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Methods Manual:

The reaction of reference-condition data bases for
benthic invertebrate community structure

By:

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Methods manual:
I. The creation of
Reference-Condition data bases
for benthic invertebrate community structure

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Prepared by:

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Aquatic Ecosystem Restoration Branch

National Water Research Institute

Environment Canada

MANAGEMENT PERSPECTIVE

The assessment of aquatic ecosystem health is an essential component of two departmental business line results. The Institute has been developing the use of the reference -condition approach for determination of effects on aquatic ecosystem health and has been promoting the implementation of a National aquatic ecosystem health programme. This report provides an important component of such a programme.

A critical part of a national programme to measure ecosystem health are standard field and laboratory methods. Through two large projects the Institute has calibrated and tested methods that are optimal for the aquatic assessment using benthic invertebrates. This report describes those methods currently in use in collecting data on benthic invertebrate community structure for either adding to or using reference data bases for both running and standing waters in Canada.

Application of methods in future ecosystem initiatives.

SOMMAIRE À L'INTENTION DE LA DIRECTION

L'évaluation de la santé d'un écosystème aquatique est un élément essentiel des résultats de deux secteurs d'activités ministériels. L'Institut travaille au développement de l'approche des conditions de référence pour la détermination des effets sur la santé des écosystèmes aquatiques et il préconise la mise en oeuvre d'un programme national d'évaluation de la santé des écosystèmes aquatiques, dont ce rapport est un élément important.

Les méthodes normalisées utilisées sur le terrain et en laboratoire constituent une partie critique d'un programme national visant à mesurer la santé des écosystèmes. Grâce à deux grands projets, l'Institut a étalonné et testé des méthodes optimales pour l'évaluation aquatique, basées sur des invertébrés benthiques. Ce rapport décrit les méthodes utilisées actuellement pour la collecte de données sur la structure des communautés d'invertébrés benthiques, afin d'accroître les bases de données de référence (ou de les utiliser) tant pour les eaux courantes que pour les eaux stagnantes du Canada.

Application de méthodes pour les initiatives futures touchant les écosystèmes.

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Introduction

Over the past ten years the National Water Research Institute has conducted major programmes in both the Great Lakes and Fraser River as part of a programme to develop a national reference database on benthic invertebrates for Canada. A critical part of this programme is the establishment of a standard set of protocols and methods for all phases of data collection and processing. This document attempts to provide that written record of the methods being used at the institute.

There are three components to the database. Data sets describing the invertebrate fauna, data sets describing the toxicity of sediments based on four invertebrate tests and data sets describing the environmental attributes of sites. In this first protocols document we have described the methods being used for establishing data on the invertebrate community structure. The first section deals with field procedures, collection of both biological and environmental data, in both lentic and lotic habitats. The second section addresses the laboratory procedures regarding sample handling and sample processing for biological samples. In addition we have provided a set of tables and forms used by the programme.

We hope that this will provide a useful resource for others involved in this type of work.

Field Procedures

Aquatic environments fall into two types: lentic environments such as lakes and ponds, and lotic environments such as rivers and streams. Each type of environment has a varied and diverse benthic community that responds to any number of environmental parameters. It is therefore critical to any study that as many of these parameters are measured as is feasible. This will allow for determination of which variables are important in structuring benthic communities.

Before any study is undertaken, serious thought should be given as to what parameters might be important to that environment. Standard sampling methods enable scientists to compare data from different studies with much more accuracy and fewer problems. NWRI has established procedures for sampling in both types of aquatic environments.

Lake Sampling

In studying benthic communities from lentic environments measurements of both water and sediment are important. In many studies initial data can often be overlooked or forgotten but can prove to be critical at a later date. When several procedures are required in the field and several different samples are being taken, things can accidentally be overlooked. Once critical parameters are determined, creation of a field data sheet will help to make sure these parameters are measured and properly recorded.

A copy of the NWRI lentic field sheet is included to illustrate these points (Appendix A).

Water column sampling

Using a Van Dorn sampler, obtain a water sample ½ meter from the bottom and take the following samples for analysis:

- i. 125 mL for nutrients: measure nitrates/nitrites and total Kjeldahl nitrogen.
- ii. 125 mL for total unfiltered phosphorus. Preserve with 1 mL of 30 % sulfuric acid. This can be added to the bottle before going into the field.
- iii. 125 mL for alkalinity.
- iv. A pH and temperature measurement (measure immediately in the field).
- v. A dissolved oxygen measurement (measure immediately in the field).

The samples are obtained and stored according to Environment Canada's National Laboratory for Environmental Testing (NLET) procedures (available on request). This involves keeping all samples cool until submitted for analysis. When taking a sample this close to the bottom it is very important that the depth is known. Water samples that are extremely silty should be discarded and retaken, as the Van Dorn was probably dropped onto the surface, disturbing the sediment. Proper, legible labels, with the appropriate Site Code marked using a water- and solvent-proof marker make sample identification easier later.

Benthic Community Sampling

Box corers are gravity corers used to collect large, relatively undisturbed, rectangular sediment cores. A box core consists of a stainless steel box of various sizes. Larger models include some type of stabilizing frame. The core retainer can easily be damaged by penetrating into substrates which contain rocks or boulders. Therefore it should be used only where there is at least ½ to 1 meter minimum of soft sediment. Due to their large sizes, weights and lifting capacity the box core can only be operated from a vessel which can meet these requirements.

The model used by NWRI, referred to as a minibox core, is 38 x 38 x 46 cm and weighs 80 kg empty. When full it can weigh upwards of 175 kg, requiring the use of a vessel with a winch. When sampling benthic community structure using either the box or minibox core the following steps should be observed:

- i. once the box core has been secured on deck, 5 core tubes (10 cm in depth and 5.5 cm in diameter) are initially placed approximately ½ their depth into the sediment. Each core tube represents a replicated sample unit. The sample consists of the 5 replicates.

Note: See Sediment Sampling protocol for concurrent actions at this stage.

- ii. the over lying water in each core tube is then siphoned off to expose the sediment surface.
- iii. sample processing consists of 5 steps, on a sample by sample basis:

Sample handling

When the replicates are obtained they may be processed immediately or stored.

- i. the replicates can be immediately sieved using a 250 micron mesh sieve and stored in 5% formalin, or
- ii. the replicates can be stored for sieving at a later date. If this is to occur then the replicates should be placed in a sealable container eg: whirl pak bag, etc. and 100 to 150 mls of 5% formalin should be added.

Note: The formalin must be used to harden soft tissue organisms, and replicates need to be in formalin at least 48 hrs (72 hrs is ideal). Replicates should then be removed and placed in 70 % ethanol. The reason for the solution switch is that the formalin causes decalcification of snail and Bivalvia shells, making them impossible to identify.

Problems can arise if the sediment is extremely soft; the box core passes through it and the sediment interface is destroyed. Controlling the winch speed can often solve this problem when depth is a known factor. Sample storage is often where most problems will arise. The concentration of the formalin is very important as samples can often be destroyed when too little or too high a concentration is used.

Sediment Sampling

In sampling for the benthic community, sediment attributes play a key role and must be looked at. To obtain this sample the following steps are taken:

i. before removal of the core tubes, the top 5 cms of sediment is removed from around the tubes and place in a glass dish. Approximately 1 liter of sediment should be removed.

ii. the 1L of sediment is homogenized.

Note: The type of equipment used to stir the sediment should be appropriate for the analysis, i.e. metals analysis requires a non-metal stirring apparatus.

iii. a 125 ml sample is removed for organic analysis. This sample is placed in a hexane rinsed glass bottle and covered with a hexane rinsed piece of tin foil before the lid is placed on.

Note: Remember to use tweezers to handle the tin foil.

iv. a 100 ml sample is removed for particle size

v. a 500 ml sample is removed for chemical analysis.

Sample Handling

Samples should be kept cool until returned to the lab for analysis. The sample taken for chemical analysis are tested for the following parameters:

- major ions
- metals
- loss on ignition
- total organic carbon
- total Kjeldahl nitrogen
- total phosphorus

River Sampling

In studying benthic communities from lotic environments many more physical factors must be taken into account than in the lentic environment. Thus the researcher must collect and measure more physical and chemical variables and characteristics in the field. In many studies initial data are often overlooked or forgotten which proves to be critical at a later date. Once critical parameters are determined, creation of a field data sheet will help to make sure these parameters are properly measured and recorded. A copy of the NWRI field sheet is included (Appendix B).

NWRI follows the following procedures when sampling lotic systems.

Initial procedures

Upon arrival at the site, parameters such as latitude, longitude, and altitude are obtained using a GPS or other similar equipment. The station number, stream name, and date should also be recorded on the field sheet.

Site photographs

Due to the expense of field collection, it is often impossible to go back to a site. Photographs of the location can often help to solve any problems that may arise during laboratory analysis. After the sampling area (reach) is determined, a photograph of the field sheet is taken to identify the ensuing series of photographs. These include: an upstream, downstream and an across the stream photograph, a dry substrate photograph, and finally an aerial photograph (where possible).

Habitat descriptors

Map variables

These are landscape scale variables and can be obtained from topographic maps:

Ecoregion

Latitude and longitude

Altitude

Stream order: Stream order refers to a quantitative method of assigning a number to stream segments, which indicates the relative importance of the segment within the drainage basin. Stream order is determined prior to arriving at a sampling location as follows:

- a) The entire watershed for the river in question is mapped out, using 1:250,00 scale topographical map sheets.
- b) First order streams are defined as those segments having no tributaries. First order streams are assigned a value of 1.

- c) Subsequent stream orders are assigned values according to the method established by Strahler (1957, p914), Figure 1 and 2.
- d) where stream segments of the same order come together, the resulting segment is assigned the next highest order. E.g: where first order streams join, the resulting stream segment is elevated to the second order (2).
- e) where segments of differing orders come together, the resulting segment retains the order of the highest contributing tributary.
- f) where a stream enters a lake, the lake is treated as part of the stream. If more than one stream enters a lake, the outflow of the lake retains the order of the highest contributing tributary segment (Figure 1). If two streams of the same order run into a lake, the outflow increases to the next stream order (Figure 2).

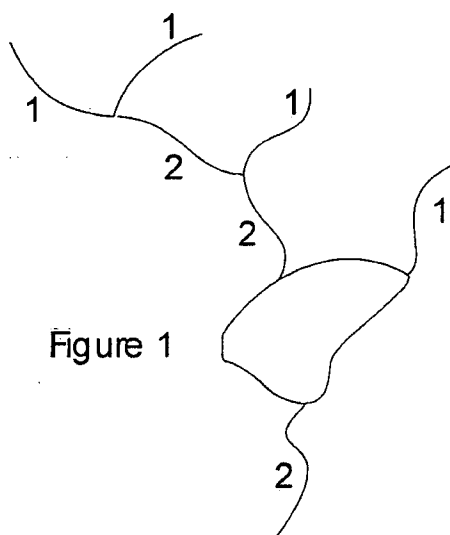


Figure 1

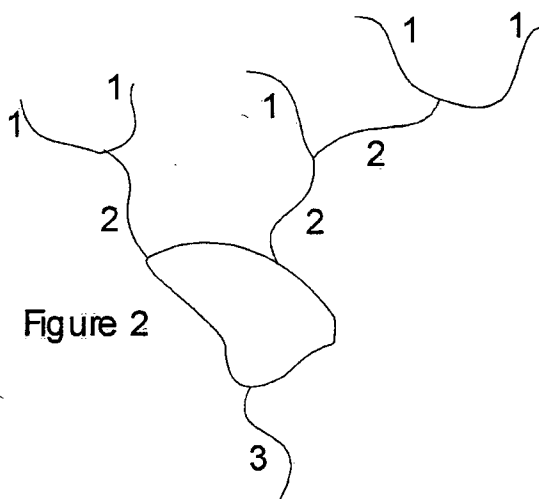


Figure 2

Site/Reach variables

Flow state: This describes the stream and categorizes it into one of the following:

- slow-subcritical: a state where the stream is flowing over a fairly level topography at a fairly constant rate of speed.
- rapid-accelerating: a state where the stream is flowing over a slope in the topography at an accelerated rate of speed.
- supercritical-shooting: a state where the stream is flowing over a slope in the topography and is bubbling over rocks and boulders at a very fast rate of speed.
- hydraulic jump area: a state where the stream drops down a series of steps in the topography.

Canopy coverage: This is an approximation of the amount of stream area the canopy covers.

Macrophyte coverage: This is an approximation of the amount of the stream bed that is covered by macrophyte vegetation.

Riparian zone vegetation: This describes the vegetation found in the riparian zone along the stream sampling site. There are four distinct categories:

- Grasses Score - 1
- Shrubs Score - 2
- Coniferous Trees Score - 3
- Deciduous Trees Score - 4

Riparian zone scoring: The presence of any of the four riparian vegetation categories results in a related score (above). Total score for a site is ranked with higher values representing greater stability and allochthonous food input, on a subjective basis:

- grass - deciduous increasing bank stability
- grass - deciduous - higher allochthonous input
- deciduous > coniferous as higher quality food and higher stream temperatures

Riparian zone logging: This describes and quantifies the level of disturbance to the riparian zone due to logging operations.

Channel variables

These characteristics are concerned with the specific area being sampled and include:

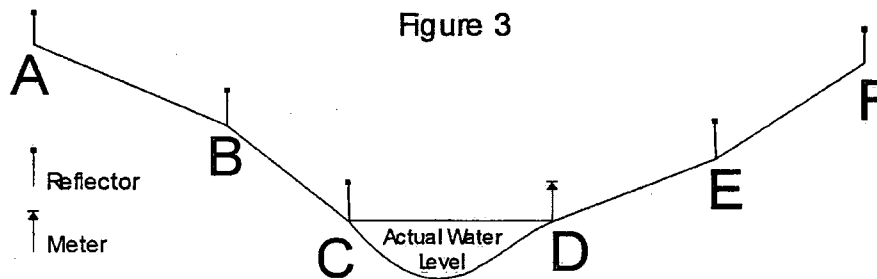
Stream cross-section and flow: This involved measuring across the stream or section sampled using a standard metric tape measure. To calculate the total stream flow the total stream width is divided into equal measurements and flow rates are counted for each of these points. This involves using a Price pygmy (Price 622A) or similar flow meter, and following the procedures outlined in the operation of these meters.

Note: These readings are done on the downstream end of the reach so as not to disturb the benthic community.

Bankfull and channel width: This involves creating a cross section of the stream through the reach (Figure 3). Bank measurements are taken using the Total Station surveying equipment to determine height and distance.

- anchor the meter at one edge of the existing water level (D).
- place the reflector at the two extreme bank edges, representing the High Water level (A, F).
- take measurements at the existing water edge, opposite the meter (C).

- take measurements at each location on the cross-section where slope changes significantly (B, E).



Substrate: A 0.09 m² grid is randomly tossed and a photo taken by standing directly over the grid. Cobble size is divided into categories (Appendix B) and the major size components scored. The amount of embeddedness is also determined and scored (Appendix B).

The ratio of framework (> 2mm) to matrix (< 2mm) is also established both with and without the armour layer. A sample of dry substrate is shovelled onto a 2mm mesh sieve covered with plastic. The total weight is obtained. The plastic sheet is removed and the material washed to remove the < 2mm fraction and re-weighed. The ratio of framework to matrix can then be established. This procedure is followed for the substrate with and without the armour layer.

Slope: This is determined using Total Station surveying equipment. The meter is anchored at the beginning of the reach and the reflector is placed at the end of the reach.

Water column variables

These characteristics are concerned with the specific area being sampled and include:

Field measurements: This includes temperature, pH, dissolved oxygen and conductivity. Water samples are also taken for total phosphorus, nutrients and alkalinity. Water is taken directly from the stream by submerging the sampling container to the middle of the water column. Water parameters (DO, pH, conductivity, temperature) are obtained using a hydrolab or similar device, following the procedures described for operation of said devices. Samples include:

- 125 mls for nutrients: measured are nitrates/nitrites and total Kjeldahl nitrogen
- 125 mls for total unfiltered phosphorus preserved with 1 ml of 30 % sulfuric acid. This can be added to the bottle before going into the field.
- 125 mls for alkalinity
- 2 L for total suspended solids
- 2 L for periphyton preparation

Sample Handling.

The samples are obtained and stored according the NLET procedures. This involves keeping the samples cool until submitted for analysis. Each container must be marked with proper, legible labels, with the appropriate Site Code and replicate number marked using a water- and solvent-proof marker.

Benthic invertebrate sampling:

When sampling benthic community structure using a D-net or kick net, the area, number of kicks and kicking time should already be determined prior to starting the sampling. Once determined the procedure should be carried out as follows:

- a) the area to kick should be defined and kicking should start at the downstream end.
- b) stepping into the stream the net should be placed firmly on the substrate surface and as close to the stream border as is possible.
- c) kicking begins by moving your feet through the substrate, rolling over rocks and stones, large rocks or those deeply embedded should be rubbed using your hand.

Note: the net should always be in contact with the substrate and should always be directly downstream of the operator.

- d) the area to be sampled should be traversed in a zigzag pattern going from bank to bank and always heading upstream. In large rivers or very fast flowing streams, bank to bank sampling may not be possible. In these cases the same procedure is followed, zigzagging through the area that has been defined for sampling.
- e) the sample is checked, with large rocks and sticks being removed after they have been washed and carefully check for organisms.
- f) the sample is then washed into a container and the net checked carefully for organisms clinging to it.

Note: Seams and folds should be checked carefully for hidden organisms.

- g) 10% formalin is then added to the sample (approximately in a 1:3 ratio).
- h) containers are labeled outside and a label is also placed inside each sample.
- i) records of the person doing the kicking and the typical depth are noted on the field sheets.

Sample handling

When the samples are obtained they may be processed immediately or stored.

- a) the replicates can be immediately sieved using a 250 micron mesh sieve and stored in 10% formalin.
- b) or the replicates can be stored for sieving at a later date. If this is to occur then the replicates should be placed in a labeled sealable container eg: whirl pak bag, etc. and 100 to 150 mls of 10% formalin should be added.

Note: The formalin must be used to harden soft tissue organisms, replicates need to be in formalin at least 48 hrs (72 hrs is ideal). Replicates should then be removed and placed in 70 % ethanol. The reason for the solution switch is that the formalin causes decalcification of snail and Bivalvia shells making them impossible to identify.

Periphyton sampling:

In lotic environments periphyton is the major primary food source for many organisms and therefore needs to be considered when studying such environments. This involves collecting samples from the substrate surface as follows:

- a) 3 rocks, of similar size, are selected from the left, middle and right areas of the stream within the sample area.
- b) a 4 cm diameter template is placed firmly on the rock surface and water is added using a syringe.
- c) using a modified battery operated toothbrush the rock surface is completely brushed and the water placed into a labeled container. This step is repeated 3 times to ensure that the rock surface is clean. The procedure is repeated on the other two rocks and the sample is composited in the same container.
- d) measurements are then taken on each rock; length, width and depth.
- e) 2 L of stream water are collected and returned with the sample.

Sample handling

Periphyton samples must be prepared for analysis as soon as possible after collection in the field:

- a) at the field station the periphyton sample is placed in a graduated cylinder and the sample container is thoroughly washed with stream water from the 2 L taken at the site.
- b) the volume is then made up to 680 ml and poured into a 1L beaker. This is stirred using a hand held Braun mixer for 3 seconds, and stirred using a stir bar and stir plate at a setting of 7.
- c) using a 30 ml syringe, two 20 ml samples are taken from the 680 ml suspension; one for biomass and the other for chlorophyll-a. The samples are filtered through a pre-ignited, pre-weighed filter paper. The pump should be run at less than 15 psi.
- d) after the samples are filtered, both filters are removed using forceps and placed in labelled petri dishes. Both samples are then desiccated for 24 hrs. After 24 hrs both samples are removed, capped and frozen. The sample for chlorophyll-a is wrapped in aluminium foil before freezing.

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- e) from the suspended solution a 10 or 20 ml sample is removed and placed in a labelled scintillation vial and four drops of Lugol's solution is added. This sample is for algal taxonomy.
 - f) from the remaining suspended solution two 200 ml samples are removed and placed in labelled whirlpaks. One sample is frozen and the other is preserved with 10 % formalin. These samples are for backup in case problems arise in analysis or taxonomy.

Leaf pack sampling:

This involves the collection of leaf pack samples from the sampling area. Leaf packs consist of organic material that has collected in areas of the stream.

Sample Handling

These packs of vegetation are removed and placed in labelled whirl paks. Both water and 10% formalin are added in 1:2 ratio water to formalin.

Laboratory Procedures for Sample Management and Analysis

Just as the sampling process for any study is critical to the success of the research, so is the management and analysis of those samples once they arrive at the laboratory from the field. A carefully structured framework for managing both the samples themselves, as well as data generated from their analysis is required. Without such a framework, samples can be lost or unanalyzed and results can become misplaced or misconstrued. By identifying specific responsibilities for each individual involved in a project, significant reductions in wasted time and resources can be achieved.

NWRI has established guidelines for sample and data management. These guidelines are broken down into sections, each identifying the proper procedure(s) and personnel responsible at each stage.

Project Initialization

Upon the initialization of a new project, a hard-copy Project Folder is started by the project co-ordinator. The Project Folder acts as a data warehouse for raw field sheets and any other hard-copy data generated during a project, until its entry into the electronic data base (see Laboratory Protocols for Data Management). Individuals responsible for specific results must ensure that their data sheets are placed in the Project Folder once analysis has been completed. Project Folders should be stored in one specific area so all personnel involved in the project know their location.

Arrival of Samples

Delivery

When project samples collected in the field arrive, they are delivered to the appropriate laboratory. The project co-ordinator is responsible for the initial inspection and subsequent re-distribution of samples to their appropriate laboratories for analysis. The inspection of samples includes:

- i. Entering each sample into the Project Folder log.
- ii. Checking against the project field sheets to ensure samples from all sampling locations are accounted for.
- iii. Identifying any samples which have been damaged/lost during transport. Damaged/lost samples are noted, and a comment must be placed in the Comments section of the benthic data information system (see Laboratory Protocols for Data Management).
- iv. Separating samples into their specific components for delivery to appropriate laboratories for analysis.

Distribution

Sample components are sent to the following laboratories for analysis:

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- i. **Community Structure Samples:** send to the appropriate laboratory for storage and processing.
 - ii. **Sediment and Water Chemistry Samples:** store and subsequently deliver to appropriate laboratories for processing.

Laboratory Sample Protocols

Community Structure

Samples arriving for community structure analysis must be checked against the Project Folder log to ensure that no samples have been lost during delivery to the laboratory. Once all of the samples have been verified, they must be prepared for storage until processing can be done.

Preparation for Storage

Preparation consists of exchanging the 5% formalin (see Field Protocols) in each sample with 70% Ethanol. Stored samples must be checked every three months, to ensure the replacement of any ethanol lost due to evaporation.

Sample Processing

- i. **Sub-Sampling** - depending on the density of organisms presumed to be in a sample, the time required to process a single benthic sample can be extremely long. For this reason, a method of sub-sampling has been adopted, based on the Marchant (1989) sub-sampling device and procedure. The sub-sampler is a box (35 x 35 x 10 cm) divided into 100 equal cells.
 - a) the need to sub-sample a sample is the subjective expert decision of the project co-ordinator, based on expected yield of organisms.
 - b) an unsorted sample is placed into the box, the box is covered and shaken to distribute the sample evenly among the 100 cells.
 - c) cells are randomly sampled until at least 200 organisms are picked (see Sample Processing). Cells which have been started **MUST** be finished, after the 200 organism level has been achieved.
 - d) the number of organisms in the entire sample is then estimated by extrapolation to the full 100 cells in the sub-sampler.

ii. Processing

Sample processing consists of 5 steps, on a sample by sample basis:

- a) Samples are picked and sorted according to Family, using a low power stereo microscope (16X with 10X eye piece).
- b) Each family is enumerated, and these counts are placed on bench sheets (Appendix C)
- c) Sorted samples and bench sheets are stored in the Project Folder until Genus/Species level identification can take place.

-
- d) During identification, each family is identified to Genus/Species and tallies are recorded on the bench sheets.
 - e) As required (*Chironomidae* and *Oligochaeta*) slide mounts are made for high power microscopic identification. Appropriate identification guides must be used, and voucher specimens of all identified specimens submitted to experts for confirmation. The confirmed voucher specimens should be maintained as a reference collection.

Tallies are transferred from bench sheets to final data sheets, and placed in the Project Folder.

iii. Quality Assurance/ Quality Control (QA/QC)

The picking and sorting of benthic organisms is an optically demanding task. There is a possibility that in some samples, organisms will be missed due to sheer numbers, or other confounding factors such as detritus in the sample. For this reason, quality assurance/quality control procedures (QA/QC) must be carried out on a regular basis, in order to establish a standard sorting efficiency for the sampling process. QA/QC involves:

- a) the remaining material from each sample picked within a single month is replaced in its sample container and stored.
- b) one sample is randomly selected from the total at the end of each month and re-picked; the number of new organisms found is counted.
- c) the % OM (organisms missed) is calculated using the equation:
$$\frac{\#OrganismsMissed}{TotalOrganismsFound} * 100 = \%OM$$
- d) if %OM > 5%, 2 more samples are randomly selected and %OM calculated for both.
- e) The average %OM is calculated based on the 3 samples re-picked, and represents the standard sorting efficiency for that month (based on only one sample if %OM < 5%).

Sediment and Water Chemistry Samples

Sediment and Water Chemistry Samples are delivered, inspected and sorted by the responsible personnel for delivery to the appropriate laboratory for analysis. Each standard sample is comprised of 3 sediment chemistry containers: 1-500ml tub, 1-125ml contaminant jar, 1-100ml pill jar. The sediment containers are labeled top and side when the sediment is collected. Each standard sample is also comprised of 3 water chemistry containers: 1 for alkalinity, 1 for total phosphorus and 1 for nutrients. Water bottles are also labeled at time of collection.

i. Water samples

A sample submission form for NLET with the sample ID's, the analysis to be done (SCHEMA) and sample date is filled out (Appendix D). Samples are then sorted such that the three bottles for each site are together, and delivered to the NLET submission office.

ii. Sediment Chemistry

Sediment chemistry samples are prepared for freezing and freeze drying. Preparation consists of removing each sample container lid and replacing it with a Kimwipe[®] which is held on by an elastic. Samples are then either: 1) placed in the freezer until a sufficient number of samples have accumulated for submission for freeze drying, or 2) taken directly for freeze drying. A submission form must then be filled out and given to whoever is responsible for the actual freeze drying process.

Once samples have been freeze dried, they are picked up by the responsible personnel and the Kimwipes[®] are replaced with proper lids for each sample. Three types of analysis are then carried out on the freeze dried samples:

- a) Particle Size Analysis - samples are sent for analysis, and a submission form is filled out for particle size analysis (Appendix D).
- b) Contaminant Analysis - samples are stored until required. If contaminant analysis is requested, samples are submitted to the appropriate laboratory.
- c) Major Elements and Total Metals - the large tubs of sediment which have been freeze dried are packaged and shipped for analysis.

Data sheets generated by sediment analysis are returned from two sources. First, the particle size analysis sheets are delivered to the project co-ordinator. Data sheets for the major elements, etc. are returned and placed in the appropriate Project Folder to await entry to the electronic data base.

Laboratory Procedures for Data Management

The management of data resulting from field collection and laboratory analysis is the final step in establishing a complete project data base. The success or failure of a project can be determined as much by the management of project data, as by the success or failure at any other stage of a project's duration. Like the collection and analysis of samples, resulting data must be handled within a well-established framework.

During the field sampling and laboratory analysis portion of a project, raw data sheets are warehoused in the Project Folder initiated by the project co-ordinator, which represents the first stage in data management. However, the Project Folder is not the final storage media for data collected during a project. Standard data storage methods enable scientists to manipulate and analyze data with much more accuracy and fewer problems. NWRI has established a computer-based information storage and retrieval system for use during projects involving Benthic Community Structure and Environmental Attributes in Aquatic Ecosystems.

ELECTRONIC BENTHIC DATA INFORMATION SYSTEM

Electronic Project Initialization

The Project Folder represents the hard-copy equivalent of the final electronic data base. Unlike the Project Folder, however, the Benthic Data Information System (BDIS) is designed to store data from multiple projects. For this reason, the first stage in transferring data from hard-copy to electronic format is establishing a unique Study Name for identification in BDIS (Appendix E). The initialization of a new study within BDIS can be undertaken at any time during a project. However, the most efficient routine is to establish a new electronic project at the same time that field-work is begun. New projects are initiated by the project co-ordinator.

Study Name Protocol

Any alphanumeric name which uniquely identifies a project and associated data within the data base.

Site Code Entry

Individual sample sites for a specific project are identified by unique Site Codes within BDIS. These are established before field work is undertaken, and are used to identify samples as they are collected in the field. Each Site Code must be entered into the information system (Appendix E), and this is the responsibility of the project co-ordinator. The entry of site codes is done only once during a project. Once these codes have been established, they are automatically maintained by BDIS as new data for each site is entered into the system from the Project Folder. This eliminates data entry errors, and the possibility of data being lost due to conflicting or contradicting site codes. No data entry can take place until the project co-ordinator has entered Site Codes for a particular project.

Site Code Protocol

- i. **Lake Projects** - projects involving lentic environments are assigned a ten digit numeric code consisting of a 4 digit number identifying the station, and 6 digits for the date a sample was taken, in the format dd/mm/yy.
- ii. **River Projects** - projects involving lotic environments are assigned a nine digit alpha-numeric consisting of: three letters identifying the major river on which samples were taken, 2 digits for the sample number for that river, 2 digits for the replicate number at the site, and 2 digits for the year of the sample.

Tracking Project and Sample Status

BDIS is capable of tracking the status of six different sample types typically taken during field collection (Appendix E). These are:

- i. **Community Structure**
 - a) Sample Location - where are the samples are currently stored.
 - b) Picked - are the samples picked yet.
 - c) Identified - are the samples identified yet.
 - d) Entered to BDIS - have the hard-copy data sheets been entered to BDIS.
- ii. **Periphyton Structure (River Projects only)**
 - a) Sample Location - where are the samples are currently stored.
 - b) Entered to BDIS - have the hard-copy data sheets been entered to BDIS.
- iii. **Particle Size Analysis**
 - a) Sample Location - where are the samples are currently stored.
 - b) Entered to BDIS - have the hard-copy data sheets been entered to BDIS.
- iv. **Sediment Chemistry**
 - a) Sample Location - where are the samples are currently stored.
 - b) Entered to BDIS - have the hard-copy data sheets been entered to BDIS.
- v. **Water Chemistry**
 - a) Sample Location - where are the samples are currently stored.
 - b) Entered to BDIS - have the hard-copy data sheets been entered to BDIS.

Once a study has been initiated in the system, project members may use the tracking system to determine a project's status. Project members must also update the tracking system if changes to the status of samples or data which they are responsible for occur.

Data Entry

i. Hard-Copy Study Binder

As data for each stage is entered into the benthic data information system, the raw data sheets are removed from the Project Folder, and placed into a permanent binder for the project. These binders are stored by the project co-ordinator.

ii. Field Sheets (Primary and Physical Data)

Field Sheets for benthic assessment include the site number, date sampled, location, and other physical parameters collected while in the field (Appendix A or B). Field sheets are placed in the Project Folder immediately after a return from the sampling trip, and are the responsibility of the project co-ordinator. The entry of data from the Field sheets is also undertaken by the project co-ordinator, and must be completed before any other data can be entered to BDIS. Once field data has been entered, field sheets are moved from the Project Folder to the Hard-Copy Study Binder.

Note: Since Primary data is the first stage of data entry completed from raw data sheets, it is the project co-ordinator's responsibility to create a new project Hard-Copy Study Binder at this point, to store all raw data sheets from subsequent data entry stages.

iii. Chemistry Data

Chemistry data is entered from the data sheets returned by the analysis labs listed in appropriate sections above. The entry of chemistry data is the responsibility of the person responsible for data entry. Once data for a particular analysis (e.g. Water Chemistry) for a project has been entered, data sheets are moved from the Project Folder to the Hard-Copy Study Binder.

iv. Particle Size Analysis

Particle size data is entered from the data sheets returned from the Sediment Lab. The entry of particle size data is the responsibility of the person responsible for data entry. Data sheets are moved from the project folder to the project binder once all data has been entered into the data base.

v. Community Structure

Community structure data is entered from the final tally sheets compiled by the project co-ordinator (Appendix C). The entry of community structure data is the responsibility of the person responsible for data entry. Data sheets are moved from the project folder to the project binder once all data has been entered into the data base.

vi. Special Cases or Procedures

a) Additions to the Master Species List

The benthic data information system maintains a master list of all species identified. As new species are identified, they can be added to the master list (Appendix E), and become available to the user during benthic data entry. The master list

provides order, family, and species name information automatically to the user, limiting data entry errors related to spelling and taxonomic placement.

b) Species Reference Number

A Key aspect of the Master Species list is the Reference Number code assigned to each entry. In order to facilitate search speeds and automatically group entries according to taxonomic level, a numeric coding system was developed. The code consists of two digits to represent the Order, the two digits to represent the family, and last four digits to represent the Genus/Species.

00	00	0000
Order	Family	Formal Name (Genus-Species)

For a new entry at any one of the taxonomic levels, the next available number in the sequence is assigned. Thus, if 9 orders have been identified, and a new one is found, the new order is assigned the digit 10. For organisms which can not be identified below a certain taxonomic level, 00 is used for the following taxonomic levels. Thus, an entry for an organism identified only to the Order in the above example would be 10 00 000. An organism identified to Family, but not to Genus/Species would be 10 01 0000.

Unidentified Taxonomic Level Naming Conventions

The data base requires that a textual entry be made for every benthic organism for Order, Family, Genus, and species. In conjunction with the numeric convention above, organisms unidentified below Order (10 00 0000) are entered as '*Order family*' for Family, and '*Unknown Order*' for Genus/Species. Organisms identified to Family (10 01 0000) are entered as '*Unknown Family*' for Genus/Species.

c) Automated Computations

Several forms in the data entry system automate the computation of values:

Decimal Degrees

Location data in the field is typically recorded using a Global Positioning System (GPS), and is measured in Degrees, Minutes, and Seconds of Latitude and Longitude. For statistical analysis, however, Latitude and Longitude must be converted to Decimal Degrees. BDIS automatically computes Decimal Degrees for the user, as Degrees, Minutes and Seconds are entered.

Mean Counts for Benthic Organisms

During sample collection in the field, up to 5 replicates at a single site can be taken. Statistical analysis typically requires a single value for each site, requiring a mean to be computed. BDIS automatically computes a mean count for each organism during data entry.

Note: If less than 5 replicates are taken, blanks must be used for each unsampled replicate (e.g. only three were taken, 4 and 5 are blank). If a zero is left in these boxes, BDIS assumes a replicate was taken, and bases the mean on the number on replicates filled in, causing an erroneous entry to the data base. The same is true for means calculated during the entry of Bio-Assay results.

Appendix

Appendix A: Lentic Sampling Field Sheet

Sediment Sampling Field Sheets

*** General Section:**

Site No.: _____ Lake: _____ Ecodistrict: _____

Latitude: _____ Longitude: _____

Sample Date (DD/MM/YR): ___/___/___ QA/QC: Yes No

*** Biological Section:**

1. Water depth: _____

2. Benthic community samples:

Operator: _____ No. of Replicates: _____

Sampling device: _____ Sediment depth in box core: _____

3. Bioassay samples:

Operator: _____ No. of Replicates: _____

Sampling device: _____

*** Physical/Chemical Section:**

1. Field measurements:

Water surface temperature: _____ Water bottom temperature: _____ DO: _____ pH: _____

2. Water samples:

Nutrients: _____ Total phosphorus: _____ Alkalinity: _____

3. Sediment samples:

Chemistry (450mls): _____ Organics (125 mls): _____ Particle size (50 mls): _____

*Comments.

Sediment type (eg: sand, clay, mud, etc.)

sediment colour:

No. of attempts with sampling device:

Appendix B: Lotic Sampling Field Sheet

I: General & Biological Sections

A. General Information

River: _____ Station Number: _____

Geographical description _____

Latitude: _____ Longitude: _____ Altitude: _____

Sampling Date(D.M.Y) ___/___/___ QA/QC site (circle one): Yes No

B. Reach Characteristics

1. Stream Order: _____

2. Flow State(Circle one):
riffle/rapids straight run pool/back eddy

3. Canopy Coverage (Approx. %): _____

4. Macrophyte Coverage (circle one):
0% 0-25% 25-50% 50-75% 75-100%

5. Riparian Zone Score (circle those present):
grasses - 1 shrubs - 2 deciduous trees - 3 coniferous trees - 4

6. Extent of Logging in riparian zone (circle one):
0% 0-25% 25-50% 50-75% 75-100%

C. Sample Site Characteristics

1. Flow State (circle one):
slow-subcritical rapid-accelerating supercritical-shooting hydraulic jump area

2. Benthic Invertebrate Samples

<u>Variables</u>	<u>Sample No.</u>				
	1	2	3	4	5

Operator _____
sampling time _____
No. of bags/sample (if>1) _____
typical depth _____

D. Reach Characteristics

1. Photos:

a) Station Sheet: _____ Site: Upstream _____ Downstream _____ Across _____ Aerial _____

b) Substrate (0.09 m2 grid): Dry (exposed bar) _____ Aquatic _____

E. Water/Substrate Samples:

TSS(2L) _____ total P _____ Nitrate _____ Major Ions _____

Station Number: _____

F. Typical Substrate Composition at Sample Sites:

Values for particle sizes and embeddedness used to calculate substrate score.

PARTICLE TYPE/SIZE		EMBEDDEDNESS	
Category	Score	Category	Score
organic cover (>50% of surface)	1	Completely embedded	1
<0.1-0.2 cm	2	3/4 embedded	2
0.2-0.5 cm	3	1/2 embedded	3
0.5-2.5 cm	4	1/4 embedded	4
2.5-5 cm	5	unembedded	5
5-10 cm	6		
10-25 cm	7		
> 25 cm	8		

Substrate Score (See above table)

i) Particle Type or Size

1. Size of predominant particle _____
2. size of 2nd most predominant particle _____
3. size of material surrounding 1 & 2 _____

ii) Embeddedness _____

Total _____

G. Periphyton Samples:

Rock Dimensions (cm)

Rock No.	Length	Width	Depth
1			
2			
3			
4			
5			
6			
7			
8			
9			

H. Leaf-Pack Samples

(circle one): Yes No

II: Physical/Chemical Section

Station No. _____ Team: _____

A. Field Measurements:

Temp. _____ pH _____ Cond. _____ DO = _____ %

B. Channel Measurements:

1. Channel Slope (m): Horizontal Dist. _____ Vertical Dist. _____

2. Bankfull measures:

LOC	A	B	C	D	E	F	G	H	I	J
Horiz. Dist.										
Vert. Dist										

3. Stream Cross-Section and Flow:

Total Stream Width: _____ (m) Meter No: _____

DIST. (M)							
Depth (cm)							
Revs. *	40	40	40	40	40	40	40
Time (sec)							

- indicates counts other than 40 revs

C. Substrate measures:

Weight of equipment: _____

	Substrate with armour layer	Substrate without armour layer
1. Total weight - Equipment wt = Framework + matrix		
2. Weight after sieving - Equipment wt = Framework		
3. Sum of 1-2 = Matrix		

Appendix C: Benthic Ecology Laboratory Bench Sheets

SITE:		cell #																		
#CELLS:	CELL																			
TAXA:	TOTALS																			

BENTHIC COMMUNITY DATA SHEET

Project:

Site ID:

Order:

Family:

Genus:

species:

Rep number: 1 2 3 4 5

Order:

Family:

Genus:

species:

Rep number: 1 2 3 4 5

Order:

Family:

Genus:

species:

Rep number: 1 2 3 4 5

Order:

Family:

Genus:

species:

Rep number: 1 2 3 4 5

Order:

Family:

Genus:

species:

Rep number: 1 2 3 4 5

Appendix D: Analytical Laboratory Submission Forms

Environment Canada / Environnement Canada
SAMPLE SUBMISSION FORM - NAQUADAT/MISCELLANEOUS
EXPEDITION D'ÉCHANTILLONS PRÉLEVÉS DANS L'ENVIRONNEMENT

NATIONAL LABORATORY FOR ENVIRONMENTAL TESTING
 ECDC HEALTH BRANCH
 LABORATOIRE NATIONAL DES ÉTATS ENVIRONNEMENTAUX
 DIRECTION DE LA SANTÉ DES ÉCOSYSTÈMES

NLET Project No. / Projet de LNEÉ		Project Leader / Chef de projet		Sample Custodian / Responsable		Site No. / Site n°			
Date Shipped / Expédié le		Date Rec'd / Reçu le		NAQUADAT Sample ID / ID NAQUADAT (if client)					
Sl. No.	Sample No. / N° de l'Échantillon	No. of Liters / N° de Litres	Client Sample No. / N° de l'échantillon (over 10 above)	NLET Sample Name(s) and/or Schema Name(s) / Nom(s) de l'échantillon / schéma	NAQUADAT Sample ID / ID de l'échantillon NAQUADAT (if client)	Collection Information / Informations de Collecte			Remarks / Remarques
						Date / Date	Time / Heure	Zone / Zone	

**SRP Analytical: Sample Submission Form
For
MAJOR AND TRACE METAL
AND
TRACE ORGANIC ANALYSIS**

Submitted by:

Branch/Study #:

of Samples:

Sample Type:

Analysis Type / Organics

- | | | |
|--------------------------------------|---|--|
| <input type="checkbox"/> PAHs | <input type="checkbox"/> n-ALKs | <input type="checkbox"/> Congener PCBs |
| <input type="checkbox"/> PCB total | <input type="checkbox"/> OCs | <input type="checkbox"/> Chlorobenzenes |
| <input type="checkbox"/> Pesticides | <input type="checkbox"/> Herbicides | <input type="checkbox"/> Custom Target Compounds |
| <input type="checkbox"/> Qualitative | <input type="checkbox"/> Method Development | <input type="checkbox"/> Confirmation |
| <input type="checkbox"/> Others | | |

Analysis Type/ Metals

- | | | |
|-----------------------------------|------------------------------------|-----------------------------------|
| <input type="checkbox"/> Aluminum | <input type="checkbox"/> Copper | <input type="checkbox"/> Silicon |
| <input type="checkbox"/> Barium | <input type="checkbox"/> Iron | <input type="checkbox"/> Sodium |
| <input type="checkbox"/> Calcium | <input type="checkbox"/> Lead | <input type="checkbox"/> Thallium |
| <input type="checkbox"/> Cadmium | <input type="checkbox"/> Magnesium | <input type="checkbox"/> Vanadium |
| <input type="checkbox"/> Cobalt | <input type="checkbox"/> Manganese | <input type="checkbox"/> Zinc |
| <input type="checkbox"/> Chromium | <input type="checkbox"/> Nickel | <input type="checkbox"/> |

Comments

Date:

Signature:

SAMPLE SUBMISSION FORM
For
PARTICLE SIZE ANALYSIS
AND
OTHER RELATED ANALYSIS

Submitted by:

Branch/Study #/Leader

of Samples:

Sample Container Type:

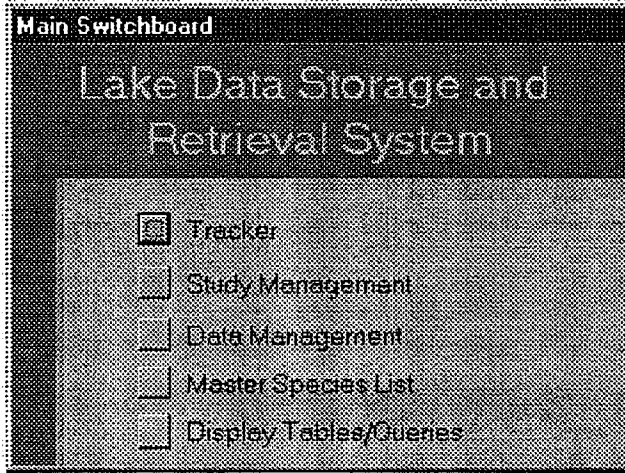
ANALYSIS TYPE/		PROCEDURE TYPE	
<input type="checkbox"/> SEDIGRAPH/SIEVE	<input type="checkbox"/> ORGANIC CONT:(LOI)	<input type="checkbox"/> FRACTIONATION	
<input type="checkbox"/> SIEVE	<input type="checkbox"/> DENSITY	<input type="checkbox"/> CORE SECTIONING	
<input type="checkbox"/> CARBON/ORGANIC	<input type="checkbox"/> MOISTURE CONTENT	<input type="checkbox"/> PHOTOGRAPH CORES	
<input type="checkbox"/> CARBON/INORGANIC	<input type="checkbox"/> FREEZE DRYING	<input type="checkbox"/> ATTERBERG LIMITS	
<input type="checkbox"/> CARBON/TOTAL			
		Analysis Type/ other/and Equipment usage	
<input type="checkbox"/> Fractionation for XRD	<input type="checkbox"/> Equipment usage	<input type="checkbox"/>	
<input type="checkbox"/> Fractionate for carbonate	<input type="checkbox"/> General Lab usage	<input type="checkbox"/>	

Comments

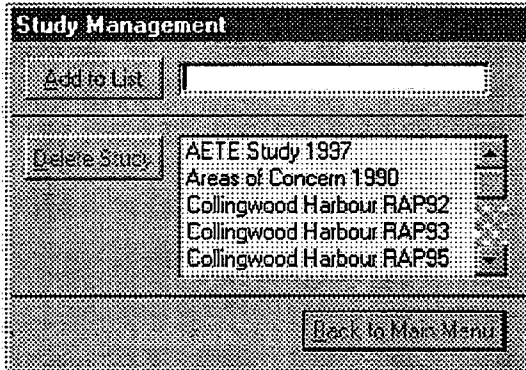
Phone # for Contact person:

Appendix E: Benthic Data Information System Forms

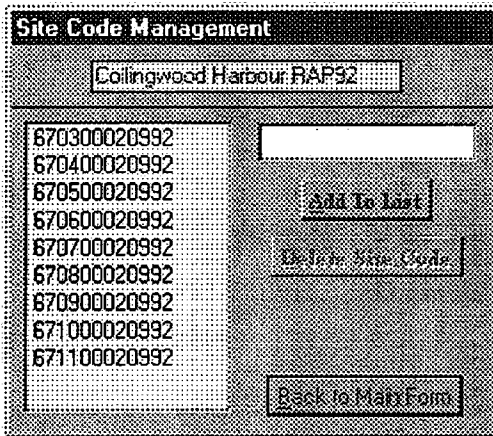
Main Menu:



New Project Name:



Site Code Entry:



Primary Site Information Entry:

Primary Entry : Form

Collingwood Harbour RAP92 Record Deleted Unlink

670300020992	Site Number: 6703	Sample Device: <input type="text"/>
670400020992	Lake Name: Georgian Bay	Mesh Size (µm): 250
670500020992	Eco Region: 7	# of Replicates: 5
670600020992	Date (d/m/yr): <input type="text"/>	Referenced: <input type="checkbox"/>
670700020992	Julian Day: 244	Repeat: <input type="checkbox"/>
670800020992	Sample Year: 1992	QA/QC: <input type="checkbox"/>
670900020992	Description: <input type="text"/>	
671000020992		

Location	Physical Data	Sediment Chemistry	Select Other Study
Community Structure	Bio-Assay	Water Chemistry	

Bio-Assay Data Entry:

Study Name: Collingwood Harbour RAP92

Site Code: **670800020992**

Chironomus riparius:	Survival	Growth		
Hyalella azteca:	Survival	Growth		
Hexagenia limbata:	Survival	Growth		
Tubifex tubifex:	Survival	cc/d	yg/d	Hatched

Appendix F: Taxonomic References

Available on request

References

Marchant, R. 1989. A subsampler for samples of benthic invertebrates. *Bull. Aust. Soc. Limnol.* Vol. 12. Pp. 49-52.

Strahler, A.N. 1957. *Quantitative Analysis of Watershed Geomorphology*. Am. Geophys. Union Trans. Vol. 38. Pp. 913-920.

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Environnement
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Canada

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Burlington, Ontario
L7R 4A6 Canada

National Hydrology Research Centre

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Saskatoon, Saskatchewan
S7N 3H5 Canada

St. Lawrence Centre

105 McGill Street
Montreal, Quebec
H2Y 2E7 Canada

Place Vincent Massey

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Gatineau, Quebec
K1A 0H3 Canada

Centre canadien des eaux intérieures

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