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Laboratory Methods

Processing, Taxonomy, and Quality Control
of Benthic Macroinvertebrate Samples

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Aussi disponible en français

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1 Introduction

The Canadian Aquatic Biomonitoring Network (CABIN) is the national biomonitoring program developed by Environment 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 (<http://ec.gc.ca/rcba-cabin/>).

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: www.ec.gc.ca/cabin-rcba

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):

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

Australian Government, Department of the Environment and Heritage. WATER
ECOscience Report Number: 543. 45 p.
Program link: <http://ausrivas.canberra.edu.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
- for river/stream 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
- 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.*

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 (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 (www.nabstcp.com).

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
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 $\geq 95\%$ sorting efficiency	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 Interacts with other taxonomists through professional societies or workshops Maintains appropriate literature
QC Auditor: Taxonomy	Re-identify 10% of samples (or a minimum of 3) to ensure $\leq 5\%$ identification error rate	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

Shipping of preserved biological samples requires 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>.

3.2.1 Shipping samples to a taxonomy laboratory

Preserved² samples should be shipped to the taxonomic laboratory as soon as possible. Timely shipping and processing can reduce damage caused by preservation.

3.2.2 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 70% ethanol upon receipt. A sieve with a mesh size of 400 µm or less should be used. Inspect samples and replace evaporated ethanol every three months.

3.2.3 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. Reference collections and 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.

3.2.4 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 must ensure voucher specimens are forwarded to the National CABIN Laboratory for verification and archiving.

3.2.5 Sample storage by a project authority

Storage time for archived samples depends on the goal of the project. CABIN recommends keeping all samples from reference sites including the sorted and unsorted residues in the event that further analysis is required. Test samples are generally held for three years past the publication of data. Inspect samples and replace evaporated ethanol every three months.

² Fix samples in buffered formalin for 48 hours before transferring to ethanol. Formalin is acidic; long term storage can lead to the decalcification of shells. Use buffered formalin to reduce the degree of degradation. Transfer samples fixed in formalin to ethanol within 7 days, if possible.

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 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 and streams 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 are required. If more than 50% of the sample is needed to obtain 300 organisms, the entire sample is processed.

Recently, the number of cells needed to complete the sample has changed from one to five. 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.

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

4.2.1 Equipment and materials

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

Table 2. Materials required for sub-sampling and sorting.

(✓)	Equipment or materials
	Marchant box
	U.S. 35 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

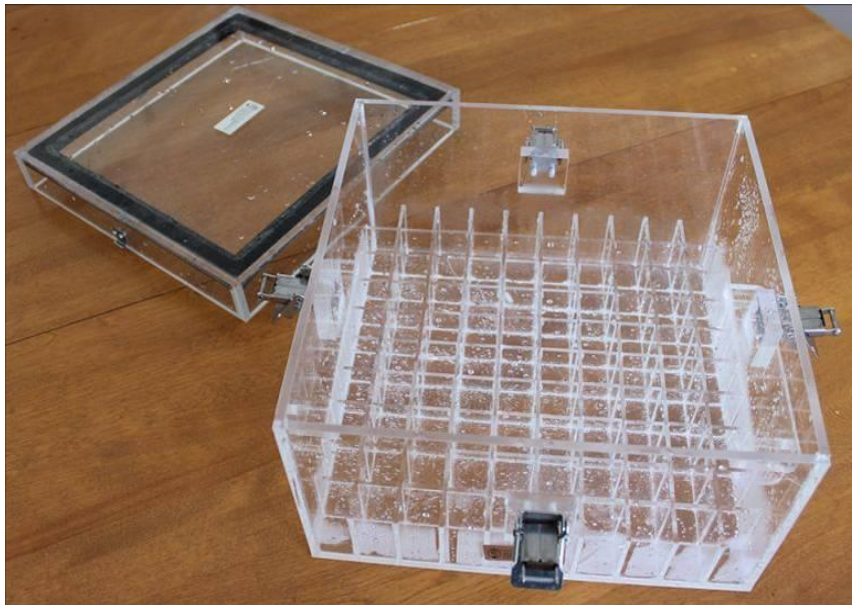


Figure 1. The Marchant Box.

4.2.2 Subsampling and sorting protocol

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

1. Wash the sample into a sieve 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 water tight.
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.

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.**
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.

Subsample by Weight

Samples may also contain large amounts of filamentous algae or moss, making the Marchant box ineffective in subsampling. Instead, the mass of algae or moss 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.
5. Record the total number and weight of subsamples selected and the number of subsamples needed to reach the individual count of 300.

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/Rotifera	Taxa not generally benthic and can bias samples collected in close proximity to lakes
Copepoda, Harpacticoida	Taxa can be pelagic and benthic. Benthic taxa are not adequately sampled using a 400 μm kicknet
Porifera	Taxa are colonial and cannot be quantified as number of individuals per sample like other benthic taxa
Nematoda, Nematomorpha, Nemertea	Taxa are not adequately sampled using a 400 μm kicknet
Platyhelminthes	Taxa are not adequately sampled using a 400 μm kicknet
Non-aquatic taxa	Terrestrial drop-ins such as earth worms, spiders, and some beetles and bugs are not part of the benthic community

4.2.3 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. Samples should be selected by someone other than the original sorter.
2. Re-sort the residue from 10% of the project samples (minimum of 3).
3. Record the number of organisms found.
4. Calculate %SE using the equation:

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

Total organisms found include 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 < 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				

Criteria for Sorting Efficiency Corrective Measures

All samples in the project should be re-sorted if:

a) the sorting efficiency rate is $\leq 95\%$

OR

b) an entire class of organisms are overlooked

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 are 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 taxonomy 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
	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 important to also 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, and in the case of Oligochaeta, sufficient number of segments. 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. A standard for nomenclature improves consistency

among taxon names and in the level of identification (Appendix B). There is a comments option when entering benthic data into the CABIN database where any additional information can be recorded, such as slash taxa, species groups and tribes.

4.3.4 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.

Samples are randomly selected by the project authority. Audits should be performed by someone other than the original taxonomist. 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 potential errors related to taxonomy

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.

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 QC 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 2 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

- missing specimens (e.g. retained in a reference collection and not sent with rest of sample)

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

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

The average error rate of audited samples should be $\leq 5\%$. All samples that exceed a 5% 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.

If specimens are not sent with the audit sample (e.g. slides missing or left with a reference collection), they will be identified as an identification error. All organisms must be accounted for in the audit.

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) $\geq 5\%$, 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	<i>Promoresia</i>	103	102			Enumeration (-1)
Diptera	Ceratopogonidae	<i>Mallochohelea</i>	1	0		1	Misidentification; identified as <i>Probezzia</i> ; anterior of head capsule narrowed
	Ceratopogonidae	<i>Probezzia</i>	1	2			Confirmed by second taxonomist
	Chironomidae	<i>Cardiocladius</i>	1	1			
	Chironomidae	<i>Eukiefferiella</i>	2	1			1 specimen identified to other taxon
	Chironomidae	<i>Labrundinia</i>	1	1			
	Chironomidae	<i>Micropsectra</i>	11	9			2 specimens identified to other taxon
	Chironomidae	<i>Orthoclaadiinae</i>	3	0			Insufficient resolution; 3 specimens identified to other taxon
	Chironomidae	<i>Orthocladus</i>	19	16			3 specimens identified to other taxon
	Chironomidae	<i>Rheocricotopus</i>	2	3			1 specimen from other identified taxon
	Chironomidae	<i>Stempellina</i>	3	3			
	Chironomidae	Tanypodinae	13	0	13		Insufficient resolution; identified as <i>Rheopelopia</i> ; Enumeration (-1)
	Chironomidae	<i>Cricotopus</i>		3		2	Misidentification; 2 specimens from other identified taxon, Enumeration (+1)
	Chironomidae	<i>Rheopelopia</i>		12			
	Chironomidae	<i>Polypedilum</i>		1		1	Misidentification; 1 specimens from other identified taxon
	Chironomidae	<i>Pseudosmittia</i>		4		4	Misidentification; 4 specimens from other identified taxon
	Chironomidae	<i>Zalutschia</i>		1		1	Misidentification; 1 specimens from other identified taxon
	Simuliidae	<i>Simulium</i>	1	0		1	Misidentification; identified as <i>Prosimulium</i> ; distal article of antennae pigmented in contrast to anterior colourless articles
	Simuliidae	<i>Prosimulium</i>		1			Confirmed by second taxonomist
Ephemeroptera	Baetidae			3	3		Questionable resolution

Order	Family	Genus	Raw Count	Audit Count	Audit Flags	IE error	Comment
	Baetidae	<i>Acerpenna</i>	28	26			2 rolled up to family, no cerci or gills visible
	Baetidae	<i>Baetis</i>	10	9			1 rolled up to family, no cerci or gills visible
	Ephemerellidae		6	28	22		Questionable resolution; Enumeration (+1)
	Ephemerellidae	<i>Ephemerella</i>	25	4			Questionable resolution; 21 rolled up to family, early instar
	Heptageniidae		8	8			
	Leptophlebiidae	<i>Paraleptophlebia</i>	17	17			
Plecoptera	Leuctridae		3	0			3 identified as Leuctra; corners of pronotum with 4 long hairs, paraprocts with long bristles
	Leuctridae	<i>Leuctra</i>		4	4		Insufficient resolution; Enumeration (+1); confirmed by second taxonomist
	Perlodidae		9	3			6 identified as Isoperla
	Perlodidae	<i>Isoperla</i>		6	6		Insufficient resolution; confirmed by second taxonomist
	Taeniopterygidae	<i>Taeniopteryx</i>	2	2			
Trichoptera	Brachycentridae		1	1			
	Hydropsychidae	<i>Hydropsyche</i>	4	4			
	Hydroptilidae	<i>Hydroptila</i>	1	1			
	Lepidostomatidae	<i>Lepidostoma</i>	7	7			
	Rhyacophilidae	<i>Rhyacophila</i>	7	7			
Veneroida	Pisidiidae		5	0	5		Identified as Pisidium
	Pisidiidae	<i>Pisidium</i>		5			Insufficient resolution; confirmed by second taxonomist
		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, the following protocols should be followed. Additional information (such as special species designations) gathered during the identification process are not accepted in the CABIN Database (Appendix B). This information should be reported to the project authority and can be appended to the data using the notes field of the taxonomy data entry page.

Taxonomists can enter data directly into the CABIN Database. The Data Entry Module of the Online CABIN Training Program should be completed in order to obtain a username and password for the CABIN Database.

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 bench sheets 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. Reference collections for individual CABIN projects are not required by the National CABIN Laboratory. 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

A reference collection may be required by the project authority for a CABIN project. 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 should be preserved in 70% ethanol. All specimens should be 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.

Sample processing Guidance 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 to ensure the national consistency of taxonomic records in the CABIN database. The CABIN taxonomist audits samples based on 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 national 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 reference samples collected that may be used for reference model development are audited. Samples may come from regional Environment Canada offices, other federal departments or provincial and territorial departments.

The National CABIN laboratory performs audits on reference samples that may be used for reference model development and available to all network participants.

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 provided 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. Project sorting efficiency audits are carried out on a regular basis in order to establish a standard sorting efficiency. An average sorting efficiency of 95% should be achieved on 10% of samples or a minimum of 3 samples in a project.

Project Sorting Efficiency Corrective Measures

Samples 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 (%IE) and should not exceed 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 %IE 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 and provided to the project authority. 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 report can assess whether taxonomic errors are isolated or recurring. Assessing taxonomy 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 National Reference Collection 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

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 taxa may be included despite poor condition if they represent the only specimen 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

Email: cabintaxonomy@ec.gc.ca

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Appendix A: Standard Taxonomic Effort for practical level identifications

Table A. Standard Taxonomic Effort and excluded taxa used by CABIN.

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
	Polychaeta	Family/Genus
Excluded Taxa	Cladocera/Rotifera	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
	Nemata	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	
	Platyhelminthes	Taxa are not adequately sampled with a 400 um kicknet

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., and 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 Synonyms

CABIN recognizes that ITIS has some limitations; it may not be in agreement with the most recent findings in taxonomic research. Due to this limitation, a 'synonym' field in the Database will list any nomenclature changes in that field until they are reflected in the ITIS Database. 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.

Synonym suggestions will not be accepted in the Database directly from the taxonomist. Taxonomists can submit suggestions for name changes by sending a justification, with rational and appropriate references, by email to the National CABIN Laboratory cabintaxonomy@ec.gc.ca

EXAMPLE: *Oligochaeta* vs. *Clitellata*

Propose to change name to *Oligochaeta* and list *Clitellata* as a synonym.

Justification:

After the DNA confirmation that the traditional classification of *Clitellata* into *Hirudinea* and *Oligochaeta* was no longer appropriate, the names *Clitellata* and *Oligochaeta* became synonyms. Either name is optional as they refer to a rank above family group level and therefore have no priority rule as outlined in ICZZN 1999 (Erseus et.al, 2008).

Decision:

Leave name at *Clitellata* as in ITIS and list *Oligochaeta* as a synonym.

B.2 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. Slash taxa designations can be entered into the taxonomy notes section of the database.

B.3 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. *Orthocladus 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:

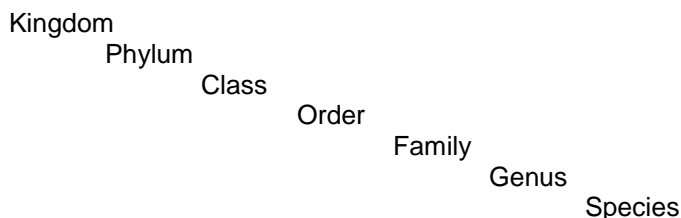
Mysis, *Baetis*, *Paracladopelma doris*, *Orthocladius rivlorum*. Provisional names or designations can be entered into the taxonomy notes section of the database.

B.4 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.5 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:



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.6 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 for National audit of sorting efficiency and identification error to National CABIN laboratory, **including any specimens set aside for a reference collection**
- 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 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 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 (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 that should 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 (cabintaxonomy@ec.gc.ca) 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
- Taxonomic level (Family or Genus) identified as specified in the contract

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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 (e.g., have never been entered into the CABIN Database previously) to the National CABIN laboratory for expert verification with all relevant data (CABIN study name, site code, date collected, date identified, taxonomist) when requested by the automated CABIN notification during data entry. 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 samples from potential reference sites. Samples received from the taxonomist or project authority will be audited for sorting efficiency (SE) and identification errors (IE) according to the methods describe 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 <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, regardless if the average SE is >95%.
2. %IE will be calculated as an average IE from all samples received for a single project. Only misidentification 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.