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CABIN LABORATORY METHODS:

Processing, Taxonomy, and
Quality Control of Benthic
Macroinvertebrate Samples

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1. Introduction

The Canadian Aquatic Biomonitoring Network (CABIN) is the national biomonitoring program developed by Environment and Climate Change Canada. It provides a standardized biomonitoring sampling protocol and data analysis for the comparability of biomonitoring data from across the country and various agencies. CABIN provides the tools necessary to conduct consistent and scientifically credible biological assessments of freshwater.

Each organization or laboratory participating in the CABIN program should implement the prescribed quality assurance and quality control (QAQC) procedures. This ensures that precision, accuracy, completeness, comparability, and representativeness of the data are known and documented (Barbour et al. 1999). The **quality assurance (QA)** component provides data users and project authorities with the confidence that the accuracy and quality of data is within controlled and acceptable limits. The **quality control (QC)** component provides users with standard procedures to reduce the error rate in sample sorting and identification. All handling and processing of taxonomic samples should proceed according to appropriate occupational health and safety training, certification and procedures including but not limited to the Transportation of Dangerous Goods (TDG), and Workplace Hazardous Materials Information System (WHMIS)¹.

The objectives of this document are to provide:

- Requirements to assure quality in the sorting and taxonomic identification of benthic macroinvertebrates.
- Descriptions of quality control procedures for the sorting and taxonomic identification of benthic macroinvertebrates.

To maintain data quality in the National CABIN Database all taxonomy laboratories should process samples and provide data using the methods outlined in this document. Details of field sampling procedures and associated QAQC procedures can be found in other CABIN documentation:

- <https://www.canada.ca/en/environment-climate-change/services/canadian-aquatic-biomonitoring-network.html>.

The preparation of this manual is based on other developed protocols and is adapted from existing QAQC programs. In particular:

Canadian Aquatic Biomonitoring Network

Reynoldson TB, Logan C, Pascoe T, Thompson SP. 2001. CABIN (Canadian Aquatic Biomonitoring Network) Invertebrate Biomonitoring Field and Laboratory Manual. Burlington (ON): Environment Canada, Science and Technology Branch, National Water Research Institute. 47 p.

- Program link: <https://www.canada.ca/en/environment-climate-change/services/canadian-aquatic-biomonitoring-network.html>

¹ Health and safety requirements and regulations may vary between jurisdictions and projects.

Australian River Assessment System

WATER ECOscience. 2004. National River Health Program, AusRivAS Quality Assurance and Quality Control Project. Appendix B, Literature Review QA/QC Methodology for Rapid Bioassessment Programs. Mt Waverley, Victoria (AUS): Australian Government, Department of the Environment and Heritage. WATER ECOscience Report Number: 543. 45 p.

- Program link: <https://ausrivas.ewater.org.au/>

United States Geological Survey

Moulton, SR, Carter, JL, Grotheer, SA, Cuffney, TF, Short, TM. 2000. Methods for Analysis by the U.S. Geological Survey National Water Quality Laboratory – Processing, Taxonomy, and Quality Control of Benthic Macroinvertebrate Samples. Denver (CO): U.S. Department of the Interior, U.S. Geological Survey. Open-File Report 00-212. 49 p.

United States Environmental Protection Agency

Barbour, MT, Gerritsen, J, Snyder, BD, Stribling, JB. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Invertebrates and Fish-Second Edition. Washington (DC): U.S. Environmental Protection Agency, Office of Water. EPA 841-B-99-002.

2. Taxonomic Services

The taxonomic services section outlines expectations of taxonomic laboratories working on CABIN projects. Services required from a taxonomy laboratory are as follows:

- receive samples and maintain chain of custody
- transfer samples from field preservative to 70% ethanol upon receipt, if required (Section 4.1)
- for river/stream/wetland samples, subsample using a Marchant box (Marchant 1989, Figure 1) to a minimum of 300 organisms (lake samples are not subsampled)
- sort benthic invertebrates from the sample matrix
- identify specimens to the required taxonomic level or according to the Standard Taxonomic Effort (Appendix A)
- implement QC protocols for sample sorting and identification
- create a reference collection, if required (section 4.5)
- enter taxonomic data into the CABIN Database, if required
- provide voucher specimen(s) to the National CABIN Laboratory in a timely fashion, if required*
- return identified samples, reference collection and sorted and unsorted residues to the project authority

**The project authority is responsible for ensuring the submission of required voucher specimens. See section 4.6 for more information.*

3. Quality Assurance

The quality assurance section specifies qualifications of taxonomic laboratories, and outlines sample shipping and storage protocols.

3.1 Taxonomic laboratory requirements

The requirements of a qualified taxonomic laboratory are to:

- maintain and/or have access to adequate technical and taxonomic literature
- maintain and/or have access to adequate sample processing equipment
- have established standard operating procedures
- have QAQC measures for sorting, subsampling and identification
- have a minimum of two people involved in providing taxonomic services; one to process samples and one to perform QC audits (Table 1). Auditors may be from different laboratories
- have completed the CABIN Data Entry module (if required to enter data)

The laboratory should be able to **provide proof** of the following:

- a combination of experience and training that demonstrates current knowledge and professional development in benthic macroinvertebrate taxonomy
- experience or expertise in the subsampling, sorting and identification of taxa within the specified study region

CABIN recommends the use of certified taxonomists. The Society for Freshwater Science or SFS (formerly known as the North American Benthological Society) offers a Taxonomic Certification Program for family and genus level taxonomy. A list of certified taxonomists and information on how to become certified can be found on the Taxonomic Certification Program website (<https://stroudcenter.org/sfstcp/>).

Table 1. Personnel, responsibility and qualifications required of taxonomic laboratories

Person	Responsibility	Qualifications
Sample Processor/ Subsampler	Transfer and wash sample Subsample using a Marchant box	Trained with Marchant Box Awareness of current WHMIS guidelines and Safety Data Sheets (SDS) while handling dangerous goods. Provisions of WHMIS training is the responsibility of the employer.
Sorter	Pick benthic macroinvertebrates out of residue Sort samples into various order/family groups	Ability to recognize benthic macroinvertebrates Ability to classify organisms into groups of similar taxa
QC Auditor: Sorting	Check samples to ensure > 95% sorting efficiency (see section 4.2.6)	Experienced in sample sorting Must be someone other than the original sorter
Taxonomist	Identify samples according to contract requirements	Trained in taxonomic identifications of benthic macroinvertebrates Appropriate SFS certification taxonomic resolution (recommended) Interacts with other taxonomists through professional societies or workshops Ongoing professional development to keep up-to-date with current taxonomic changes Maintains appropriate literature
QC Auditor: Taxonomy	Re-identify 10% of samples (or a minimum of 3) to ensure < 5% identification error rate (see section 4.3.4)	Trained extensively in identifying benthic macroinvertebrates to a minimum of family level Must be someone other than the original taxonomist

3.2 Shipping, receiving and storage protocols

3.2.1 *Safe handling of chemicals*

CABIN adheres to federal guidelines on the safe handling of chemicals for the goal of protecting human health and the environment from harmful toxic substances. Most samples are preserved in the field using one of two preservatives:

- A. **Ethanol**, either pure or denatured, in concentrations of 70% to 95%. Field staff may use a higher of concentration of ethanol to account for water dilution in the sample. The optimal final concentration for preservation in the field is 50%. Denatured ethanol is mixed with additives like methanol to make it poisonous and discourage recreational consumption. Note that pure ethanol and denatured ethanol have different UN shipping numbers that are required for TDG documentation.
- B. **Formalin** is a traditional preservative that uses dilute formaldehyde in an aqueous solution with a final concentration of 10%. Since formaldehyde is only 37% in solution, 10% formalin contains approximately 3.7% formaldehyde. Formalin is naturally acidic and can break down calcium-based organisms so a phosphate buffer is added to create a neutral solution. Note that there is no UN shipping number for 10% Formalin and TDG documentation is not required for formaldehyde concentrations of less than 25%.

Shipping of preserved biological samples requires knowledge of and training in TDG. It is prohibited to send dangerous and/or flammable substances without training and certification. Information for TDG training and regulations can be found at <http://www.tc.gc.ca/tdg/menu.htm>. It is the responsibility of persons handling, transporting and shipping chemicals to be aware of safe handling, disposal and shipping requirements. Proper TDG training is the responsibility of the employer.

3.2.2 *Shipping samples to a taxonomy laboratory*

Preserved samples should be shipped to the taxonomic laboratory as soon as possible. Formalin preserved samples can become acidic and break down organisms in the sample. It is recommended to only leave samples preserved in formalin for 7 days before switching to ethanol for long-term storage. Timely shipping and processing can reduce damage caused by preservation.

3.2.3 *Sample receiving by a taxonomy laboratory*

Samples received by the taxonomic laboratory should be verified against the sample submission form to ensure the shipment is complete. Wash samples and transfer into fresh 70% ethanol upon receipt. A sieve with a mesh size of 400 µm or less is required to collect the sample while draining the waste preservative. Rinse the sample with water to remove all traces of formalin and dispose of waste chemical in appropriate manner. Inspect samples and replace evaporated ethanol every three months.

3.2.4 Sample shipping to a project authority

All samples and sample residues (sorted and unsorted) should be returned to the project authority and shipped in 70% ethanol, unless otherwise specified. If reference collections are required, vials should be carefully packaged, labeled and returned to the project authority. Voucher specimens should be sent to the National CABIN Laboratory for verification and archiving, if required (refer to section 5.5).

3.2.5 Sample receiving by a project authority

Samples received from the taxonomic laboratory should be checked against the sample submission form to ensure the shipment is complete. The project authority is to ensure voucher specimens are forwarded to the National CABIN Laboratory for verification and archiving (refer to section 5.5).

3.2.6 Sample storage by a project authority

Storage time for archived samples depends on the goal of the project. Reference sample data are shared by the network and used frequently. CABIN recommends keeping all samples from reference sites for 10 years, until models for the region are completed and published and the potential need to revisit benthic identification no longer exists. This includes the sorted and unsorted residues in the event that further analysis is required. Test samples, on the other hand, are specific to a given project. CABIN recommends holding test samples for three years past the publication of data. However, this decision will be dependent on the project and the ability of the project authority to store and maintain samples.

Storage of flammables and hazardous chemicals in a workspace will require good ventilation, a space designated to keep chemicals separate (e.g. flammables cabinet) and proper WHMIS labels. Flammables like ethanol must be kept away from ignition sources and out of direct sunlight. Check with your building administration for specific regulations and fire codes.

Inspect samples and replace evaporated ethanol every three months. To properly dispose of samples, all chemical trace must be safely removed before drying and discarding the debris. Waste ethanol and formalin should be collected and disposed of by a chemical waste management company.

4. Quality Control

Quality control procedures reduce the level of error in transferring, subsampling, sorting, identification, and data entry. This section outlines the procedures and protocols for each QC component.

4.1 Sample transferring and storage

When received, samples should be transferred to fresh 70% ethanol, including samples originally preserved in ethanol. Ethanol used to preserve samples in the field may have become diluted by water loss from organism tissues, resulting in an unknown concentration of preservative; this could compromise the intactness of individual specimens in the sample.

Carefully wash samples over a sieve with a mesh size no larger than 400 µm. Dispose of residual preservative in accordance with local bylaws and provincial hazardous waste regulations. Formalin neutralizing agents are available from laboratory equipment suppliers. Inspect stored samples every three months to replace evaporated ethanol.

4.2 Subsampling and sorting

Subsampling refers to fractioning of a sample to achieve a desired fixed count that is representative of the whole sample. **Sorting** refers to the removal of benthic macroinvertebrates from the sample matrix and organization into coarse taxonomic groupings (Moulton et al. 2000).

Subsampling for CABIN river, stream and wetland samples is done with a Marchant Box (Marchant 1989, Figure 1), following the protocol outlined below. Samples are not separated into different size fractions. A minimum count of 300 individuals and a minimum of 5 subsamples is required. If more than 50% of the sample is needed to obtain 300 organisms, the entire sample is processed. Lake samples are typically not subsampled since the abundance of organisms from a single sample is usually less than 300 and the Marchant Box is an unnecessary step.

In 2014, the CABIN program implemented a requirement to subsample a minimum of 5% of the sample or 5 cells of the Marchant box. Therefore, even if a count of 300 individuals is reached before completing the 5 subsamples, the remainder of subsamples must be sorted and identified.

Prior to subsampling, assess the need to subsample by placing the sample in a shallow pan or tray. Scan the sample to determine if subsampling will be required.

4.2.1 Receiving elutriate or washed vegetation QC samples from the field

If a sample was elutriated in the field using the bucket swirl and the elutriate (e.g. heavy inorganic residue, such as sand and pebbles) was submitted for QC purposes, the elutriate **SHOULD BE** examined before continuing with the main sample. Any organisms removed from the QC audit of elutriate should be recorded (at the order/family level) and added to the main sample for subsampling. A record of the organisms missed should be submitted with the QAQC report to the project authority.

Similarly with wetland samples, large macrophytes, organic debris and algal masses are usually collected with the sweep method and provide challenges subsampling with a Marchant box. Depending on the sampler, the vegetation may have been removed in the field prior to preservation and retained for QC purposes. The retained material **MUST BE** examined before continuing with the main sample. Any organisms found in the vegetation will be recorded (at the order/family level) and added back to the main sample for subsampling with a Marchant box. A record of the organisms missed should be submitted with the QAQC report to the project authority.

4.2.2 Equipment and materials

Table 2 lists essential materials and equipment for sample subsampling and sorting.

Table 2. Materials required for subsampling and sorting

(✓)	Equipment or materials
	Marchant box
	U.S. 45 sieve (400 µm or smaller)
	Spoons
	Random numbers table or ten-sided die
	Pipette (or suction device)
	Petri dishes
	Scissors
	Waterproof paper for labels
	White sorting trays
	Bench or tally sheets
	Dissecting microscope with light source (10–40X)
	Forceps
	Squeeze bottles (for water and ethanol)
	Specimen vials, caps or stoppers
	Plastic container for sorted residue
	Probes (fine tipped and blunt)
	70% ethanol

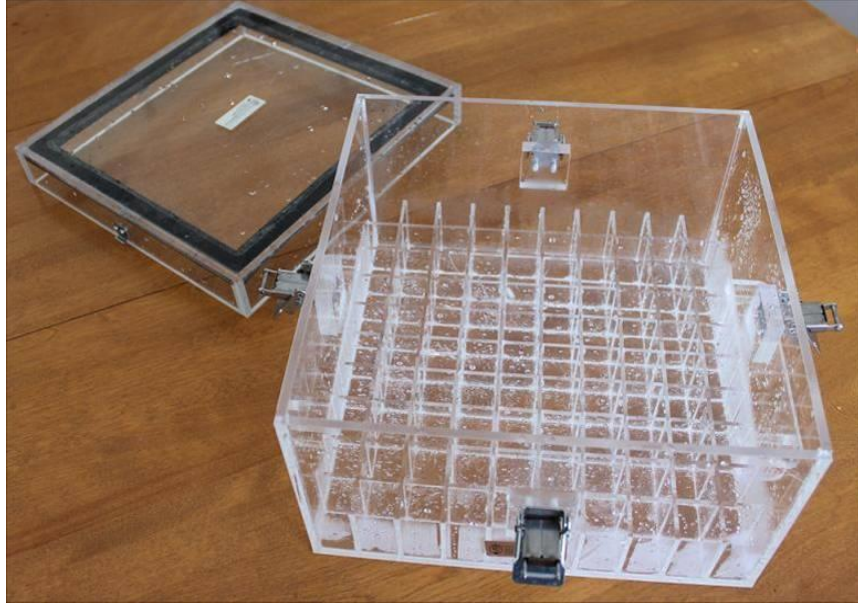


Figure 1. The Marchant Box.

4.2.3 Stream and river subsampling and sorting protocol

The subsampling and sorting protocol using a Marchant Box is outlined below.

1. Wash the sample into a sieve (400 micron or less) to remove preservative.
2. Wash large material, rocks, twigs, and macrophytes gently and thoroughly over the sieve. Return washed material to a sorted residue container or discard; do not add material to the Marchant box.
3. Transfer the sample into the Marchant box.
4. Fill cells with water but **do not overfill the** cells. The water level should be below the top of each cell.
5. Secure the lid to the Marchant box so that it is watertight.
6. Flip the Marchant box over (180 degrees, top to bottom).
7. Gently agitate the sample in the open space of the lid to equally distribute the sample.
8. Quickly flip the box back over (180 degrees, bottom to top) so the sample is evenly distributed in each of the 100 cells.

Note: This step takes practice; several attempts may be required to achieve an even distribution. Practice with other material before using a CABIN sample.

9. Repeat steps 6 to 8 if the sample is not evenly distributed.

Tip: Be sure to flip the box quickly so that the majority of the sample does not settle into one corner.

10. Randomly select a cell using a ten-sided die or a random number generator.
11. Extract the subsample from the cell using a vacuum pump or suction device, and transfer into petri dish or sorting tray.
12. Count the number of organisms extracted from the cell and estimate the approximate number of cells that will be required to achieve 300 organisms. There must be at least 5 cells (5% of the sample) sorted and identified, no matter the organism count. If the 300 count is reached part way through a cell, the entire cell should be completed. The final count may be slightly higher than 300. **If more than 50% of the sample will be required to reach 300+ organisms, then the entire sample is sorted and identified.**
13. Remove specimens and separate into coarse taxonomic groupings. Use a dissecting microscope to sort samples.
14. Tally and record each organism removed on a bench sheet.

IMPORTANT NOTE: Certain taxa are not used in the CABIN analysis and are not included in the 300 count. Record these taxa as 'present' only. Excluded taxa are listed in Table 3 and Appendix A.

15. Record the number of cells extracted to achieve the 300 organism count.
16. Ensure all vials and sorted residue (extracted cells) are labeled, preserved and retained for QC audits of sorting efficiency. Do not recombine the sorted residue with the original sample. Be sure to also preserve, label and retain unsorted residue for future reference.

Table 3. Taxa not normally included in the 300-organism count for CABIN river/stream samples

Taxa Groups	Rationale
Ostracoda	Taxa can be found in extremely high numbers and bias a sample
Cladocera	Taxa not generally benthic and can bias samples collected in close proximity to lakes
Copepoda	Taxa can be pelagic and benthic. Benthic taxa are not adequately sampled using a 400 um kicknet
Rotifera	Taxa not generally benthic and are not adequately sampled using a 400 um kicknet
Porifera	Taxa are colonial and cannot be quantified as number of individuals per sample like other benthic taxa
Nemata, Nematomorpha, Nemertea	Taxa are not adequately sampled using a 400 um kicknet
Non-aquatic taxa	Terrestrial drop-ins such as earth worms, spiders, and some beetles and bugs are not part of the benthic community

4.2.4 Subsample by Weight

Samples may also contain large amounts of filamentous algae, making the Marchant box ineffective in subsampling. Instead, the mass of algae is separated by weight and the subsample is sorted (Sebastien et al. 1988). The protocol for subsampling by weight is outlined below.

1. Mix sample in a large glass beaker thoroughly with a stirring rod to ensure an even distribution.
2. Drain sample into a pre-weighed 400 µm (or smaller) sieve and let drain for 15 to 20 minutes.
3. Stir sample gently and weigh sieve and sample on an electronic balance to the nearest 0.1 g.
4. Randomly separate algal mat into equally weighted subsamples of 4 or more and proceed with sorting in step 13 (section 4.2.3).
5. Record the total number and weight of subsamples selected and the number of subsamples needed to reach the individual count of 300.
6. A minimum of 5% of the total mass should be searched for organisms.

4.2.5 Wetland subsampling and sorting protocol

Wetlands now have their own set of CABIN standardized methods for collection of benthic invertebrates. The collection method and field protocol can be found in the CABIN Wetland Macroinvertebrate Protocol

(http://publications.gc.ca/collections/collection_2019/eccc/CW66-571-2019-eng.pdf).

The steps for subsampling wetland samples are the same as for streams and rivers in section 4.2.3. Subsampling with the Marchant Box may prove difficult, even with all larger debris removed. Where a large mass cannot be subsampled effectively, use the subsampling by weight method described in the previous section 4.2.4.

One of the important differences in the sorting protocol between streams and rivers and wetlands is the inclusion of certain taxa. Large zooplankton (Copepoda, Ostracoda, Cladocera) are an important and often abundant group in wetland communities. The CABIN Wadeable Streams Protocol does not count these taxa in the 300 organism count. For CABIN Wetland samples, the taxonomist should note zooplankton presence and provide associated subsample counts and identifications. **At this stage, however, zooplankton including Copepoda, Ostracoda and Cladocera will not be considered toward the 300 organism count for wetlands but will be enumerated per cell and identified to the lowest practical taxonomic level.** See Table 3 above for other non-counted taxa rationale.

Abundance levels of total organisms may also be very high in some productive wetlands. For expedient processing, only a certain number of taxa will be picked out and identified in these cases. If the total number of organisms (including both counted and non-counted taxa) is greater than 2,500 individuals before reaching 5% of the sample, the remainder of the cell is finished and no further cells need to be sorted. In some cases, only one species is found in high abundance, which is common for Amphipoda. If the count for one species is greater than 1,000 individuals before reaching 5% of the sample but it is still feasible to pick out and count other species, the abundant taxa are no longer picked out and counted. A note must be made on the lab sheet concerning the number of cells counted with the abundant taxa.

4.2.6 Sorting auditing protocol

Sorting precision is calculated as **percent sorting efficiency (%SE)**. The QC auditor estimates sorting efficiency by examining randomly selected sample residuals. Quality control audits should be carried out on a regular basis to establish a standard sorting efficiency.

The sorting auditing protocol is as follows:

1. Randomly select samples to be audited. Someone other than the original sorter should select samples.
2. Re-sort the residue from 10% of the project samples (minimum of 3).
3. Record the number of organisms found in each sample residue.
4. Calculate %SE for each sample using the equation:

$$\%SE = \left(1 - \frac{\# \text{ Organisms Missed}}{\text{Total Organisms Found}}\right) * 100$$

Total organisms found includes the original count and the number found from the re-sorting of the residue. The average %SE is calculated based on the number of re-sorted samples, and represents the standard sorting efficiency for that project (Table 4).

Additional criteria for evaluating sorting efficiency:

- **If the average sorting efficiency is $\leq 95\%$, all samples in the project should be re-sorted.**
- Notify the sorter of organisms missed to rectify the problem.
- If an entire class of benthic macroinvertebrates is overlooked by the sorter (for example, molluscs are consistently not identified and left in the sample residue) all samples in the project should be re-sorted even if the missed organism may be less than 5% of the total sample (Table 5).

Table 4. Example of sorting audit results for samples and a project that met required sorting efficiency criteria

Sample #	Original count	QA audit count	Comments	% SE
1	323	323	Clean	100%
2	313	332	Missed heads, some Chironomidae	$(1 - 20/332)*100 = 94.0\%$
3	303	305	No comments	$(1 - 2/305)*100 = 99.3\%$
Average %SE				97.9% PASS

Table 5. Example of sorting audit results for samples and a project that do not meet sorting efficiency criteria

Sample #	Original count	QA audit count	Comments	% SE
1	323	323	Clean	100%
2	313	332	Missed a variety of different organisms	94.0%
3	303	305	Missed all mollusks	99.3%
Average %SE				97.9%
FAIL due to consistent omission of mollusks				

<p align="center">Criteria for Sorting Efficiency Corrective Measures</p> <p>All samples in the project should be re-sorted if:</p> <p>a) the sorting efficiency rate is $\leq 95\%$</p> <p align="center">OR</p> <p>b) an entire class of organisms is overlooked</p>
--

4.3 Identification

This section describes standards for taxonomic identifications and for data entry into the CABIN Database. The preparation of reference collections and voucher specimens is outlined. Details of Standard Taxonomic Effort, nomenclature and the auditing protocol for identification error rates are also provided.

The essentials for identification are as follows:

1. Identifications should be based on current published taxonomic references.
2. Nomenclature should conform to the Integrated Taxonomic Information System (ITIS), available on the United States home page <http://www.itis.gov> or on the Canadian partner home page <http://www.cbif.gc.ca>.
3. A list of literature used to identify organisms should be submitted with the processed samples.

4.3.1 *Equipment and materials*

Table 6 lists the minimum equipment required for taxonomic identifications.

Table 6. List of essential equipment and materials for taxonomic identification

(✓)	Equipment or materials
	Dissecting microscope 10-80x with fiber-optics or other adequate light source
	Petri dishes
	Euparal, Kahle's solution or CMCP-10 (or other appropriate mounting medium)
	Forceps
	Sample labels
	Appropriate taxonomic literature
	Hand tally counter
	Compound microscope 60-1500 x for slide mounted organisms
	Microscope slides and cover slips (appropriately sized)
	Dropper
	70% denatured ethanol
	Plastic squeeze bottle
	Specimen vials, with caps or stoppers
	Bench Sheet

4.3.2 Standard Taxonomic Effort

Taxa should be identified to the Standard Taxonomic Effort (STE) as outlined in Appendix A. The minimum level of identification required (where possible) is family level as CABIN analyses are routinely performed on data at this level. With reference site data, it is recommended that identifications be taken to the lowest practical level. It is also important to note that some taxa are not included in the sample counts (Appendix A).

Identify specimens to genus only if undamaged and mature organisms are available. Use caution when identifying early instar or juvenile specimens to lower levels. Specimens should have the features necessary to be verified by a third party.

Damaged specimens are only identified if the fragment includes the head. Some benthic macroinvertebrate groups require slide mounting for genus level identification.

4.3.3 Nomenclature

CABIN uses ITIS as the standard for taxonomic nomenclature and classification. The ITIS database is reviewed periodically to ensure valid classifications through revisions and additions to species lists. It represents a fair consensus of modern taxonomic opinion. ITIS is supported by the Society of Freshwater Science and is the official source of current nomenclature for aquatic benthic macroinvertebrate taxa associated with their Taxonomic Certification Program tests.

CABIN recognizes that ITIS has some limitations. ITIS may not always be in agreement with the most recent findings in taxonomic research. Synonyms for a given taxon may be reflected in the CABIN Database until they are confirmed in the ITIS database. However it is the responsibility of the taxonomist and the person entering the data to be familiar with current taxonomy and use the taxon name as found in ITIS.

In addition to the use of ITIS for standard nomenclature, CABIN does not accept a number of naming conventions to improve consistency between taxon names (Appendix B).

4.3.4 Identification auditing protocol

For each project, 10% of samples (or a minimum of 3) should be audited. The audit is not simply a comparison of taxon lists; it is a complete re-identification and enumeration of the selected samples.

The project authority randomly selects samples and someone other than the original taxonomist should perform audits. The audit may be performed by a taxonomist from the same laboratory or by a taxonomist from a separate laboratory.

There are several types of errors related to taxonomy including:

1. **Misidentification** occurs when the specimen is incorrectly identified (Example 1).
2. **Enumeration** errors occur when the count for a particular taxon is incorrect. Enumeration errors can contribute to elevated uncertainty about data quality (Stribling et al, 2003).
3. **Questionable taxonomic resolution** occurs when a specimen is identified to a level that cannot be validated by its features (Example 2).
4. **Insufficient taxonomic resolution** is the identification of a specimen at a different taxonomic level than identified by the QC audit. This could be a higher or lower level of classification.

Example 1. Misidentification: Incorrect genus

A specimen is identified as **genus *Sweltsa***. The QC auditor identifies the specimen as **genus *Suwallia***. A third party confirms the QA auditor's identification; the original identification is recorded as an identification error.

Example 2. Questionable taxonomic resolution: Genus to family

A Perlodidae is identified to the **genus *Frisonia***. The QC auditor notes that the insect is an early instar with only the head and first two segments of the thorax intact. Key features, such as the cerci, cannot be reviewed. The QC auditor leaves the identification at **family *Perlodidae*** and records the identification as an error.

Errors could be the result of:

- operational factors; poor lighting, poor microscope
- inadequate training
- recording error
- inexperience with benthic macroinvertebrates
- poor counting protocols (e.g., bodies counted)
- specimen degradation

The identification error rate (%IE) is calculated by summing the number of identification errors in a sample.

$$\frac{\text{\#Incorrect Identifications}}{\text{Total Organisms Found in Audit}} * 100 = \% \text{ Identification Error}$$

The average error rate of audited samples should be < 5%. All samples with a 5% or higher error rate are examined for repeated error or patterns, regardless of the average error rate of the audited samples.

Enumeration, questionable taxonomic resolution and insufficient taxonomic resolution are not included in the %IE. **CABIN recommends the documentation and reporting of these errors in the QC report.** See Table 7 for examples of errors related to taxonomic data.

Disagreements between original and QC identification should be communicated to the original taxonomist. If no consensus can be reached between the original and QC identifications, a third party should be consulted for verification. All third party results should be reported in the QC audit report.

Criteria for Identification Error Corrective Measures

If the identification error rate (%IE) ≥ 5%, the entire project should be re-identified by someone other than the original taxonomist.

Table 7. Example identification QC audit report.

Order	Family	Genus	Raw Count	Audit Count	Audit Flags	IE error	Comment
Coleoptera	Elmidae	Promoresia	103	102			Enumeration (-1)
Diptera	Ceratopogonidae	Mallochohelea	1	0		1	Misidentification; identified as Probezzia; anterior of head capsule narrowed
	Ceratopogonidae	Probezzia	1	2			Confirmed by second taxonomist
	Chironomidae	Cardiocladius	1	1			
	Chironomidae	Eukiefferiella	2	1			
	Chironomidae	Labrundinia	1	1			
	Chironomidae	Micropsectra	11	9			
	Chironomidae	Orthoclatiinae	3	1			
	Chironomidae	Orthoclatidius	19	16			
	Chironomidae	Rheocricotopus	2	3			
	Chironomidae	Stempellina	3	3			
	Chironomidae	Tanypodinae	13	0	13		Subfamily not accepted in database, 12 identified as Rheopelopia; Enumeration (-1)
	Chironomidae	Cricotopus		3		2	Misidentification; 2 specimens from other identified taxon, Enumeration (+1)
	Chironomidae	Rheopelopia		12			
	Chironomidae	Polypedilum		1		1	Misidentification; 1 specimen from other identified taxon
	Chironomidae	Pseudosmittia		3		3	Misidentification; 3 specimens from other identified taxon
	Chironomidae	Zalutschia		1		1	Misidentification; 1 specimen from other identified taxon
	Simuliidae	Simulium	1	0		1	Misidentification; identified as Prosimulium; distal article of antennae pigmented in contrast to anterior colourless articles
	Simuliidae	Prosimulium		1			Confirmed by second taxonomist
Ephemeroptera	Baetidae			3	3		
	Baetidae	Acerpenna	28	26			2 rolled up to family, no cerci or gills visible

Table 7. cont'd.

Order	Family	Genus	Raw Count	Audit Count	Audit Flags	IE error	Comment
	Baetidae	Baetis	10	9			1 rolled up to family, no cerci or gills visible
	Ephemerellidae		6	28	22		Enumeration (+1)
	Ephemerellidae	Ephemerella	25	4			Questionable resolution; 21 rolled up to family, early instar
	Heptageniidae		8	8			
	Leptophlebiidae	Paraleptophlebia	17	17			
Plecoptera	Leuctridae		3	0			3 identified as Leuctra, corners of pronotum with 4 long hairs, paraprocts with long bristles); confirmed by second taxonomist
	Leuctridae	Leuctra		4	4		Enumeration (+1)
	Perlodidae		9	3			6 identified as Isoperla; confirmed by second taxonomist
	Perlodidae	Isoperla		6	6		
	Taeniopterygidae	Taeniopteryx	2	2			
Trichoptera	Brachycentridae		1	1			
	Hydropsychidae	Hydropsyche	4	4			
	Hydroptilidae	Hydroptila	1	1			
	Lepidostomatidae	Lepidostoma	7	7			
	Rhyacophilidae	Rhyacophila	7	7			
Veneroida	Pisidiidae		5	0	5		Identified as Pisidium; confirmed by second taxonomist
	Pisidiidae	Pisidium		5			
		TOTAL	312	313	63	10	
		Identification Error				3.19%	PASS

4.4 Data entry

The CABIN Database houses national data with contributions of taxonomic information from many laboratories. In order to maintain data consistency, standardised protocols should be followed. Supplemental information (such as special species designations) gathered during the identification process are not accepted in the CABIN Database (Appendix B).

Taxonomists can enter data directly into the CABIN Database provided they are trained in CABIN Data Entry and have a CABIN username and password. For information about the CABIN Training program, refer to the [Training and Certification link](#) on the CABIN webpage.

Data entry criteria should follow the naming conventions outlined in Appendix B

4.4.1 Data auditing

Taxonomic hierarchy and nomenclature are the largest sources of error in data entry. Taxonomy laboratories should submit raw data (e.g. bench sheets or electronic files) to the project authority for verification of data entry accuracy. The project authority is responsible for ensuring data entry accuracy and completeness. CABIN recommends an audit of 10% of bench sheets for each project. In cases where an error is identified, the source of error should be investigated and corrected for all samples as appropriate.

4.5 Reference collections

A reference collection is a collection of vials and slides that contain all reported taxa for a particular project. Each vial or slide contains one or more specimens of a single taxon collected together at one place and time. The National CABIN Laboratory does not require reference collections for individual CABIN projects. The decision to obtain and maintain a reference collection is made by the project authority. Reference collections are used to:

- ensure taxonomic consistency
- assure repeatability and independent verification, or re-evaluation of the study result
- allow for historical comparisons

The project authority for a CABIN project may require a reference collection. If so, the reference collection and associated documentation should be submitted to the project authority at the completion of a project.

Specimens in a reference collection are to be preserved in 70% ethanol and stored in sealed vials with appropriate labels. Labels should be printed on waterproof paper with pencil or laser ink. **The collection should be accompanied by a spreadsheet that**

includes all information on the specimen labels. The required information for each label and for the spreadsheet is listed below and shown in Figure 2.

Required reference collection specimen information:

1. Specimen name
2. CABIN study name (Project name)
3. Site code (from which the specimen was taken)
4. Province or Territory
5. Taxonomist responsible for the identification
6. Date collected (DD/MM/YY)
7. Date identified (DD/MM/YY)
8. Number of individuals

<i>Baetis</i> Columbia Basin RCA Site: ABC123 Prov: BC	ID: H. McDermott Collected: 12/09/08 Identified: 23/01/09 No. of indiv.: 3
Front label	Back label

Figure 2. Example specimen label.

4.6 Voucher specimens

Voucher specimens provide a documented, permanent record of taxonomic identifications and are critical to quality control. The voucher specimen may be separate and in addition to the specimen required for the reference collection. Voucher specimens are required by the National CABIN Laboratory to verify the identification of any taxa that are new to the CABIN Database. The CABIN user entering the new taxon will receive an automatic notification that a voucher specimen is required. Each vial contains one or more specimens of a single taxon collected together at one place and time. If only one specimen is submitted, the voucher specimen will serve as the reference specimen in the National Reference Collection.

Voucher specimens should be submitted to and verified by the National CABIN Laboratory before they are marked as valid on the CABIN taxa list. Unverified taxa can be entered into the Database but the taxon counts will not be pooled with the other data until a voucher specimen is received and verified by the National CABIN Laboratory.

As of 2014, the National CABIN Laboratory will no longer be verifying voucher specimens at species level. If a specimen has been identified to species, it can still be recorded in the CABIN Database, but no voucher request notification will be issued. A request for a voucher will be sent only when a new genus or family level identification is entered into the CABIN Database.

Voucher specimens should be preserved in 70% ethanol and labeled in the same way as a reference collection specimen, shown in Section 4.5. **Each vial should be labeled and specimens should be accompanied by a spreadsheet detailing the information**

provided on the specimen labels. Additionally, a reference to the literature used to identify the specimen should accompany the voucher submission. All voucher specimens are sent directly to the National CABIN Laboratory by the contracting taxonomy lab or by the project authority. Voucher specimens will be verified in the National CABIN Laboratory by the National Taxonomist. Any taxa that require expert opinion (e.g., genus level identifications) will be sent to the appropriate recognized expert.

All voucher specimens should be sent to:

**National CABIN Laboratory
Pacific Environmental Science Centre, Rm 113
2645 Dollarton Highway
North Vancouver, BC
V7H 1B1**

Each voucher specimen should be accompanied by a reference of the literature used for the identification.

Unverified taxon counts are not pooled with the other data until a voucher specimen is received and verified by the National CABIN Laboratory.

An overview of the sample processing requirements for the taxonomist and project authority are found in Appendix C.

5. The National CABIN Laboratory

The National CABIN Laboratory provides the taxonomic QAQC function for the CABIN program for samples that may be shared within the network for assessment (e.g. reference samples for assessment models). The CABIN taxonomist audits and processes samples using the methods outlined in the previous sections.

The laboratory houses an extensive collection of taxonomic literature to support the identification of benthic macroinvertebrates for QC audits. Literature is routinely reviewed and updated. The CABIN taxonomist attends taxonomy workshops and maintains working relationships with experts in the field.

The National CABIN Laboratory performs QC audits to verify sorting efficiency (Section 3.2) and taxonomic identifications (Section 3.3) to ensure the accuracy of contracted laboratories. The QC audit does not replace the third party verification performed by the contract taxonomist. A minimum of 10% of all samples collected as part of reference model development are audited. Samples may come from regional Environment and Climate Change Canada offices, other federal departments or provincial and territorial departments.

The National CABIN Laboratory only performs audits on samples that are collected as part of reference model development.

The QAQC audits conducted by the National CABIN Laboratory do not replace the QC requirement of 10% re-identification by a third party for individual CABIN projects.

An overview of the responsibilities of the National CABIN Laboratory is shown in Appendix C.

5.1 Verification of sorting efficiency

Sorting precision is calculated as percent sorting efficiency (%SE) and evaluated by examining randomly selected sample residues from a project, as described in section 4.2.6. Project sorting efficiency audits are carried out on a regular basis in order to establish a standard sorting efficiency. An average sorting efficiency over 95% should be achieved on 10% of samples or a minimum of 3 samples in a project.

Project Sorting Efficiency Corrective Measures

Projects that do not meet the sorting efficiency criteria (>95%) are reported to the project authority and sent back to the taxonomist for re-sorting.

Sample sorting efficiency will be reported in project QC reports and summarized in the National QC Report.

5.2 Verification of taxonomic identifications

The CABIN taxonomist performs random re-identifications of samples. Slide mounted specimens are also re-identified. New bench sheets are produced for each sample. The results generated by the contract taxonomist are compared to the audit and discrepancies are evaluated. The identification error rate is calculated ($%I/E$) and should be below 5%.

Four types of taxonomic error are evaluated:

1. Misidentification
2. Enumeration
3. Questionable taxonomic resolution
4. Insufficient taxonomic resolution

Only misidentification is included in the $%I/E$ calculation (see Section 4.3.4). The CABIN taxonomist examines all taxonomic errors and determines the corrective action. All errors are reported to the project authority and in project QC reports.

Project Identification Error Corrective Measures

Errors and corrective measures are reviewed and reported to the project authority.

The project authority will review errors as necessary and contact the contract laboratory.

If there is disagreement between the CABIN taxonomist and the contract laboratory, the specimen is sent to a recognized expert for verification.

5.3 Reporting

A QC report is generated for each project by the National CABIN laboratory. Reports will quantify aspects of taxonomic precision, assess data acceptability and highlight taxonomic problem areas. Recommendations for improving precision may also be offered.

The National QC Report summarizes trends in taxonomic error and taxonomic efficiency on a national scale. The National Report can assess whether taxonomic errors are isolated or recurring. Assessing the taxonomy on a national level aids in decision making regarding diagnosis and correction of taxonomic errors (Moulton et al. 2000). The result is taxonomic consistency on a national scale.

5.4 Data management

The National CABIN Laboratory maintains and updates nomenclature of the CABIN Database as required. The CABIN taxonomist verifies all new taxa and ensures that the data are consistent with ITIS. Routine audits are performed to ensure that the data conform to standard CABIN naming conventions (Appendix B). Errors are discussed with the project authority and database manager to determine the necessary corrective action.

5.5 Reference collection and voucher specimens

The National CABIN Laboratory houses a physical National Reference Collection and digital catalogue with one or more taxonomic specimens for each taxon in the CABIN Database.

The purpose of the collection is to:

- hold a permanent record of specimens collected as part of the CABIN program
- ensure that future taxonomic comparisons are accurate and consistent
- allow users to search and view particular taxon images and where they have been collected across Canada.

The digital catalogue contains multiple images of a specimen and important features of its group. A map showing all sample locations where specific taxa have been collected in Canada can also be viewed in this catalogue. This catalogue will be routinely updated with new and improved images as additional specimens enter the collection.

About the TAENIOPTERYX Genus

Details

ITIS TSN	102789	CABIN Status	Valid	Site Occurrences	531
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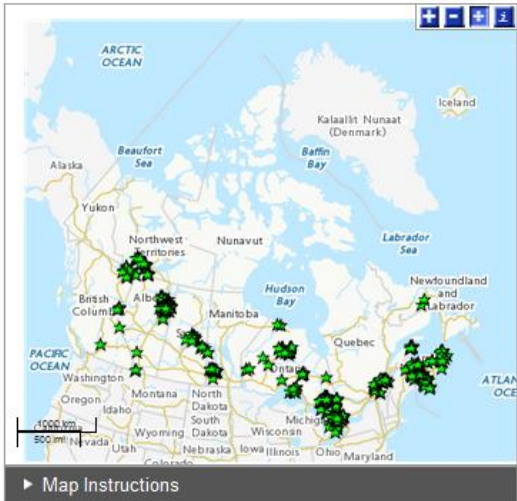


Image Details



Map Instructions

Hierarchy

- [Phylum: Arthropoda](#) (ITIS Record)
 - [Class: Insecta](#) (ITIS Record)
 - [Order: Plecoptera](#) (ITIS Record)
 - [Family: Taeniopterygidae](#) (ITIS Record)
 - [Genus: Taeniopteryx](#) (ITIS Record)
 - [Species: burksi](#) (ITIS Record)
 - [Species: ionicera](#) (ITIS Record)
 - [Species: nivalis](#) (ITIS Record)

Voucher specimens received from CABIN projects are verified in the laboratory or sent to recognized experts, and added to the National Reference Collection. Ideally, specimens in the collection are mature and intact with all key features visible. Certain specimens may be included despite poor condition if they represent the only example of that taxon. All specimens will be verified by the National taxonomist or a recognized expert before addition to the collection. The collection is catalogued and maintained routinely.

Contact Information:

National CABIN Laboratory
 Pacific Environmental Science Centre, Rm 113
 2645 Dollarton Highway
 North Vancouver, BC
 V7H 1B1

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Appendix A: Standard Taxonomic Effort for practical level identifications

Table A. Standard Taxonomic Effort and excluded taxa used by CABIN streams/rivers. Note that wetland samples still require zooplankton identification (refer to section 4.2.5)

Group	Taxa	Level of Identification
Insects	Coleoptera	Genus
	Chironomidae	Genus (Note: require slide mounts)
	Diptera	Genus
	Ephemeroptera	Genus
	Heteroptera	Genus
	Lepidoptera	Genus
	Megaloptera	Genus
	Odonata	Genus
	Plecoptera	Genus
	Trichoptera	Genus
Non-Insects	Amphipoda	Family/Genus
	Bryozoa	Phylum
	Bivalvia	Genus
	Cnidaria	Family/Genus
	Collembola	Family/Genus (with caution)
	Decapoda	Family/Genus
	Gastropoda	Genus
	Hirudinea	Family/Genus
	Hydrachnidae	Family/Genus
	Isopoda	Family/Genus
	Clitellata (Oligochaeta)	Family/Genus
	Platyhelminthes	Class
	Polychaeta	Family/Genus
Excluded Taxa	Cladocera	Taxa are not generally benthic and in some cases can bias samples collected in close proximity to reservoirs or lakes
	Copepoda	Some taxa are small and not adequately sampled using a 400 um kicknet and can be found in extremely high numbers and can bias a sample
	Ostracoda	Taxa can be found in high numbers and can bias a sample
	Rotifera	Taxa are not generally benthic and are not adequately sampled using a 400 um kicknet
	Nematoda	Taxa are not adequately sampled using a 400 um kicknet
	Non-aquatic taxa	Terrestrial drop-ins such as earth worms, spiders and some beetle and bugs are not part of the benthic community
	Porifera	

Appendix B: Errors in taxonomic nomenclature

Table B. Unacceptable naming conventions for taxonomic nomenclature

Designation	Description	Example	Instruction
sp.	Species place holder <ul style="list-style-type: none"> Species place holder for identification to Genus level only 	<i>Baetis sp.</i>	Not accepted
sp.1 or sp. A	Provisional name <ul style="list-style-type: none"> Provisional taxa reported in the literature where a specific identity remains unknown Usually followed by authors name and year in parenthesis 	<i>Cladotanytarsus sp. B</i> <i>Micrasema spA</i> <i>Oecetis sp. A</i> (Floyd, 1995)	Not accepted
()	Additional taxonomic units <ul style="list-style-type: none"> Sublevel taxonomic units included in the taxon names, for example sub genus included in the entry Sublevel taxonomic units entered in incorrect hierarchical position, e.g. taxa entered at tribe level 	e.g. Sublevel inclusions, <i>Nanocladius (Nanocladius) rectinervis</i> e.g. Tribe level designation, <i>Tanytarsini</i>	Not accepted
Group or gr.	Group designations <ul style="list-style-type: none"> Denote a group of more than two closely related species that cannot be separated or A taxon that can be reliably placed in a species group where the determination to species is unsupported 	<i>Rhyacophila vofixa gr.</i> <i>Parachironomus vitiosus group</i>	Not accepted
Provisional or out-of-date names	Incorrect nomenclature	Unpublished name changes or name not recognized in ITIS	Not accepted
Slash Taxa	A/B <ul style="list-style-type: none"> A taxon that has previously been separated and now thought to be inseparable. Sometimes used to communicate uncertainty in the identification but still noted as it can help to determine what the specimen is 'not'. 	<i>Bezzia/Palpomyia</i>	Not accepted

Adapted from Mouton *et al*, 2000

The following is adapted from: Rogers, D.C., A. B. Richards. 2006. *Southwest Association of Freshwater Invertebrate Taxonomists (SAFIT) Rules for the Development and Maintenance of the Standard level of Taxonomic Effort*.

B.1 Slash Taxa

Using a slash (/) to separate two taxon names is a common naming convention. The slash combines two taxa that are inseparable but were at one time considered different or when an individual specimen can only be identified as two possible taxa. This usually happens at the genus level. The CABIN Database cannot distinguish between valid (published) slash taxa and taxonomic opinion and therefore will not accept any slash naming conventions. **Elevate any taxa that cannot be identified with certainty to the next higher level.** Slash taxa designations can be entered into the notes section of the data entry sheet or communicated on bench sheets.

EXAMPLE: Use of slash (/) naming convention

Identification:

Family Ceratopogonidae Genus *Bezzia/Palpomyia*

Decision:

Leave at Ceratopogonidae.

B.2 Provisional names: Group and Species designations

Provisional names are those where the taxonomist has added a 'var. 1', 'sp. A' or 'sp. 1' to the end of the taxon name. The CABIN Database will not accept provisional names, species (e.g. *Baetis* sp.B) or group (e.g. *Orthocladius rivulorum* gr.) designations. Personal identifiers or tags on taxon names cannot be entered into the Database. Any provisional names or designations can be entered into the notes section of the data entry sheet or communicated on the bench sheet.

EXAMPLE: Provisional names

Mysis sp.1, *Baetis* sp.B, *Paracladopelma doris* group, *Orthocladius* (*Euorthocladius*) *rivulorum* gr.

Decision:

Mysis, *Baetis*, *Paracladopelma doris*, *Orthocladius rivulorum*

B.3 Spelling errors

Any taxon that is not recognized from the ITIS Database will automatically be flagged in the CABIN Database and data will be invalid. Often the flag is due to a spelling mistake; please ensure that the taxon name entered has the most current spelling. The structure of the CABIN Database is designed to eliminate typographic errors.

B.4 Taxonomic arrangement and classification

CABIN uses ITIS as the standard for taxonomic arrangement and classification (<http://www.itis.gov>). The CABIN Database will only accept the following taxonomic hierarchies:

Kingdom
 Phylum
 Class
 Order
 Family
 Genus
 Species

Do not enter other units such as *subfamily*, *tribe*, *subgenus*, or *subspecies*.

Additional information regarding taxonomic units can be entered into the notes field within the Database.

B.5 Invalid taxa

New taxa entered into the CABIN Database that have not been previously entered or reported should be accompanied by voucher specimens, which are sent to the National CABIN Laboratory for verification. Every voucher specimen submitted should be accompanied by a copy of the appropriate literature to support the identification. Without a voucher specimen, the data associated with that taxon will remain unverified and will not be included in analyses conducted using CABIN analytical tools.

In cases where the current taxon name is absent from ITIS, the previous name will be used as a default until ITIS is updated. It is advised that a taxon be entered as the last reported name as listed in ITIS and the 'new' name recorded in the notes section. ***Adding this name to the notes section ensures that when ITIS is updated the data can be changed accordingly.*** There will be a period of delay from new publications, to updating of ITIS and the subsequent updating of the CABIN Database.

EXAMPLE: Addition of taxa (not in ITIS)

Identification:

Helodon (Simuliidae)

Some subgenera of the genus *Prosimulium* s.l. have been removed from that genus and reassigned to a new genus - *Helodon* s.l. Within this new genus, we now recognize three subgenera (*Distosimulium*, *Parahelodon*, and *Helodon* s.s) based on Adler, P.H., D.C. Currie, and D.M. Wood. 2004. The black flies (Simuliidae) of North America. Cornell University Press. 941 pp.

Decision:

The genus name *Helodon* will be added to the notes column of *Prosimulium*. CABIN advises that the taxon be entered as the last reported name as listed in ITIS and that the 'new' name be recorded in the notes section.

Appendix C: Guidance for project authorities and taxonomists – a summary of taxonomic responsibilities

C.1 Project Authorities

The project authority shall clearly identify in the taxonomy contract the activities and deliverables they require of the taxonomist which may include any or all of the following:

- Proof of taxonomic certification or relevant experience
- Acknowledgement of sample shipment
- Comparison of samples received against sample manifest
- Transfer of samples from formalin to ethanol
- Maintenance and storage of samples
- Subsampling according to the CABIN protocol
- Identification of organisms to specified level of resolution
- Identification of samples according to acceptable CABIN nomenclature
- Data upload to the CABIN Database or data reported in a specified format
- Report of internal QAQC
- Compilation of reference collection with appropriate labels and contents summary documentation
- Shipment of voucher specimens to National CABIN Laboratory
- Shipment of samples to Project Authority
- Shipment of 10% of reference samples (or a minimum of 3 per project) to the National CABIN Laboratory for audit of sorting efficiency and identification error
- Responsibility for corrective action identified by the National CABIN Laboratory audit

Additional recommendations for the project authority include:

Sample labels: Ship all sample material in clearly labelled containers to the taxonomist. Labels should include: site code, date, number of jars, and preservative.

Sample manifest: Include inside the shipment a sample submission form or chain of custody with all benthic samples and relevant information such as the CABIN study name where data will be entered, sampling date, number of sample jars, preservative, and notes about the samples that the taxonomist should be made aware of. An electronic copy may also be sent to the taxonomist.

Preparing for data upload: Enter all site visit and sample information into the CABIN Database so that taxonomic data can be quickly uploaded when completed. Taxonomy data cannot be uploaded if the site visit and sample information is not entered into the CABIN Database.

Data upload: If the taxonomist is not contracted to upload the data, the project authority must ensure that the data meet the nomenclature standards for CABIN and adhere to ITIS. The project authority will be responsible for sending any voucher specimens or correcting invalid taxon names that are identified in the data entry process.

National CABIN audits: Samples from Reference sites may be used in reference model development and therefore used by others in the network. As such these samples will undergo an audit by the National CABIN Laboratory. Ten percent of reference samples or a minimum of 3 per

project (sorted residue and identified organisms) should be sent to the **National CABIN Laboratory** for QAQC audits on sorting efficiency and taxonomic errors upon receipt of the completed samples from the taxonomist. The taxonomist may be instructed to send 10% of the samples directly to the CABIN Laboratory. If so, the project authority should identify the samples to be sent; the taxonomist should not select the samples to be audited.

Note: If any specimens were taken out of the samples to be audited (e.g., for a reference collection or voucher specimens), this information should be documented and relayed to the National CABIN Laboratory or it may result in a failed audit as a result of missing organisms that cannot be confirmed.

Please provide advance notice to the National CABIN Laboratory that samples will be arriving for auditing with the following information:

- number of samples or vouchers (with proper labeling)
- general geographical location information for the samples (e.g., watershed)
- CABIN study name
- Taxonomist contact information
- Project authority contact information

National CABIN Laboratory

Pacific Environmental Science Centre, RM 113
2645 Dollarton Highway
North Vancouver, BC
V7H 1B1

Voucher Specimens: A new taxon name that has not been previously entered into the CABIN Database may be identified. This name should be valid in ITIS and a voucher should be sent to the National CABIN Laboratory for expert verification and addition to the National Reference Collection. The CABIN user who enters the taxonomic data will receive an automatic notification requesting a voucher if a new taxon is entered into the Database. This taxon remains unverified in the Database until it can be verified at which point the voucher will become part of the National Reference Collection.

Reference Collections: The National CABIN Laboratory does not require reference collections for each project. A collection should only be requested as part of the contract with the taxonomist if the project authority wishes to have and maintain such a collection.

C.2 Taxonomist

The taxonomist shall follow the requirements according to the signed contract, which may include some or of all of the following terms:

Sample receipt: Discrepancies or feedback regarding sample integrity should be communicated to the project authority as soon as possible. Transfer samples from field preservative to ethanol if required. Process samples in accordance with CABIN Laboratory Methods manual.

Provide a report of internal QAQC procedures and results to the project authority as specified in the contract.

Data Entry/Reporting: Enter taxonomic data into the CABIN Database, if certified and required by the contract. Taxonomic data should be entered/reported according to the CABIN nomenclature standards (refer to Appendix B). All taxon names should be valid in ITIS (<http://www.itis.gov>). Taxonomic names not currently updated in ITIS should be entered as the valid ITIS name with a note as to its recognized name so that this information can be updated in the future. CABIN does not accept sub- or intermediate classifications such as subfamilies or suborders. This information may be added as a note.

Voucher specimens: Send new taxa (those that have never been entered into the CABIN Database previously) to the National CABIN Laboratory for expert verification when requested by the automated CABIN notification during data entry. Include CABIN study name, site code, date collected, date identified and taxonomist with all voucher specimens. Ideally vouchers include more than one intact mature specimen, collected together at one place and time. If only one specimen is submitted, it will also serve as the reference specimen for the National Reference Collection.

Reference Collection: Create a reference collection of all identified organisms within the project labelled with all relevant data if required by the contract for the project authority; the National CABIN Laboratory does not require a reference collection for each project. Ensure that labels include the information identified in the CABIN Laboratory Methods manual. A spreadsheet documenting all the specimens and associated information contained in the reference collection should also be provided to the project authority.

Sample completion: Send all material (sorted and unsorted residues, identified organisms, reference/voucher specimens) to the project authority in clearly labelled containers as required by the contract.

If required to send samples or vouchers directly to the National CABIN Laboratory, please provide advance notice samples/specimens will be arriving for auditing with the following information:

- number of samples or vouchers (with proper labeling)
- general geographical location information for the samples
- CABIN study name
- Taxonomist contact information
- Project Authority contact information

C.3 The National CABIN Laboratory

The National CABIN Laboratory will conduct audits on 10% of all reference and potential reference samples (or a minimum of 3) in a project. Samples received from the taxonomist or project authority will be audited for sorting efficiency (SE) and identification errors (IE) according to the methods described in the CABIN Laboratory Methods manual.

Sample reception: The project authority should contact the National CABIN Laboratory to indicate that samples will be submitted for auditing.

QAQC reports: The National CABIN Laboratory will provide QAQC audit reports to the project authority and taxonomist with all final results and recommendations for corrective actions required.

1. %SE will be calculated as an average SE from all samples received for a single project. A failed average SE is $\leq 95\%$ and corrective actions will be outlined by the CABIN taxonomist in the QAQC report. A project may also fail the audit if one group of organisms is consistently missed, even if the average SE is $>95\%$.
2. %IE will be calculated as an average IE from all samples received for a single project. Only identification errors will count towards the %IE. Identification errors are counted for each organism not each taxon. A failed average IE is $\geq 5\%$ and corrective actions will be outlined by the CABIN taxonomist in the report. Each sample that is $\geq 5\%$ IE will be examined further for repeated errors and patterns.
3. Other taxonomic flags such as enumeration or resolution errors will be identified in the report with recommendations where appropriate but do not count towards the final %IE.

Verifications: Conflicting identifications determined by the CABIN taxonomist in the audit will be verified by a second taxonomist or expert.

Sample storage: The National CABIN Laboratory will return audited reference samples to the appropriate project authorities after the audit has been completed. If the project manager does not wish to store and maintain these samples, the unsorted residual will be retained at the National CABIN Laboratory for a minimum of 3 years. When space becomes limited, older samples will be discarded. Voucher specimens will be incorporated into the National Reference Collection.