Environment Canada Environnement Canada

CABIN
CANADIAN AQUATIC BIOMONITORING NETWORK

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 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 office or laboratory participating in the CABIN program must implement the prescribed quality assurance and quality control (QA/QC) 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 managers 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.

The objectives of this document are to provide:

- Requirements to assure quality in the processing and 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 must process samples and provide data using the following methods.

Details of field sampling procedures, data analysis and associated QA/QC procedures can be found in other CABIN documentation.

The preparation of this manual relied on protocols developed by other authors and is adapted from existing QA/QC programs. In particular:

CABIN: Reynoldson, T.B., C. Logan, T. Pascoe and S.P. Thompson. 2001. CABIN (Canadian Aquatic Biomonitoring Network) Invertebrate Biomonitoring Field and Laboratory Manual, National Water Research Institute, Environment Canada 47 pp.

AUSRIVAS: 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. Prepared for the Australian Government, Department of the Environment and Heritage. WATER ECOscience Report Number: 543 Program: http://ausrivas.canberra.edu.au/

USGS: Moulton, S.R., Carter, J.L., Grotheer, S.A., Cuffney, T.F., and Short, T.M. 2000. Methods for analysis by the U.S. Geological Survey National Water Quality Laboratory – processing, taxonomy, and quality control of benthic macroinvertebrate samples: U.S. Geological Survey Open-File Report 00-212, 49 p.

EPA: Barbour, M.T., J. Gerritsen, B.D. Snyder and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic invertebrates and fish. Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

2 Taxonomic Services

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
- Subsample using a Marchant box (Marchant 1989) to a minimum 300 organisms (lake samples do not require this step; do not subsample lake samples)
- Identify specimens to the lowest taxonomic level according to the specified taxonomic effort
- Implement QC protocols for sample sorting and identification
- Create a reference collection if required
- Enter taxonomic data into the CABIN database if required
- Provide a voucher specimen to the National CABIN Laboratory in a timely fashion, if required *
- Return identified samples, reference collection and debris to the project authority

**NOTE: The project authority is responsible for submitting necessary voucher specimens.*

3 Quality Assurance

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

3.1 Taxonomic laboratory requirements

Sample processing and taxonomic identifications are performed by both internal and external taxonomic laboratories. The requirements of a qualified taxonomic laboratory are as follows:

- Adequate technical and taxonomic literature
- Adequate sample processing equipment
- Established standard operating procedures
- QA/QC measures for sorting, subsampling and identification
- Minimum two people involved in providing taxonomic services; one to process samples and one to perform QC audits (Table 1). These people may be from different laboratories
- CABIN Certification OR a pending registration for the online CABIN data entry module (http://www.unb.ca/research/institutes/cri/opportunities/courses/cabin-rcba/index.html)
- Completed CABIN Data Entry module (if required to enter data)

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

- A combination of experience and training that demonstrate current knowledge and professional development in benthic macroinvertebrate taxonomy
- Experience or expertise in the identification of taxa within the specified study region

CABIN does not require use of a certified taxonomist although it is strongly recommended. The North American Benthological Society (NABS) 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 NABS website (www.nabstcp.com).

Table 1: Personnel, responsibility and qualifications required by taxonomic laboratories.

Person	Responsibility	Qualifications
Sample Processor/ Subsampler	Transfer and wash sample Subsample using Marchant box	Trained with Marchant Box
Sorter	Pick macroinvertebrates out of debris Sort 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	Experienced in sample sorting **Must be someone other than the sorter**
Taxonomist	Identify samples according to contract requirements	Trained in taxonomic identifications of 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 ≤ 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

3.2.1 Shipping samples to taxonomy laboratory

Samples must be shipped to the taxonomic laboratory as soon as possible. To avoid the break down of calcified organisms and the clearing of pigments, do not store samples in formalin for long periods[1]. Timely shipping can prevent damage caused by preservation in formalin.

Shipping of biological samples requires training in the Transportation of Dangerous Goods (TDG). It is prohibited to send flammable liquids without training and certification. Information for TDG regulations can be found at http://www.tc.gc.ca/tdg/menu.htm.

[1] Fix samples in buffered formalin for 48 hours before transferring to ethanol. Formalin is acidic, long term storage can lead to decalcification shells. Use buffered formalin to reduce the degree of degradation. Transfer samples fixed in formalin to ethanol within 14 days.

3.2.2 Sample receiving by taxonomy laboratory

Samples received by the taxonomic laboratory must 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 must be used. Replace evaporated ethanol every three months.

3.2.3 Sample shipping to project authority

All samples and sample residues (sorted and unsorted) must be returned to the project authority and shipped in 70% ethanol, unless otherwise specified in the contract. Reference collections and vials must be carefully packaged, labeled and returned to the project authority. Voucher specimens must be sent to the National CABIN laboratory for verification and archiving.

3.2.4 Sample receiving by project authority

Samples received from the taxonomic laboratory must be checked against the sample submission form to ensure the shipment is complete. Voucher specimens must be forwarded to the National CABIN taxonomy laboratory for verification and archiving.

3.2.5 Sample storage by 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 debris in the event that further analysis is required. Test samples are generally held for three years past the publication of data. Replace evaporated ethanol every three months.

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 must be transferred to 70% ethanol, including samples fixed in ethanol. Ethanol used to preserve samples in the field may have evaporated, leaving an unknown concentration of preservative; this will lead to decomposition of 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.

Check 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. **Sorting** refers to the removal of benthic macroinvertebrates from the sample matrix into coarse taxonomic groupings (Moulton et al. 2000).

Subsampling for CABIN is done with a Marchant Box (Marchant 1989) following the protocol outlined below. Samples are not separated into different size fractions for processing. A minimum count of 300 individuals is required. If more than 50% of the sample is needed to obtain 300 organisms, the entire sample is processed.

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.

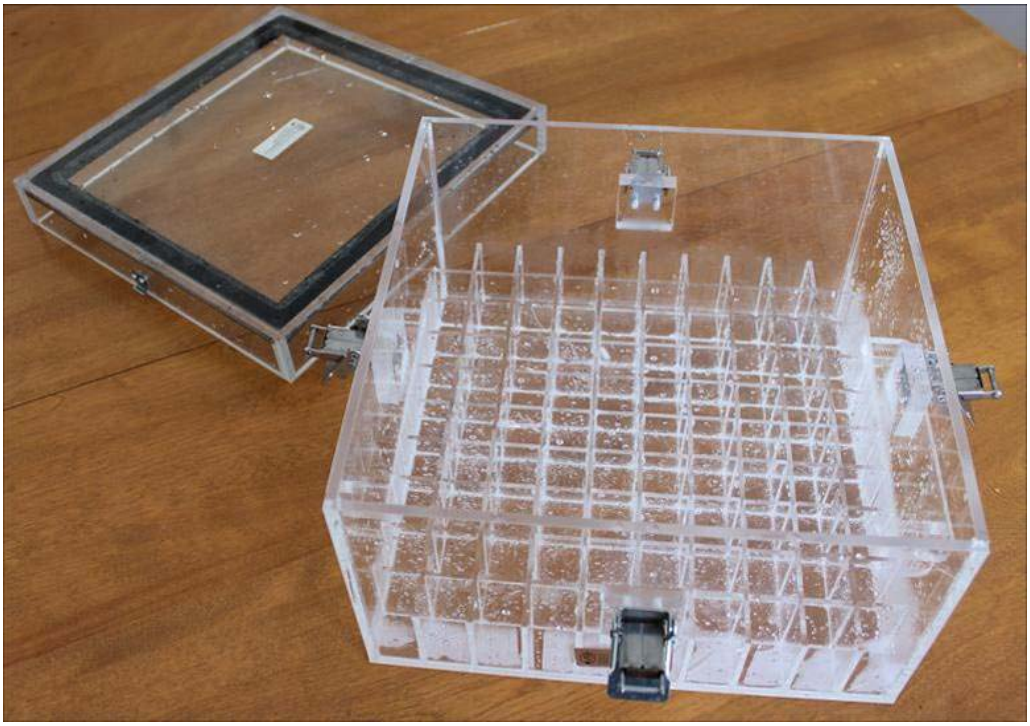
If a sample was elutriated in the field and the elutriate (i.e. heavy inorganic debris, sand and pebbles) was submitted for QC purposes, the elutriate MUST BE examined before subsampling. Any organisms removed from the QC audit of elutriate must be recorded (order/family level) and added to the sample for subsampling. A record of the organisms missed in the elutriate should be submitted with the QA/QC report to the project authority.

4.2.1 Equipment and materials

Table 2: Material required for sub-sampling and sorting.

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* *Water proof paper for labels*	*White sorting trays* *Bench or tally sheets* *Dissecting microscope with light source (10–40×)* *Forceps* *Squeeze bottles (for water and ethanol)* *Specimen vials, caps or stoppers* *Probes (fine tipped and blunt)* *70% ethanol*

Figure 1: The Marchant Box



4.2.2 Subsampling and sorting protocol

1. Wash the sample into a sieve to remove preservative.
2. Wash large material, rocks, twigs, macrophytes gently and thoroughly over the sieve. Return washed material to the 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 the first couple of rows.*
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. If the 300 count is reached part way through a cell, the entire cell must 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 processed.**
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.

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

Table 3: Taxa not included in the 300 organism count for CABIN samples.

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

4.2.3 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 must be carried out on a regular basis to establish a standard sorting efficiency.

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 2).
3. Record the number of organisms found.
4. Calculate % sorting efficiency (%SE) is using the equation:

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

The average *%SE* is calculated based on the number of re-sorted samples, and represents the standard sorting efficiency for that project (Table 4).

- **If the average sorting efficiency is < 95%, all samples in the project must be re-sorted**.
- Notify the sorter of organisms missed to rectify the problem.
- If an entire class of macroinvertebrates is overlooked by the sorter (for example, mollusks consistently not identified and left in the sample residue) all samples in the project must 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 for samples 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 for samples that did 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

For taxonomists or laboratories that have not met the minimum qualifications as outlined in Section 3.1, the following verification schedule is proposed. The sorting audit results must be communicated to the project authority as each step is completed and a pass must be achieved before proceeding with the remaining samples.

- First **5** samples are verified
- **20%** of the next 10
- **10%** of the remaining

Corrective Measures

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

a) the sorting efficiency rate is ≤ 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 national database. The preparation of reference collections and voucher specimens are outlined. Details of the level of effort, nomenclature and the auditing protocol for identification error rate are also provided in the following sections.

The essentials for identification are as follows:

1. Identifications must be based on current published taxonomic references.
2. Nomenclature must conform to the Integrated Taxonomic Information System (ITIS), available on the US 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 must be submitted with the processed samples.

4.3.1 Equipment and Materials

The following is the minimum equipment required for taxonomic identifications.

Table 6: List of essential equipment and materials for taxonomy identification.

Dissecting microscope 10-80x with fiber-optics or other adequate light source Petri dishes Euparol, 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 (STE)

CABIN analysis is currently performed on family level data, which is the minimum level of identification required. With reference site data, it is recommended that identifications be taken to the lowest practical level. Taxa should be identified to the Standard Taxonomic Effort (Appendix A). Some taxa are not included in the sample counts (Appendix A).

Identify specimens to genus/species only if undamaged and mature organisms are available. Use caution when identifying early instar or juvenile specimens to lower levels. Specimens must 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 macroinvertebrate groups require slide mounting for genus or species level identification.

4.3.3 Nomenclature

CABIN uses the Integrated Taxonomic Information System (ITIS) as the standard for taxonomic nomenclature and classification. The ITIS database is reviewed periodically to ensure valid classifications, revisions and additions to species lists; it represents a fair consensus of modern taxonomic opinion. ITIS is supported by The North American Benthological Society as its official source of current nomenclature for aquatic macroinvertebrate taxa associated with their taxonomic certification 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 taxa may be reflected in the taxonomic 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 also uses a number of naming conventions to improve consistency between taxon names (Appendix B).

4.3.4 Auditing protocol

For each project, 10% of samples (or a minimum of 2) must be audited. The audit is not simply a comparison of taxa lists; it is a complete re-identification and enumeration of selected samples.

Samples are randomly selected by the project manager. Audits must 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 an external laboratory.

There are several types of errors that contribute to misidentification (Table 7):

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 error: Incorrect genus

A specimen is identified as **genus *Swelsta***. 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 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 macroinvertebrates
- Poor counting protocols (e.g. heads only or bodies counted)
- Specimen degradation

Corrective Measures

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

The average error rate of audited samples must be ≤ 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.

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

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

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.***

For taxonomists or laboratories that have not met the minimum qualifications as outlined in Section 3.1, the following verification audit schedule is proposed for the first project. The audit results must be communicated to the project authority as each step is completed and a pass must be demonstrated before proceeding with the remaining project samples.

- First **5** samples are verified by a third party
- **20%** of the next 10 samples
- **10%** of the remaining samples

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

Table 7 : Identification QC audit report

Taxon	Laboratory counts	QC audit counts	Agreement	Mis-identification	Questionable Taxonomic Resolution	Enumeration	Insufficient Taxonomic resolution	Comments
Baetis tricaudatus	3	2	N		**X**			Two correct, one specimen elevated to *Baetis*
Baetis	0	1	N	**X**			**X**	QC to genus, no caudal filaments Elevated *B.tricaudatus* to *Baetis*
Caudatella	1	0	N	**X**				1 Ephemerella
Ephemerella	3	2	N	**X**				1 Seratella
Micrasema	30	28				**X**	**X**	Counted and confirmed at 28. Left two at Family, insufficient features to take to genus.
Isoperla	22	22	Y					
Trichoptera	3	0	N				**X**	Identified to Order
Uenoida	0	3	N			**X**	**X**	QC audit identified to Family
Sweltsa	30	28	Y			**X**		Counted and confirmed at 28
Totals	**92**	**86**		**3**	**1**	**3**	**4**	
Total misidentification rate = $\frac{3}{86}*100 = \%\ Identification\ Error$ **3.5% (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 must 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 CABIN online course must be completed in order to obtain a username and password for the CABIN database.

Data entry criteria must follow the naming conventions outlined in Appendix A.

4.4.1 Data Auditing

Taxonomic hierarchy and nomenclature present are the largest sources of error in data entry. Taxonomy laboratories must submit bench sheets to the project authority for verification of any data entry errors. The project authority is responsible for auditing and ensuring data entry errors are corrected. 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. Reference collections are invaluable to the credibility of CABIN projects. Reference collections are used to:

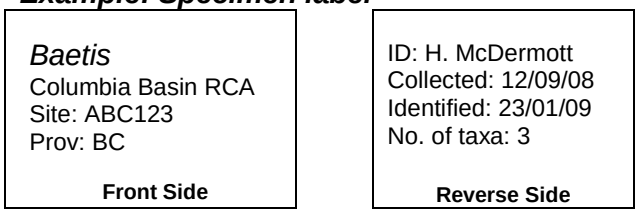
- ensure taxonomic consistency
- assure repeatability and independent verification, or re-evaluation of the study result
- allow for historical comparisons

A reference collection may be required by the project authority for every CABIN project. The reference collection and associated documentation must be submitted to the project authority at the end of the contract.

Specimens in a reference collection are preserved in 70% ethanol. All specimens must be stored in sealed vials with appropriate labels. Labels are printed on waterproof paper with pencil or laser ink. **The collection must 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.

1. Specimen name
2. CABIN Study name (Project name)
3. Site code (from which it 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

Example: Specimen label



4.6 Voucher specimens

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

Voucher specimens must be submitted to and verified by the National CABIN laboratory before they are added to the valid taxa list in the CABIN database. Unverified taxa may be entered into the database, but the taxon counts are not pooled with the remainder of the biological data until a voucher specimen is received and verified by the CABIN laboratory.

Voucher specimens are preserved in 70% ethanol and labeled in the same way as the reference collection. **Each vial is labeled and specimens must be accompanied by a spreadsheet detailing the information provided on the specimen labels.** All voucher specimens are sent directly to the National CABIN laboratory from the contracting taxonomy lab or by the project authority. Voucher specimens will be verified in the CABIN laboratory by the CABIN taxonomist. Any taxa that require expert opinion (e.g., species level identifications) will be sent to the appropriate recognized expert.

Verification of voucher specimens at the family and genus level take priority over species level identifications to expedite data analysis conducted at higher taxonomic levels.

All voucher specimens must be sent to:

National CABIN Laboratory Rm113
Pacific Environmental Science Centre
2645 Dollarton Highway
North Vancouver, BC
V7H 1B1

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

Taxon counts are not pooled with the remainder of the biological data until a voucher specimen is received and verified by the CABIN laboratory.

5 The National CABIN Laboratory

The National CABIN laboratory provides the taxonomic QA/QC function for the CABIN program in support of the reference collections, database and assessment models. The CABIN taxonomist audits and processes samples by using the methods outlined in the previous sections.

The laboratory houses an extensive collection of taxonomic literature to support the identification of macroinvertebrates for QC audits. Literature is routinely reviewed and updated. The CABIN taxonomist attends annual 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 Canada offices or from partners such as Parks Canada, Department of Fisheries and Oceans or provincial and territorial departments.

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

The QA/QC audit in the National CABIN laboratory does not replace the QC requirement of 10% re-identification by a third party.

5.1 Verification of sorting efficiency

The precision of sorting is calculated as percent sorting efficiency (*%SE*) by examining randomly selected sample residues, as described in section 4.2. Audits are carried out on a regular basis in order to establish a standard sorting efficiency. An average sorting efficiency of 95% must be achieved on 10% of samples or a minimum of 3 samples.

Corrective Measures

Samples that do not meet the sorting efficiency criteria (Section 4.2) are reported to the project authority and sent back to the taxonomist for re-sorting.

Sample sorting efficiency will be reported in regional QC audit reports and summarized in the National QC audit report.

5.2 Verification of taxonomic identifications

The CABIN taxonomist performs random whole sample re-identifications. 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 must not exceed 5%.

Four types of taxonomic error are evaluated, although only two are included in the %IE calculation (see Section 4.3.4).

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

The CABIN taxonomist examines all taxonomic errors and determines the corrective action. All errors are reported to the project authority and in annual QC reports.

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.

The CABIN taxonomist performs a follow up review with the project authority to determine that all corrections are addressed.

5.3 Reporting

The National CABIN laboratory will produce annual QC reports.

Regional and project specific QC reports

Reports are regional and project specific to assess taxonomy and the associated data. A report with QC audit results is generated for each project. Reports will quantify aspects of taxonomic precision, assess data acceptability and highlight taxonomic problem areas. Recommendations for improving precision may also be offered.

National QC report

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 needed. The CABIN taxonomist verifies all new taxa and ensures that the data are consistent with ITIS. Routine audits are performed on the database to ensure that the data conforms to standard CABIN naming conventions (Appendix B). Random checks of data entry are performed to ensure that data are successfully transferred from bench sheet to database. 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 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

New specimens are regularly added to the 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 CABIN taxonomist or a recognized expert before addition to the collection. The collection is catalogued and maintained routinely.
Voucher specimens received from CABIN projects are verified in the laboratory or sent to recognized experts, and added to the reference collection.

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Appendix A: Standard Taxonomic Effort for practical level identifications

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

Appendix B: Standard naming conventions for taxonomic nomenclature

Designation	Description	Example	Instruction
sp.	Species place holder • Species place holder for identification to Genus level only	*Baetis sp.*	Not accepted
sp.1 or sp. A	Provisional name • Provisional taxa reported in the literature where a specific identity remains unknown • Usually followed by authors name and year in parenthesis	*Cladotanytarsus sp. B* *Micrasema spA* *Oecetis* sp. A (Floyd, 1995)	Not accepted
( )	Additional taxonomic units • Sub level taxonomic units included in the taxa names, for example sub genus included in the entry • Sub level taxonomic units entered in incorrect hierarchical position, e.g. taxa entered at tribe level	e.g. Sub level inclusions, *Nanocladius (Nanocladius) rectinervis* e.g. Tribe level designation, *Tanytarsini*	Not accepted
Group or gr.	Group designations • Denote a group of more than two closely related species that cannot be separated or • A taxon that can reliably placed in a species group where the determination to species is unsupported	*Rhyacophila vofixa gr.* *Parachironomus vitiosus group*	Not accepted
Provisional or out-of-date names	Incorrect nomenclature	Unpublished name changes or name not recognized in IT IS	Not accepted
Slash Taxa	A/B • 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'.	*Bezzia/Palpomyia*	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 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 taxa 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 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 has 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 taxa is a common naming convention. The slash combines two taxa that are inseparable but were at one time been considered as 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 taxa. 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

Identification:
Family Ceratopogonidae Genus *Bezzia/Palpomyia*

Decision:
Leave at Ceratopogonidae

B.3 Provisional names: Group and Species designations

Provisional names are those that the taxonomist has added a *var.1, sp. A or sp.1* to the end. The CABIN database will not accept provisional names, species or group 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
Mysis sp.1, Baetis sp.B, Paracladopelma doris group, Orthocladius(Euorthocladius) rivulorum gr.

Decision:
Mysis, Baetis, Paracladopelma doris, Orthocladius (Euorthocladius) rivlorum

B.4 Spelling mistakes

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 taxa 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:

Kingdom
Phylum
Class
Order
Family
Sub-family
Genus
Species

Do not enter other units such as *tribe*, *sub-genus* or *sub-species*.

Any additional information regarding taxonomic units can be entered into the notes section on the data entry sheet or communicated in the bench sheets.

B.6 Invalid taxa

Any new taxa entered into the CABIN database that have not been previously entered or reported must be sent to the CABIN taxonomy laboratory for verification. Every voucher specimen submitted must be accompanied by a copy of the appropriate literature to support the identification.

In cases where the current taxa name is absent from ITIS, the previous name will be used as a default until ITIS is updated. It is advised that 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 has been updated the data can be changed accordingly.*** There may be a transition period between new publications, the updating of ITIS and the subsequent updating of the CABIN database.

EXAMPLE: Addition of taxa (not in ITIS)

Identification:
Helodon (Simulliidae)

Some sub-genera 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 taxa be entered as the last reported name as listed in ITIS and that the 'new' name be recorded in the notes section and the following form be completed and sent to the CABIN laboratory.