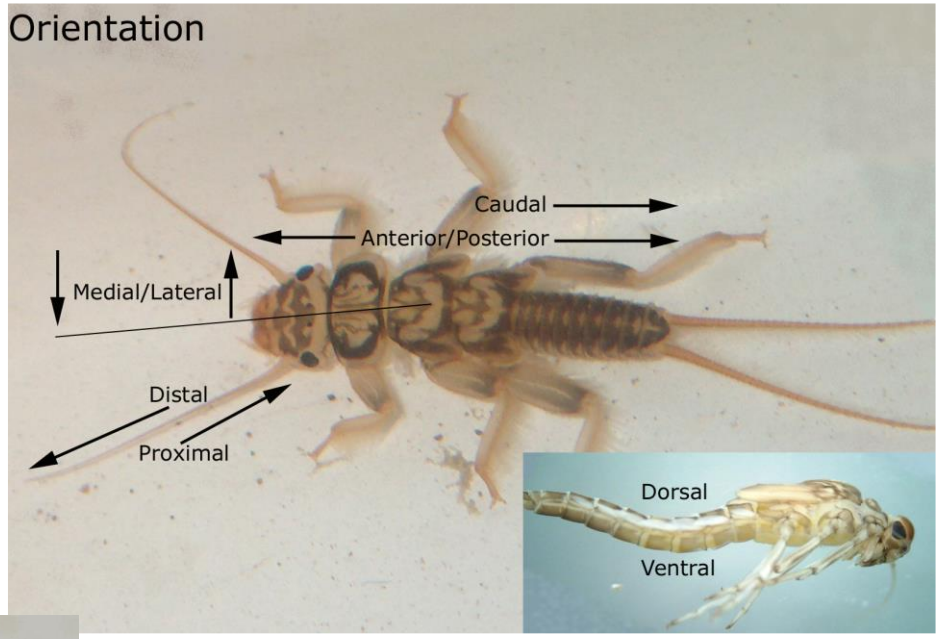
Appendix 4: Coarse Level Identification Guide for Aquatic Invertebrates

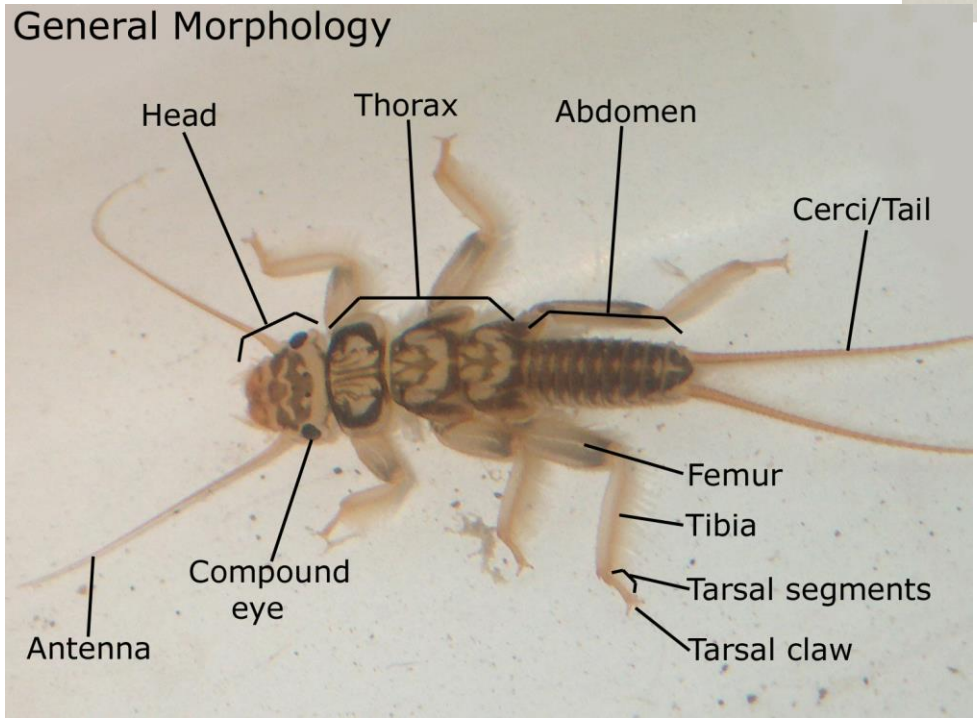
Aquatic Invertebrate Identification (coarse group level)

ABMI Training Manual

Modified from OBBN Training Course

This guide was modeled after the Bug Identification PowerPoint originally developed by Chris Jones and the Ontario Benthos Biomonitoring Network (obbn.eman-rese.ca). BIO-DiTRL and John R. Meyer provided permission to use their photos where indicated. Robert Hinchliffe created all other photographs in the guide. The guide has been reviewed and updated by Sue Salter (Cordillera Consulting).





Physical characteristics of 33 taxonomic groups are described in this guide. These groups were included because they can be separated accurately by people after a few days of training and practice. Characteristics listed in **blue** are especially important when separating the groups. Training and quality control required to maintain accuracy during identification are describe above.

List of 33 Coarse groups

Secondary Organisms

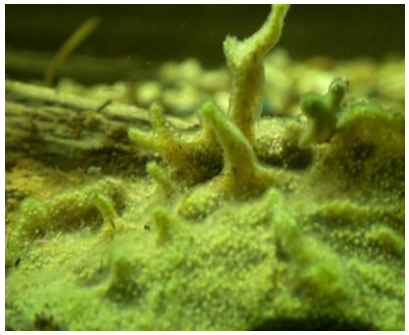
Primary Organisms

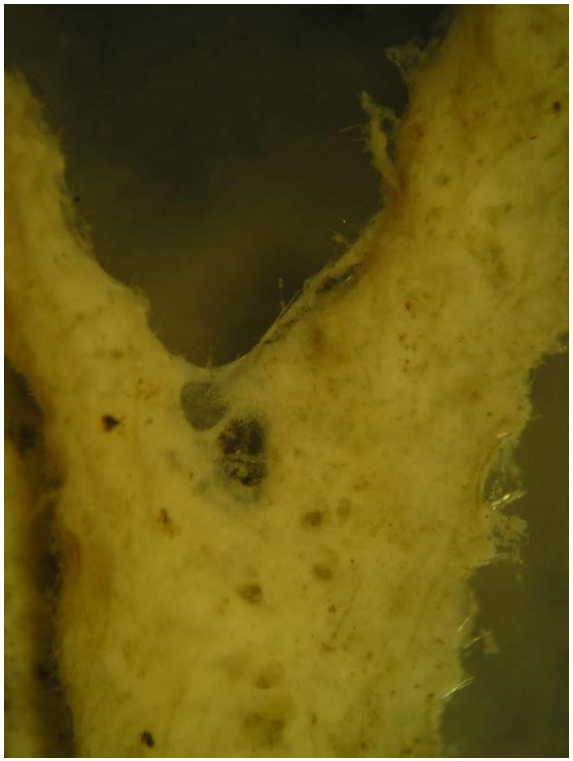
Phylum:

Porifera (Sponges)

"Secondary Organism"

- Morphology and size highly variable (some are small and inconspicuous)
- Color from tan to greenish
- Surface has many small pores; it looks like a sponge
- Usually attached to rocks or woody debris



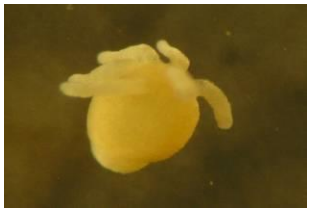


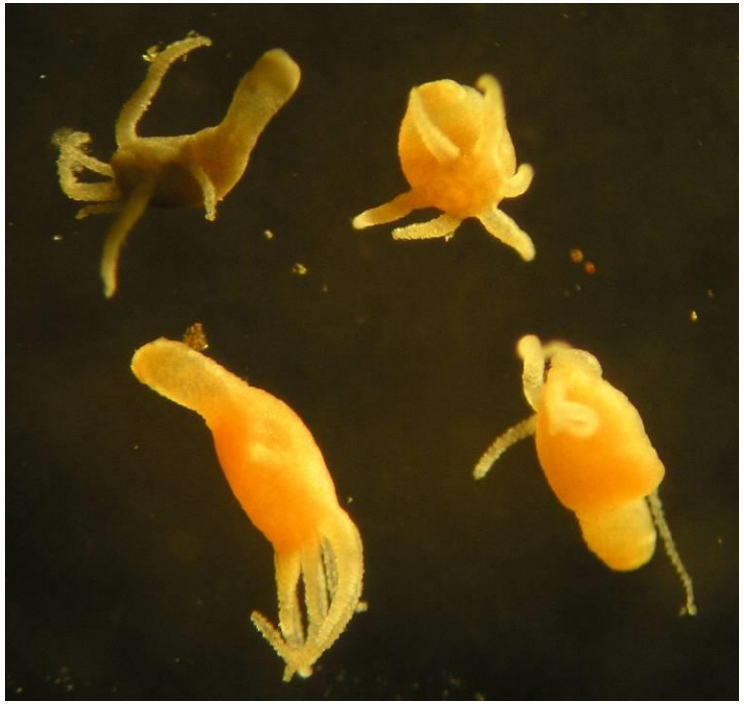
Class:

Hydrozoa (Hydras)

"Secondary Organism"

- 2 to 25 mm long (often inconspicuous)
- Clear to whitish; sometimes green
- Simple tube-like body with tentacles
- Asexual reproduction by budding
- Preserved specimens are often brown to orange in color & contracted into a ball





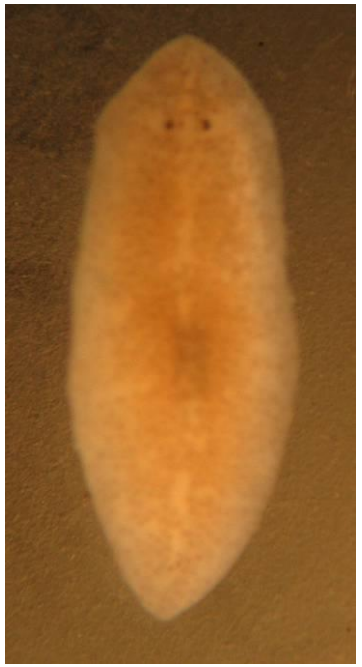
Phylum:

Platyhelminthes (Flatworms)

"Secondary Organism"

- 5 to 20 mm long
- Mottled cream to grayish-brown dorsally; lighter on ventral side
- Flat unsegmented wormlike body with no hairs or setae
- Usually with two simple eye spots but may have several small ones
- Mouth on ventral side; may have extended pharynx
- Preserved specimens may be contracted & curled up



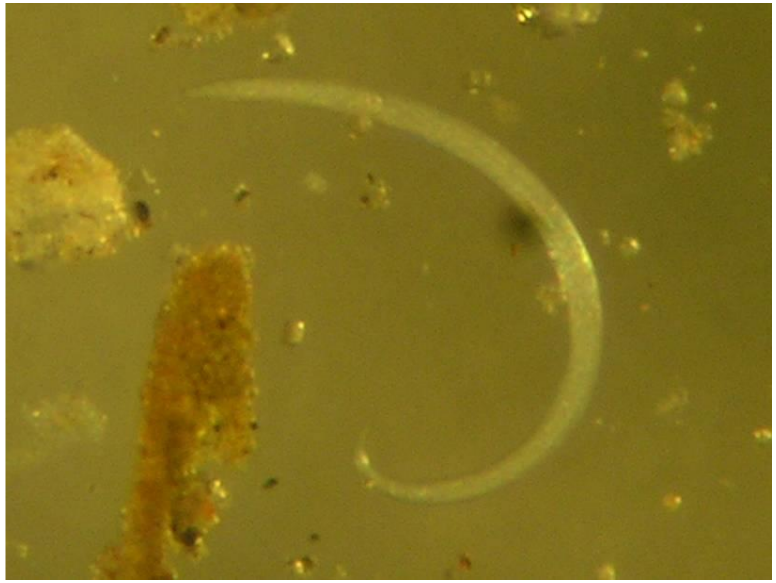



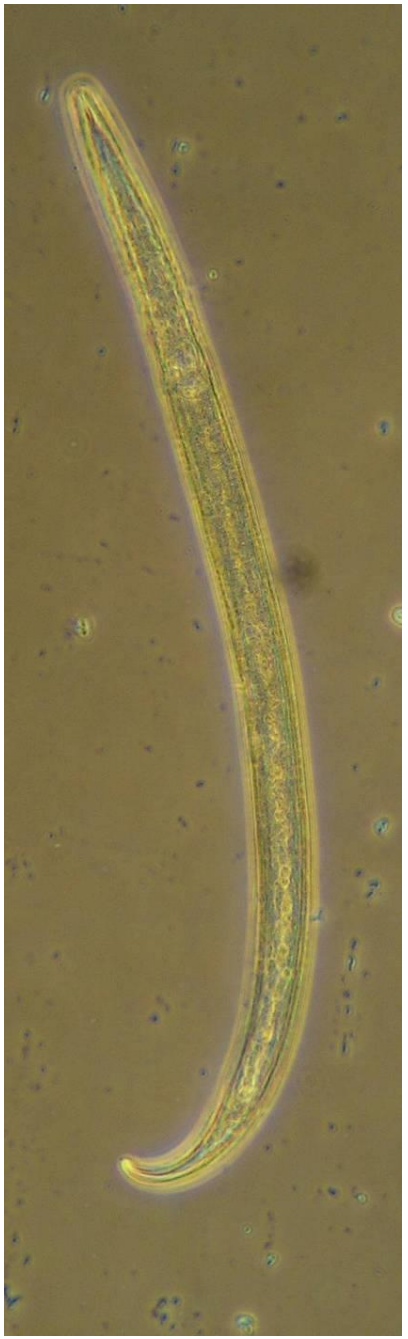
Phylum:

Nematoda (Roundworms)

"Secondary Organism"

- Often <10 mm long
- Variable coloration; body is often translucent
- Simple unsegmented wormlike body with no hairs or setae
- Head usually tapered, tail pointed



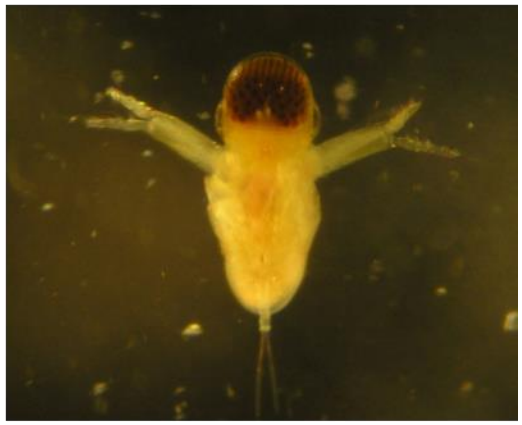


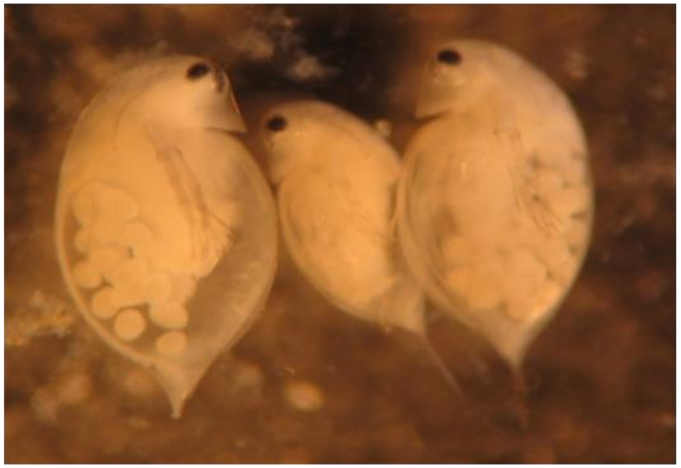
Group:

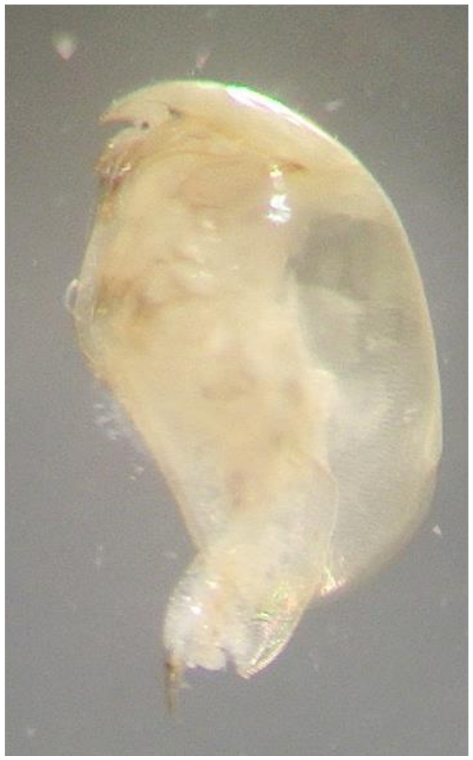
Cladocera (Water Fleas)

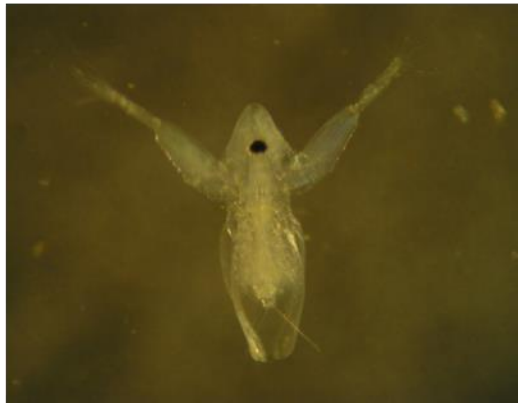
"Secondary Organism"

- Usually less than 10 mm
- Most of body (excluding head) enclosed in translucent, flexible bivalved carapace
- 5 or 6 pairs of thoracic appendages
- Usually a prominent pair of branched antennae
- Some may also be enclosed in a gelatinous case and/or lack a carapace







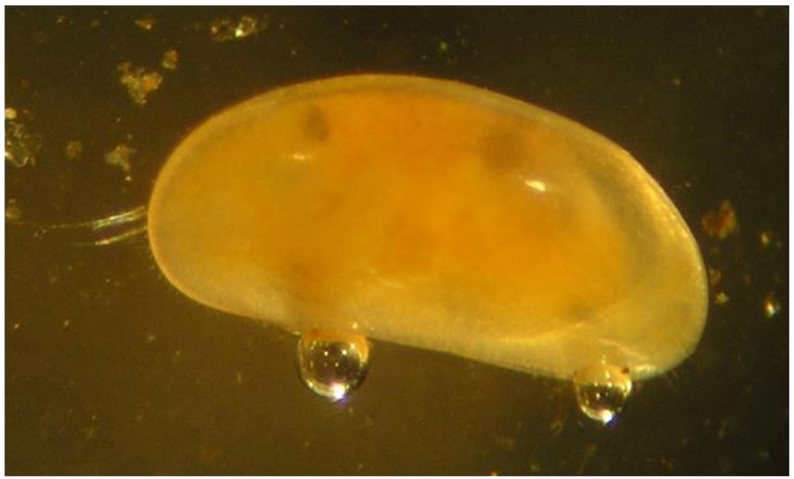


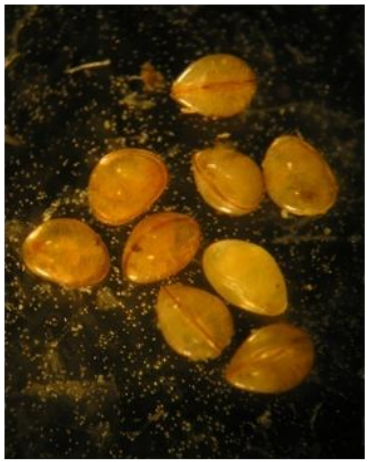
Subclass:

Ostracoda (Seed Shrimp)

"Secondary Organism"

- Usually less than 5 mm
- Color brown to translucent tan
- Entire body (including head) enclosed in a rigid, seed-like bivalved carapace
- 3 pairs of thoracic appendages that may partially protrude from shell
- Carapace without growth lines





Order:

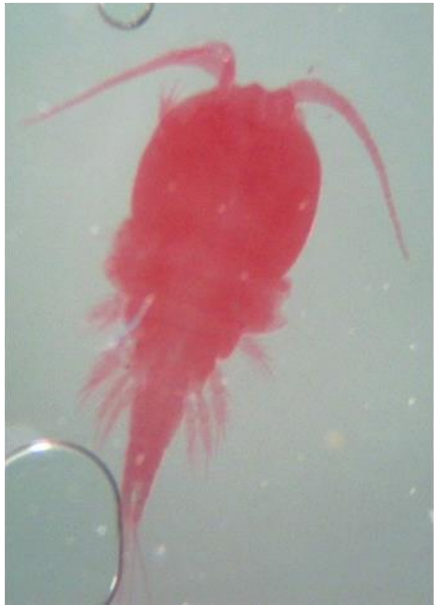
Copepoda (Copepods)

"Secondary Organism"

- Usually less than 5 mm
- Mostly cream to whitish in color
- Semi-cylindrical segmented body with 5 pairs of thoracic appendages and one pair of prominent antennae
- Paired caudal segments with terminal setae

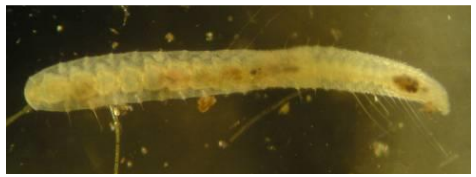




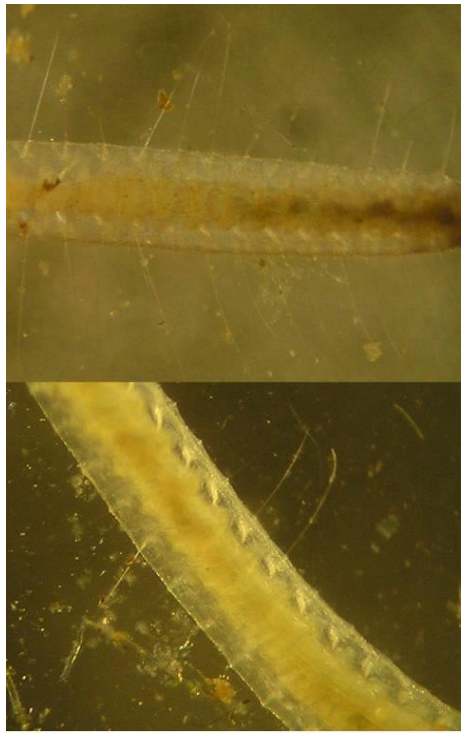



Subclass:

Oligochaeta (Aquatic Worms)



- Adults 1 to 30 mm long
- Often pinkish to white or transparent
- Wormlike segmented body with bundles of hairs or setae on each segment along the body
- No suckers present
- Usually no eyes but may have a pair of small stemmata and/or a proboscis



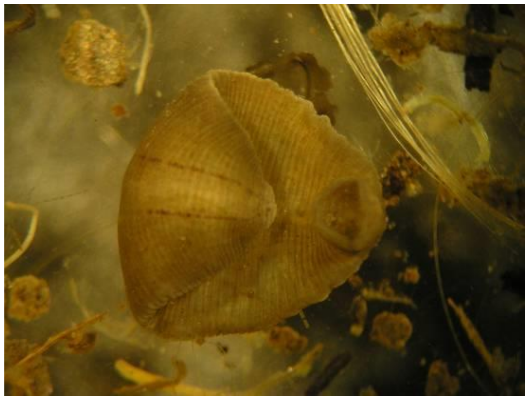
Subclass:

Hirudinea (Leeches)

- Adults 5 to 100 mm long
- Color varies; brown, olive and black common; may have color patterns on dorsal surface
- Flat segmented wormlike body with no hair or setae
- Suckers at anterior and posterior ends
- Head often with several pairs of eyes
- Preserved specimens are often contracted and curled






Class:

Gastropoda (Snails & Limpets)

- Adults 2 to 35 mm
- Hard spiral shell; some are saucer-like
- Watch for tiny specimens in bottom of dish
- Do not pick or count empty shells

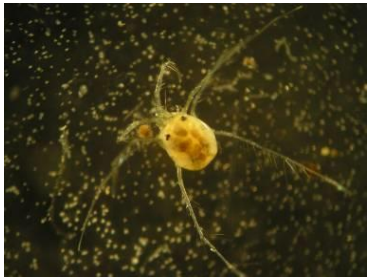
Class:

Bivalvia (Clams and Mussels)

- Adults 2 to 250 mm
- Color tan to brown
- Hard oval shell hinged in two halves with apparent growth lines
- No appendages
- Watch for tiny specimens at bottom of dish in sand or gravel
- Do not pick or count empty shells

Subcohort:

Hydrachnida (Mites)



- Adults 1 to 7 mm
- Often brightly colored (red, green, blue, brown)
- One body segment with 4 pairs of segmented legs; look similar to small spiders
- usually soft bodied but may have sclerotized plates
- Finger-like pedipalps between forelegs
- Simple eyespots and no antennae

Order:

Amphipoda (Scuds)

- Adults 6 to 20 mm long
- Usually a translucent green or olive brown; preserved specimens may be white to orange
- Laterally compressed and segmented body with 8 pairs of thoracic appendages
- 2 pairs of antennae

Order:

Isopoda (Sow Bugs)

- 5 to 20 mm long
- Alberta specimens are usually un-pigmented
- Dorso-ventrally compressed body with 7 pairs of legs ending in paired claws
- 1st antennae longer than 2nd

Order:

Decapoda (Crayfish)

- 10 to 100 mm long
- Tan to dark brown in color
- Looks like a small lobster
- Body segments are covered in hard armor plates; 5 pairs of legs; first pair with enlarged claws
- 2 pairs of antennae (one long and one short); eyes on short stalks

Order:

Ephemeroptera (Mayflies)

- 3 to 30 mm long (not including tails)
- Elongate tapered body with three regions (head, thorax & abdomen) and 3 pairs of jointed thoracic legs
- Dorsal or lateral gills (usually leaf like) on 2 or more abdominal segments and legs ending in single claws
- Usually 3 long segmented tails but sometimes with 2








Suborder:

Anisoptera (Dragonflies)

- 5 to 45 mm long
- Body with three regions (head, thorax & abdomen) and 3 pairs of jointed thoracic legs
- Abdomen is wide and dorsoventrally compressed; large head and eyes with no visible external gills or long segmented tails
- Modified labium for catching prey

Suborder:

Zygoptera (Damselflies)

- 5 to 25 mm long
- Body with three regions (head, thorax & abdomen) and 3 pairs of jointed thoracic legs
- Abdomen is narrow and tubular; large head and eyes with 3 broad gills at terminal end of abdomen
- Modified labium for catching prey

Order:

Plecoptera (Stoneflies)

- 6 to 50 mm long
- Elongate body with three regions (head, thorax & abdomen) and 3 pairs of jointed thoracic legs
- Bushy or simple (finger like) ventral gills may be seen on neck, thorax, first three abdominal segments or at base of tails; legs end in paired claws
- Always 2 segmented tails








Order:

Hemiptera (True Bugs)

- 5 to 40 mm long
- 3 body regions (when viewed from ventral side) and 3 pairs of jointed thoracic legs
- Membranous ("leathery") forewings covering most or all of the thorax and abdomen
- Sucking mouth parts (rostrum)
- Wings may be absent in immature specimens →







Order:

Megaloptera (Fishflies, Alderflies)

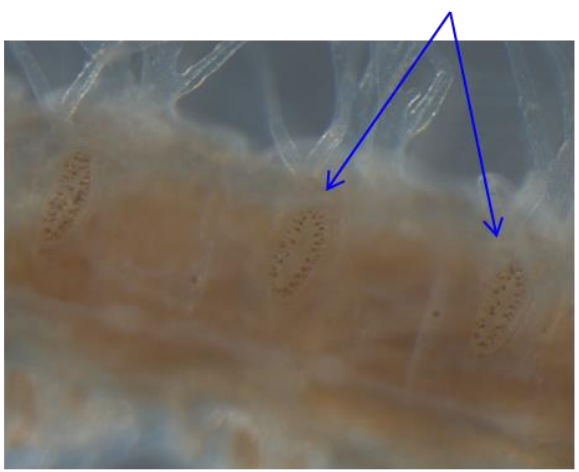
- 20 to 75 mm long
- Elongate tapered body with three regions (head, thorax & abdomen) and 3 pairs of jointed thoracic legs
- Lateral abdominal gill filaments with a long tapered terminal abdominal segment or two anal appendages with paired hooks
- Well developed mandibles

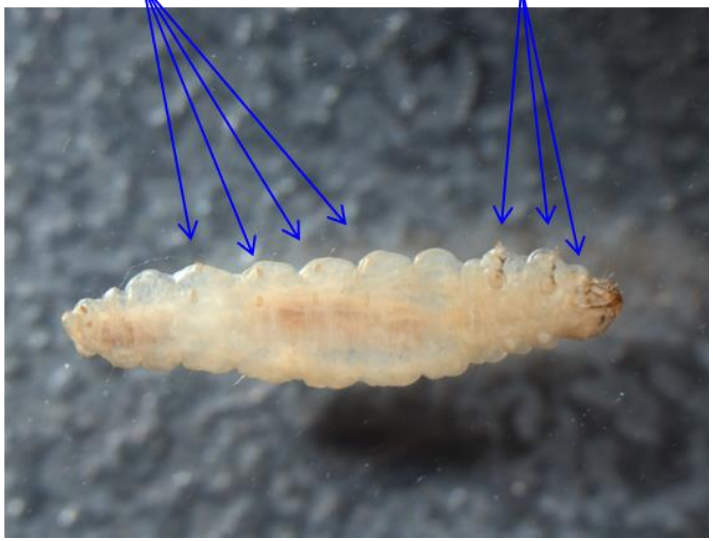
Photo by John R. Meyer, NC State University, Used with Permission

Order:

Lepidoptera (Aquatic Moth Larva)

- 10 to 25 mm long
- Caterpillar like body with 3 pairs of short, segmented, thoracic legs
- Head and thorax compressed into anterior 1/3 of body
- May have branched or filamentous gills on abdominal segments
- 4 pairs of short ventral abdominal prolegs with rows of small hooks; no hooked anal prolegs or spines

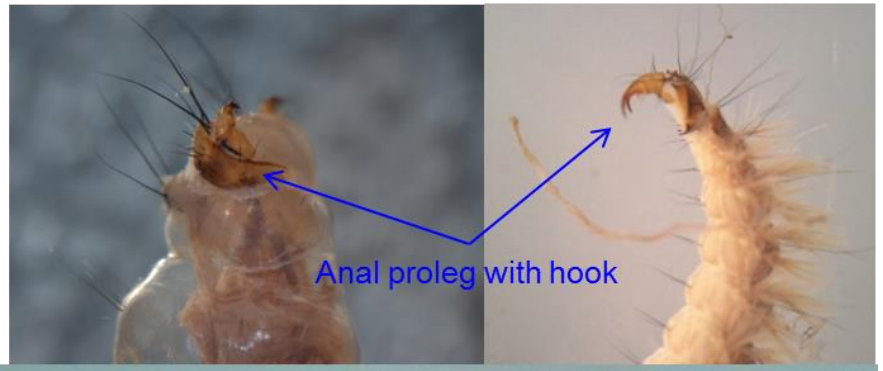


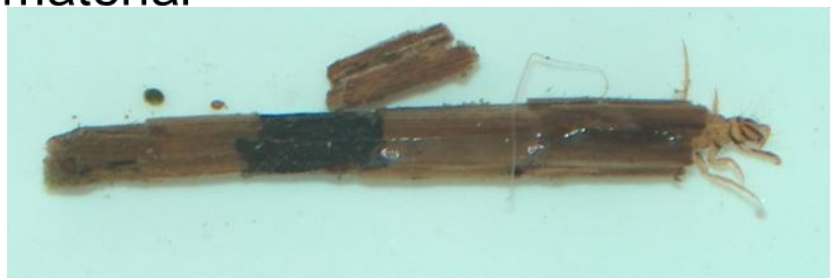


Order:

Trichoptera (Caddisflies)

- 2 to 30 mm long
- Body with three regions (head, thorax & abdomen) and 3 pairs of jointed thoracic legs
- Head and thorax compressed into anterior 1/3 of body
- Dorsal thoracic plates variously sclerotized
- Anal prolegs with hooks and generally short one segmented antennae
- May have branched or filamentous gills on abdominal segments
- Often build portable case or fixed retreat using bits of mineral or plant material





Trichoptera continued:

Order:

Coleoptera (Beetles)

- 2 to 30 mm long
- 3 body regions and (usually) 3 pairs of jointed thoracic legs

- Larvae:
  - Sclerotized head and thorax sometimes compressed into front 1/3 of body
  - May have unsegmented terminal abdominal appendages (tails)
  - No hooked anal prolegs but may have terminal hooks or spines; antennae with more than one segment
  - May have abdominal gill filaments or terminal gill tuft
  - Do not build cases

Coleoptera larvae continued:

Order:

Coleoptera (Beetles)

- 2 to 30 mm long
- 3 body regions and 3 pairs of jointed thoracic legs
- Adults:
  - Fore-wings modified into hard shell-like elytra
  - Chewing mouth parts with well developed mandibles

Family:

Chironomidae (Midges)

- 2 to 30 mm long
- Color variable; red, white, green, olive or yellowish
- Wormlike segmented body with well developed, sclerotized head and one pair of anterior and posterior prolegs
- May be in a tube made of fine dirt particles

Family:

Ceratopogonidae (no-see-ums)

- 3 to 13 mm long
- Very slender wormlike segmented body; pointed at both ends with a small pointed sclerotized head
- No abdominal appendages but may be a tuft of terminal abdominal hairs

Family:

Tabanidae (Horse Flies, Deer Flies)

- 15 - 40 mm long
- Cylindrical segmented body pointed at both ends with girdles of low prolegs on abdominal segments
- Leathery texture
- Head usually retracted into thorax
- No siphons or posterior lobes present

Family:

Tipulidae (Crane Flies)

- 10 to 50 mm long
- Reduced head is usually retracted into thorax
- Membranous wormlike body may have welt like prolegs; posterior end with fleshy lobes surrounding spiracles

Family:

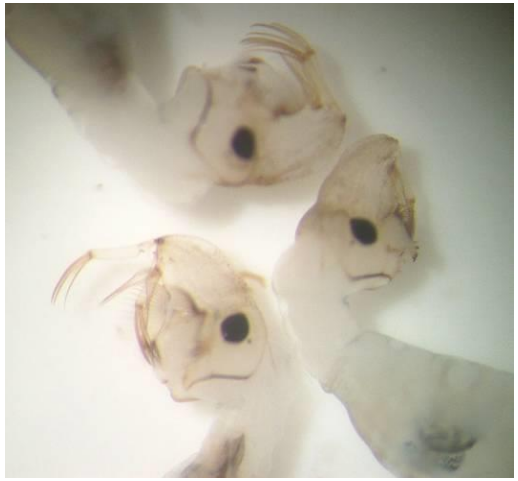
Culicidae (Mosquitos)

- 3 to15 mm long
- Fused thoracic segments are wider than abdominal segments
- Well developed head with prominent setal brushes; antennae with short terminal setae
- Anal segment at angle to abdomen
- Posterior respiratory siphon usually present

Family:

Chaoboridae (Phantom Midges)

- 3 to 20 mm long
- Fused thoracic segments wider than abdomen
- Well developed head with reduced setal brushes; antennae with long terminal setae
- Anal segment at angle to abdomen
- May have posterior respiratory siphon



Family:

Simuliidae (Black Flies)

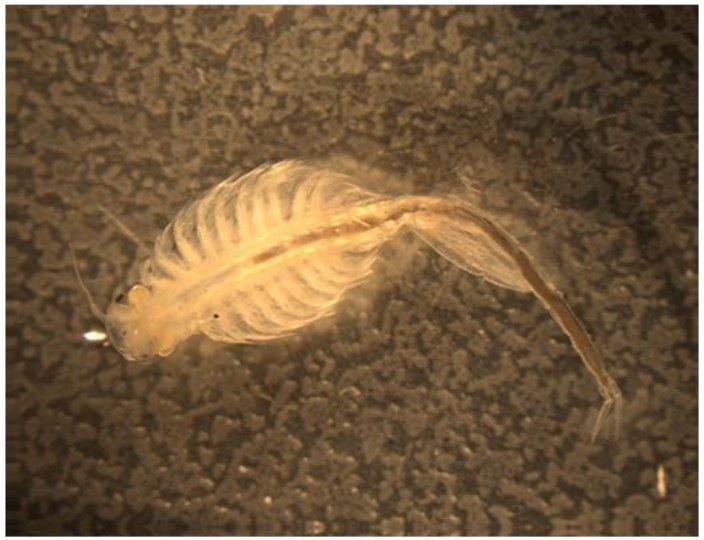
- 3 to 15 mm long
- Distinct club shaped body and well developed head with prominent labral fans (may be folded)
- One pair of prolegs may be visible behind head
- Hold fast structure at posterior end

Other *Diptera* (Other True Flies)

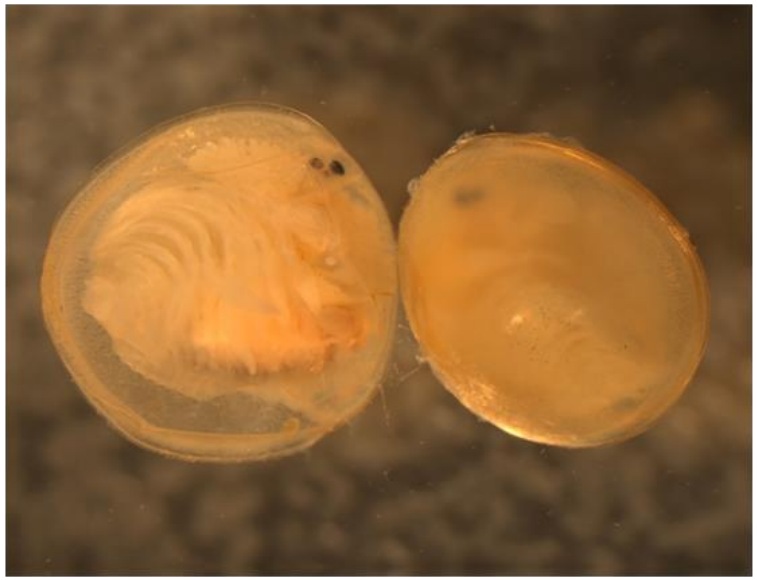
- May have parapods, pseudopods, prolegs, welts or other appendages, but **no jointed thoracic legs**
- Often wormlike; head may be retracted into thorax

"Other" Organisms you may encounter

Other: Neuroptera
(Lacewings)



Other: Anostraca
(Fairy Shrimp)



Other: Conchostraca
(Clam Shrimp)

Other: Notostraca
(Tadpole Shrimp)