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CABIN WETLAND MACROINVERTEBRATE PROTOCOL

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PREFACE

This document was developed by scientists at Environment and Climate Change Canada (ECCC) for the Canadian Aquatic Biomonitoring Network (CABIN). It contains information on and procedures for sampling macroinvertebrates in wetland habitats. It does not provide details on the development and operation of the wetland biomonitoring models, which are still being developed by the ECCC CABIN team. People who are already familiar with the CABIN wadeable streams protocol do not need additional field training in order to use this protocol.

INTRODUCTION

Aquatic biomonitoring involves tracking changes in the composition of biological assemblages (i.e., fish, macroinvertebrates, and algae) to support assessment of the condition of aquatic ecosystems over time and space. The Canadian Aquatic Biomonitoring Network (CABIN) is a national biomonitoring program, led by Environment and Climate Change Canada (ECCC), based on a networked partnership model. Up until now, CABIN has been focused on benthic macroinvertebrates in streams and rivers as well as in the Great Lakes. Coastal wetlands—which are often hazardous in terms of tidal exposure—are explicitly excluded for consideration under this protocol at present. This document presents a standard sampling approach which is intended to support biomonitoring in Canadian wetland habitats, and thus expands the monitoring scope of the current CABIN program.

Traditionally, wetland condition is assessed based on vegetation parameters or chemical analyses of the water. The presence or absence of rare species, of invasive plant species, and changes in the areal extent of different types of wetlands (i.e., marshes, swamps, or aquatic grass beds) have been and are still used to assess the integrity of wetlands. The use of invertebrates, which play an important role in food webs and in nutrient recycling, has many advantages. Invertebrates are a good indicator of ecosystem health because they are continually exposed to natural or anthropogenic disturbances, and they often show predictable responses to these disturbances, or they integrate episodic or cumulative water quality impacts in time and space (Burton et al., 1999; Uzarski et al., 2004; 2017).

This document sets out the nationally standardized CABIN field protocol for the collection of macroinvertebrate samples in the littoral zones of wetlands; it also provides information on associated habitat characteristics. More specifically, it provides guidance on:

- Using macroinvertebrates for biomonitoring
- Ensuring safety
- Determining the optimal time to sample
- Collecting required non-field data before sampling
- Collecting macroinvertebrate samples for CABIN and related water quality and habitat data
- Preparing the equipment required to carry out the field protocol

Biomonitoring using macroinvertebrates

Aquatic macroinvertebrates—freshwater organisms, including insects, molluscs, crustaceans, and segmented worms—are an important link in the food web, as they serve as food sources for other macroinvertebrates, fish, amphibians, birds, and mammals.

The CABIN wetland protocol covers the collection of both benthic and pelagic macroinvertebrates from the substrate, water column, and aquatic plant habitats. Macroinvertebrates are defined operationally as invertebrates that are retained by a net or sieve with a mesh size of at least 400 µm. Although insects are the most diverse group of macroinvertebrates in streams and rivers (accounting for almost 70% of known species in North America; Rosenberg et al., 1997), the macroinvertebrates that inhabit wetlands belong to a handful of orders: Turbellaria, Rotifera, Nematoda, Annelida, Mollusca, and Arthropoda. A worldwide inventory of 447 wetlands identified 40 dominant macroinvertebrate families, which included 25 insects, 5 annelids, 4 crustaceans, 4 molluscans, 1 acarine (mites), and 1 turbellarian. The most common insect families were distributed

as follows: 8 Diptera, 5 Hemiptera, 4 Coleoptera, 4 Odonata, 2 Ephemeroptera, and 2 Trichoptera. Two insect families, Chironomidae and Dytiscidae, were found in all 447 wetlands (Batzer and Boix, 2016). Figure 1 presents examples of common wetland taxa.

Among the many environmental factors which control invertebrate community diversity and abundance, four are determinant: hydroperiod (e.g., duration, amplitude), plants (e.g., macrophytes and algae), anthropogenic factors and predation (by fish or salamanders). However, causal links between these factors and macroinvertebrate assemblages are sometimes weak or indirect depending on wetland type (Batzer, 2013). Macroinvertebrates are highly sensitive to a range of environmental stressors and can be good indicators of pollution by toxic substances (Kenney et al., 2009). The results of a preliminary literature review suggest that indices or metrics that have been developed for rivers or lakes are not directly applicable to wetlands. Fortunately, invertebrate responses to anthropogenic stress have been well studied in rivers, and numerous wetland taxa (i.e., Cladocera) have long been considered useful in toxicological studies (Suhett et al., 2015; Sarma and Nandini, 2006).

Figure 1 Common invertebrates found in wetlands, clockwise from top left: Amphipoda, Diptera (family Chironomidae), Gastropoda and Cladocera (family Daphniidae) (Sources: Society of Freshwater Sciences, Environment and Climate Change Canada)

Biomonitoring of macroinvertebrates is not carried out in wetlands to the same extent as in rivers. For this reason, the science underpinning wetland biomonitoring (i.e., bioassessment metrics, indicator species) is incomplete. The use of macroinvertebrates for the assessment of wetland health is relatively new and presents several challenges (Table 1). For example, in order to develop a Benthic Index of Biological Integrity (B-IBI), such as that applied in the Great Lakes (Cooper and Uzarski, 2016), it is necessary to first identify the variability in community structure that is due to natural factors

and distinguish it from the variability that is due to anthropogenic factors. It is also necessary to calibrate the resulting IBI. Two possible approaches include the following: comparing reference communities to those at disturbed sites; and comparing the IBI along a disturbance gradient (Cooper and Uzarski, 2016). It should be noted, however, that there is a disparity between the dominant taxa in rivers (Plecoptera, Ephemeroptera and Trichoptera) and those in wetlands. Therefore, for wetland assessments, it would be appropriate to establish metrics based on the combined richness of Mollusca, Hemiptera and Coleoptera (Batzer and Boix, 2016). A list of environmental stressors and the metrics used to evaluate them can be found in Ruhí et al., 2016.

Table 1 Criteria for using macroinvertebrate bioassessments in typical aquatic environments in relation to wetland habitat (Batzer et al. 2001, adapted from Rosenberg and Resh, 1993).

CRITERIA FOR USING MACROINVERTEBRATE BIOMONITORING IN AQUATIC ENVIRONMENT	APPLICABILITY OF CRITERIA FOR WETLAND
ADVANTAGES	
Macroinvertebrates are ubiquitous	True
A large number of species are present, offering a wide spectrum of responses	True
Macroinvertebrates are usually sedentary, allowing for effective spatial analysis	Many wetland taxa are highly mobile (i.g., hemipterans and coleopterans), especially in ephemeral wetlands
Sampling macroinvertebrates is a simple procedure	True
The taxonomy of many groups is well understood	Less so for wetland groups (i.e., microcrustaceans)
Macroinvertebrates can be quite easily manipulated in experimental studies (i.e., bioassays)	True
DISADVANTAGES	
Macroinvertebrates may not respond to all types of pollution or all types of human impacts	Similar to lotic habitats
Large numbers of samples are often needed to achieve desirable precision	Similar to lotic habitats
There are potentially confounding seasonal effects (i.e., emergence of adult insects)	Similar to lotic habitats
Some groups can be difficult to identify (i.e., Chironomidae)	Similar to lotic habitats
Biotic indices are just beginning to be developed	Especially true for wetlands

In an effort to address these challenges, this document describes a protocol for the collection of macroinvertebrate samples, water chemistry data, and associated wetland habitat data in safely wadeable wetlands.

The sections below include discussions and recommendations for:

- Pre-sampling considerations
- Initial procedures when arriving at a wetland
- Habitat variables
    - Macrophytes
    - Other habitat measurements
- A sampling method for wetland macroinvertebrates
    - When to sample
    - Where to sample
    - How to collect samples for DNA analysis
- Sample processing and taxonomy

Optimal time to sample

The optimal time period for sampling corresponds to the time interval when the most representative macroinvertebrate communities are present and the number of immature taxa is minimal. There are several considerations for defining this sampling period, including the purpose of the study and the different types of taxonomic assemblages concerned (Barbour et al., 1992).

The hydrologic cycle and associated characteristics of wetlands are important in selecting the sampling period (Table 2). Temporary or seasonal wetlands, such as potholes, should be sampled early in the summer and even in spring when they still contain water. Semi-permanent, intermittent or permanent wetlands with a flooding duration of several months or even a full year may be sampled throughout the summer. In the American states bordering Canada, sampling periods range from April to September depending on the ecoregion (U.S. EPA, 2002b). In Alberta, wetland sampling is conducted from mid-June to late July to coincide with the presence of mature macrophytes (ABMI, 2007). In Quebec, sampling in the littoral wetlands of the St. Lawrence River is done in September (Tall et al., 2008, 2015). In addition, it is recommended that multiple sampling trips be made over the course of a year to identify the optimal sampling period for macroinvertebrate abundance and diversity. In permanent wetlands, this approach will likely yield different assemblages of macroinvertebrates, which can give an indication of seasonal fluctuations of macroinvertebrate diversity and abundance within a wetland.

Table 2 Cowardin Water Regime Modifiers (adapted from Government of Alberta, 2016)

WATER REGIME MODIFIER (WETLAND INUNDATION)	WEEKS FLOODED	WETLAND CLASS	DOMINANT VEGETATION ZONE	VEGETATION FORM
Temporary	1–4	Marsh	Wet meadow	Graminoid
Seasonal	5–17	Marsh Shallow Open Water	Shallow Marsh	Graminoid Submersed/ Floating Aquatic Vegetation
Semi-permanent	18–40	Marsh Shallow Open Water	Deep Marsh	Graminoid Submerged/ Floating Aquatic Vegetation
Intermittent	41–51	Shallow Open Water	Open Water or Bare ground	Bare
Permanent	52	Shallow Open Water	Open Water	Submerged/ Floating Aquatic Vegetation

A second important aspect to consider in selecting a site is vegetation type. Emergent and submerged plants do not support similar invertebrate communities. Some invertebrates are associated with a particular type of vegetation (i.e., Gammarus and Pisidium are strongly associated with dense, submerged vegetation beds while Isopoda are associated with emergent and floating vegetation). In littoral lake wetlands, Uzarski et al. (2017) recommend sampling vegetation zones where floating or emergent plants represent at least 75% of the vegetation. One possible strategy could involve sampling according to vegetation type, for example, targeting bulrush-dominated zones or wet meadow zones (Cooper and Uzarski, 2016).

In summary, in order to identify the optimal sampling window, it is important to have at least minimal knowledge of the hydrologic cycle of the wetland(s) covered by the study. This can be estimated from the composition of the vegetation. The presence of submerged or floating vegetation indicates a long period of inundation, while the presence of graminoids indicates a short period of inundation. It is recommended that field reconnaissance be undertaken prior to sampling. Generally, sampling will be timed according to the water regime, and will be carried out in April or May for temporary and seasonal wetlands, and in July, August and September for semi-permanent, intermittent and permanent wetlands.

Study design and data analysis

Prior to sampling, it is important to consider the objectives of the study, the question(s) the study seeks to answer and the most plausible approach. Is the purpose to assess the impact of a point source of contamination? To determine the status of macroinvertebrate communities (characteristic conditions, cumulative effects assessment)? To assess trends in macroinvertebrate communities (measurement of changes, assessment of remediation or restoration measures)? To ensure regulatory compliance (point-source measures)? These questions will help identify the best approach to adopt, such as Before-After Control- Impact (BACI), Control-Impact (CI), Simple Gradient, or Reference Condition Approach (RCA).

Each of these approaches has advantages and disadvantages, and it is up to the project manager to choose the most appropriate approach for a given study. The next three paragraphs briefly describe possible study designs. For more information on applying various designs, see Environment Canada (2010) and Mellina and Hinch (1995).

Gradient designs

Gradient designs focus on locating sampling sites across a range of exposure to a given stressor (i.g, agricultural land-use, urbanization). Gradient designs are appropriate where a clear stressor gradient exists, and will work best in landscapes with smaller, isolated wetlands characterized by well-defined catchments and relatively simple hydrology. Gradient approaches are difficult to apply in larger wetlands, as establishing stressor gradients at a larger spatial scale and obtaining adequate replication may prove challenging. For more information on applying gradient designs in wetlands, see Cooper et al. (2006).

Before-After Control-Impact (BACI)

The BACI approach was initially described by Green (1979). It involves sampling both control and impacted sites before and after a disturbance in order to control for natural variability. Sampling should be done simultaneously at control and impacted sites. In order to adequately assess the natural variability, it is preferable to sample several control sites and impacted sites.

The BACI approach is particularly well suited in cases where impacts result in significant permanent changes to macroinvertebrate communities (Mellina and Hinch, 1995).

Reference Condition Approach (RCA)

Used widely in river biomonitoring, the RCA has seen limited application in wetland ecosystems due to the length of time required to capture reference conditions once variability due to hydrology and vegetation are isolated (Cooper and Uzarski, 2016). Briefly, RCA designs rely on establishing "reference conditions" by sampling a number of minimally impacted sites and classifying them into groups based on their assemblages. Using habitat variables to develop predictive models from the reference invertebrate assemblages, test sites are then sampled and their composition evaluated against predictions. Test sites are considered impacted if their observed community differs notably from the expected reference community. The Australian Wetlands Assessment and Monitoring Program (AUSWAMP) is an example of the RCA approach applied to wetlands (Davis et al. 2006). As mentioned previously, it make take several years to establish reference conditions in wetlands, as data must be obtained to capture the natural variability in the hydrologic conditions influencing wetland structure and above-ground vegetation (conditions which may only be fully observed over decades). Accordingly, multiple years of data may be required to establish the range of variation in assemblages representing "reference conditions." Caution should also be exercised to avoid over-interpreting large inter-annual differences in the absence of relevant information on temporal variability. Indeed, the characteristics of a given wetland vary with variations in the water balance, for example, inputs from streams, runoff, precipitation, groundwater, and evaporation, which are climate controlled and vary significantly in the short and medium terms (Euliss et al., 2004; Batzer and Boix, 2016).

Metrics and indices

Metrics are summary descriptors of an assemblage or sample, for example, species richness. In certain cases, assemblage information can be related to external information about the sensitivity of taxa to stressors or pollution in order to derive a single number, an index. Perhaps the best-known index for aquatic biomonitoring is the Hilsenhoff Biotic Index (HBI), which is often used as an indicator of organic pollution.

Multi-metric indices assign a weight to and combine several metrics or indices into a single value that can be compared across different sites in a region to assess impairment. However, many of these metrics and indices were developed for riverine ecosystems, so the science underpinning their use in wetland ecosystems is incomplete, and in some cases riverine indices may not be transferable to wetlands. For example, the EPT index, calculated as the proportion (%) of larvae of Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies), in a sample, will undoubtedly have different properties in wetlands, since stoneflies are not commonly observed in wetland habitats (Burton et al., 1999; Helgen and Gernes, 2001). In addition, it is recommended that IBIs be stratified according to the zonation of the vegetation, as is suggested for lacustrine wetlands (Uzarski et al., 2017; Cooper and Uzarski, 2016).

The development of wetland-specific metrics and indices is an area of ongoing research in CABIN and elsewhere (Uzarski et al., 2004; 2017; U.S. EPA, 2002b), and the comparability of macroinvertebrate assemblages between wetlands and rivers will benefit from continued wetland index development. Cooper and Uzarski (2016) indicated that an important step in developing an IBI to assess wetland health is to distinguish between variability that is due to natural factors and variability that is due to human activities. An invertebrate IBI can be calibrated by comparing reference communities with impacted ones or by observing variations in the IBI along a disturbance gradient.

Overview of CABIN wetland sampling procedures

This section outlines the types of measurements and samples that are collected at a wetland site as part of this protocol. A field sheet is provided in Appendix A.

- **Primary Site Data:** These data are determined before field sampling begins, usually during the site selection process. The wetland name, estimate of site location coordinates, and the ecoregion, are all recorded. Classification of the wetland will also be helpful for deciding where and when to sample. For example, the hydrology of prairie potholes will dictate when to sample the wetland. Many of them will be dry in July or August so sampling should take place in the spring. The same reasoning may be applied to other types of wetland.
- **Site Selection:** The site will be representative of the wetland of interest and encompass the zone with the highest diversity of benthic macroinvertebrates. The ideal sampling location is an area of emergent and submergent vegetation, typically in water 0.2 to 1 metre deep. The aim is to provide a rapid assessment of the composition of macroinvertebrate assemblages that can be compared among sites, not an exhaustive inventory of biodiversity within sites.
- **Site Description:** This is a broad characterization of the site. It includes a site drawing and written description, GPS coordinates, and surrounding land use classification.
- **Wetland Characteristics:** This is a description of the wetland in general: canopy coverage, submerged and emergent aquatic plant coverage, and filamentous or other obvious algal growth at a defined sampling site.

- **Water and Sediment Chemistry:** This includes measurements of relevant physical-chemical water and sediment quality parameters.
- **Macroinvertebrate Sample:** This is obtained using the standardized CABIN benthic macroinvertebrate collection method. A travelling sweep technique, with a 400-µm sweep net, is used to collect the sample.

It is critical that all measurements be completed where possible. Incomplete information will compromise future data analysis.

ALWAYS check field sheets before leaving a site. Ensure that all measurements have been taken and all samples collected.

There are times when a field parameter cannot be assessed and recorded on the field sheets (for example, due to equipment malfunction). In such a case, make a clear note on the field sheet indicating why specific fields were not completed. The site survey is only complete when data has been entered in all fields on the field sheet.

Creating site codes

Site codes are important identifiers that are essential for data management, and each site must have a unique code. To avoid duplication, only the project manager should assign codes, and site nomenclature should be established prior to field sampling. The existing conventions for naming lotic CABIN sites can be applied to wetlands and are outlined below. Wetlands often do not have an established name associated with them, which poses a challenge. In the case of unnamed wetlands, ECCC recommends scaling up to the nearest unique identifier (e.g., basin/sub-basin/region). Maps of major and minor watersheds are available on the Natural Resources Canada website www.nrcan.gc.ca/earth-sciences/geography/atlas-canada/selected-thematic-maps/16888), or on provincial webpages. Some examples are provided below:

Quebec: www.mddelcc.gouv.qc.ca/eau/bassinversant/index_en.htm

Ontario: www.ontario.ca/environment-and-energy/great-lakes-watershed-locator

British Columbia: http://maps.gov.bc.ca/ess/sv/wrbc

CABIN recommended site coding system

The current conventional site nomenclature includes:

- 3 or 4 letters for a unique watershed identifier code (i.e., basin/sub-basin/wetland name)
- 2 or 3 numbers for a site number
- 1 or 2 numbers or letters for another level of sampling within a site (i.e., replication - if only benthic replicates are taken, multiple samples can be entered in the CABIN database for a given date therefore this additional numbering system is not required).
- Date is recognized by the CABIN database and does not need to be included in the site code

For example, PAD33 would be the site code for site number 33 in the Peace Athabasca Delta. If replicate samples are collected on the same date, another identifier is appended to the site code; the first sample can be designated as A (PAD33A), the second sample as B (PAD33B) and the third sample as C (PAD33C). The CABIN database allows multiple site visits (i.e., visiting the same site in different years) to be entered under the same site code, assuming the location is the same. As a result, there is no need to include the year in the site code.

Health and field safety

There are potentially unsafe conditions in certain wetlands such as bogs and fens, which can pose considerable risk for operators due to hazardous wading conditions. For example, deep holes in wetlands due to the presence of beavers can pose significant danger as the water level is often over a person's head. Personal safety and the safety of the field team must be ensured before field sampling is carried out. Sampling should never be conducted in unsafe conditions. Field safety equipment must be available and all field personnel must be adequately trained and follow health and safety procedures. No sample is worth risking the health or lives of team members. This section describes safety considerations for field sampling and proper use and handling of chemicals.

Field safety training, procedures,and equipment

The following safety recommendations apply to anyone who is conducting field work.

- Do not conduct sampling if all or some of the following conditions are unsafe: higher water than usual, weather conditions, dangerous wildlife in the area, construction.
- Never carry out sampling alone. A field crew should consist of at least two people; three-or four-person field crews are beneficial for safety and efficiency.
- Establish a check-in procedure with colleagues in the office or a call-in service.
- Always inform someone of the sampling route and sampling location along with the expected return time.
- Wear a PFD when working near or in water, no matter how safe the conditions appear.
- Additional safety considerations for remote areas include the following: egress training if site access is by helicopter; survival gear and food; and appropriate training for field sampling in remote areas.

Environment and Climate Change Canada is not responsible for safety training of CABIN participants. The mandatory safety training, procedures, and equipment for ECCC employees are listed here only as an example. CABIN participants should comply with the occupational health and safety requirements of their respective organizations.

Safety training

- Swiftwater Rescue (Rescue Canada) (river and delta wetlands)
- Wilderness Survival (remote areas)
- First Aid Level 1 (St. John Ambulance)
- Bear Awareness (depending on location)
- Wilderness first aid (remote areas)
- Boat operator training
- Underwater egress

Safety procedures

- Read and sign the task hazard analysis (THA) for small boat operation, aircraft, bear awareness, and wading.
- Read and sign the safe work procedures (SWP) for wading in wetlands.
- Fill out a site inspection sheet for each site upon arrival.
- Provide the supervisor with a field sampling itinerary prior to departure.
- Establish check-in procedure for the beginning and end of the day.
- Protect against insect pests such as mosquitos and ticks.
- Provide proper protection from toxic plants.

Safety equipment

- PFD
- Wading staff, waders, and a first aid and survival kit brought to all field sites
- A satellite phone is also recommended if enclosed in a waterproof casing

Use and handling of chemicals and preservatives

Field preservation is necessary to prevent degradation. If DNA sampling is to be carried out, preserve samples in the field with 95% ethanol (with little to no water) and transfer to fresh 95% ethanol in the lab for long-term storage in a -80°C freezer. Large amounts of organic matter in samples may jeopardize proper preservation, so the transfer to fresh 95% ethanol is recommended, especially for large sample volumes. If DNA preservation is not a primary concern, 70% ethanol can be used, again using as little water in the sample as possible. ECCC also recommends cleaning and removing large leaves and plant material from the sample in the field prior to preservation, as subsampling in the lab will be more expedient and leaching of plant pigments can affect DNA analysis. Macroinvertebrate samples can also be preserved in the field using 10% buffered formalin in a 1:3 ratio (one part formalin to three parts sample).

Samples that contain large amounts of organic matter (i.e., leaf litter, twigs) often require multiple sample containers to maintain adequate preservative ratios.

Ethanol and formalin can be hazardous and should be handled with care. Use of ethanol and formalin, like all chemicals, requires knowledge and understanding of the Workplace Hazardous Materials Information System (WHMIS) and the Material Safety Data Sheet (MSDS). Use proper personal protective safety gear (i.e., gloves, safety glasses) when handling all chemicals.

Pre-sampling water chemistry considerations

CABIN recommends that certain general physical-chemical water quality parameters be measured for each site; they are described below in the Water Quality section. Additional water quality parameters may be measured and analyzed by an analytical laboratory or on-site depending upon the available resources and the study objective.

It is important to make arrangements for analyses with the analytical laboratory prior to initiating sampling. Moreover, it is critical to maintain frequent contact with laboratory staff and notify them how many samples will need to be analyzed and when. The lab may provide the bottle sets needed and information on sampling requirements, such as preservatives or specific handling times. For more information on water quality measurements, see the Water Quality section below.

Pre-departure considerations

Check the weather forecast and recent water level information before initiating sampling (ECCC Weather - https://weather.gc.ca/canada_e.html; Water Survey of Canada - www.wateroffice.ec.gc.ca). In general, large or unusually heavy rain events can cause higher than normal water levels, which can affect all aspects of CABIN sampling such as:

- Water and substrate chemistry (i.e., suspended solids, contaminant spikes from runoff, pesticides)
- Benthic habitat and substrate characteristics
- Safety considerations

While not as important in wetlands as rivers, the influence of rain events should be taken into consideration when planning CABIN sampling. A period of time should be allowed to pass so that hydrologic conditions return to normal and stabilize.

Also important to consider is the need to obtain permission to enter private property or land where a permit is required (i.e., national or provincial parks).

Pre-departure equipment checklist

Before going into the field, ensure that all the necessary equipment is functioning properly. Water quality probes must be calibrated and batteries of electronic devices charged or replaced and checked for damage. Use a checklist to ensure that all equipment needed to implement the CABIN protocol is prepared and available (Table 3).

Table 3 Field equipment checklist for CABIN wetland sampling

General Equipment
Field sheets on waterproof paper and clipboard
Pencils and markers
Waterproof labels
Labelling tape
Ziploc bags
Tool kit and duct tape

Location Data
GPS
Compass
Map
Camera

Wetland characteristics
Meter stick with a foot/plate to prevent sinking in the mud
Measuring tape
Flagging tape
Calculator
Canoe/boat
Range finders
Turbidity tube

Water chemistry sampling
Water quality meters (temp, pH, DO, conductivity, turbidity)
Cooler with sample bottles and ice pack
Extra batteries
Water filtration equipment and filters

Macroinvertebrate Sampling
Sampling net with 400-µm mesh size
Stopwatch
Squeeze bottle
Spoon/tweezers
Bucket
Sieves
White tray
Sample jars
Preservative
Tightly sealed container for sample jars

Safety equipment
Lifejackets
First aid kits (field and vehicle)
Cell phone or satellite phone
Throw bags
Waders, boots, raingear
Gloves (rubber, neoprene)
Safety goggles
MSDS sheets for chemicals
Sunscreen, hat, bug spray
Bear spray, other deterrents
Personal locater beacon or GPS messenger (i.e., Spot, InReach)

COLLECTING CABIN WETLAND SAMPLES

Primary site data

Certain site attributes can be determined prior to field sampling using topographic maps and online resources (data sheet illustrated below). Primary site data includes a project name or CABIN study name. The study name should be unique to your project, including the region, purpose or scope.

The primary site data should also include a wetland name and local basin name. The ecoregion will be automatically assigned by CABIN when data is entered into the database; however, before going into the field it is useful to know for study design purposes what kind of landscapes will be sampled.

PRIMARY SITE DATA

CABIN Study Name: _____

Wetland Name: _____

Local Basin Name: _____

Ecoregion: _____

Arrival time: _____

Departure time: _____

Local basin and wetland name

The local basin name is based on a large or well- known river in the study area. This information is used as a reference to the region where the site is located. The local basin name is on a scale that is meaningful to the project manager. Once location information is entered in the CABIN database, a large-scale identifier will be automatically assigned from the Know Your Watershed website (www.canadiangeographic.com/watersheds/map/?path=english). This watershed scale will be consistent from study to study within CABIN.

The wetland name is the official name of the water body. Revert to the site code if no name is available. Obtain the wetland name from a map, signage in the area, or a local expert.

Method

Enter the CABIN study name and the wetland name on the field sheet.

Ecoregions

An ecoregion is a unit of space that is defined by unique landscape characteristics. Characteristics used to define entered on the field sheet the ecological framework in Canada include geology, soil, vegetation, climate, wildlife, water, and human factors (Ecological Stratification Working Group 1995). Ecoregions provide general information about the broad differences between regions. The dominant landscape characteristics differ from one ecoregion to another. The ecoregion summarizes the major environmental gradients in the study area.

When a study is being designed, ecoregions may be used to stratify site selection. Ecoregion information can be obtained from the Ecological Framework of Canada website (http://ecozones.ca), and can be.

Method
Ecoregion information can be obtained from the CABIN database by entering the GPS coordinates for the site.

Initial procedures upon arriving at site

Initial procedures upon arriving at the site include a safety inspection, determination of the area to be sampled, and the documentation of the site code, sampling crew names, and date. Stay on the margins and refrain from entering the wetland at this time. All data collection prior to macroinvertebrate sampling should be done from the shore where possible.

Safety inspection

OHS: Site Inspection Sheet Completed

All members of the field crew should be aware of potential safety hazards and be appropriately prepared. A thorough inspection upon arrival is important for preventing accidents.

Examples of safety considerations are:

- Potential hazards such as parking on the highway shoulder, bear activity in the area, beavers, hunting season, poor footing in sampling area
- Vehicle approach and parking at site
- Weather conditions such as approaching storms, heavy rain, high winds, excessive heat and cold (i.e., lightning protection at www.ec.gc.ca/foudre-lightning/defaultasp?lang=En&n=159F8282-1)
- Boating hazards if site access is via water

ECCC uses a standard site inspection sheet for all visits. Safety hazards are identified on this sheet along with emergency contact information and other safety procedures. Be sure to inform someone of your itinerary and sampling location in order to maintain a chain of communication. The site inspection sheet is provided in Appendix A.

Method
Complete the site inspection as required, and provide the necessary site inspection information on the field sheet.

Location inspection and site selection

Many wetlands are fragile habitats and they may contain rare, highly-ecological valued or delicate vegetation and/or fauna. A number of wetlands provide habitat for "at-risk" species covered by the Species at Risk Act (SARA) (www. sararegistry. gc.ca), and potential study sites should be considered from this perspective. Extreme care must be taken to ensure that any wetlands targeted for sampling do not constitute protected areas under conservation law. Registers of protected areas can be accessed on the websites of the Canadian Wildlife Service (www.ccea.org/en/google-earth-download), Parks Canada or provincial agencies (e.g.,www.mddelcc.gouv.qc.ca/index_en.asp, www.ontario.ca/environment-and-energy/find-conservation-reserve). Always obtain the necessary permits from federal and/or provincial agencies prior to carrying out sampling (i.e., www.pc.gc.ca/progs/np-pn/recherche_research/index_e.asp; www.ec.gc.ca/

default.asp?lang=En&n=9AC6536A-1; https://mffp.gouv.qc.ca/faune/formulaires/demande-permis-seg.jsp)

Site selection will differ for each type of wetland and many factors need to be considered before choosing a sampling location. Ideally, the site will be representative of the wetland of interest and encompass the zone with the highest diversity of benthic macroinvertebrates. While this may be possible from any point in a wetland, the most important factors are accessibility and safety. Some questions to think about when selecting a sampling site:

- Is there an easy access point to the wetland?
- Does the wetland contain any fragile vegetation that must be avoided?
- Is it a safe depth for wading?
- Can the team exit quickly in case of an emergency?
- Will the team disturb the samplinglocation by walking near the site?

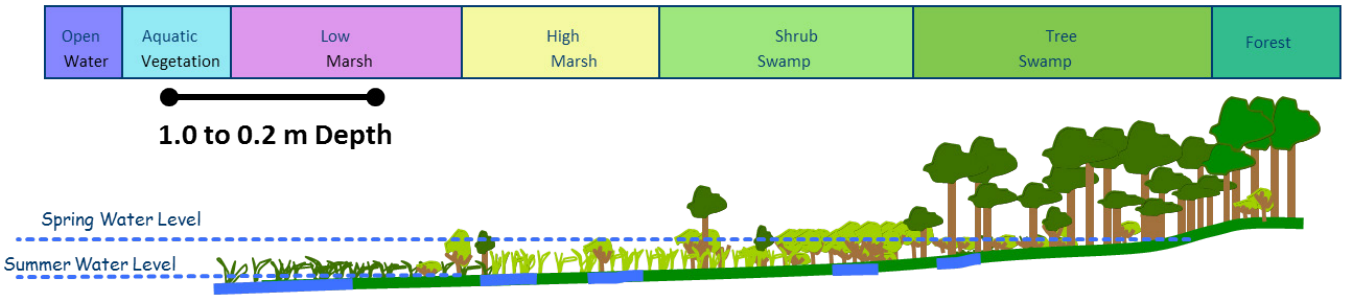
These are all questions that can be discussed by the sampling team before entering the wetland. Mapping tools and GIS can also provide an idea of how to access a wetland and where to sample.

The ideal sampling location is an area of emergent and submergent vegetation, typically in water 0.2 to 1 metre deep (Figure 2). The aim is to provide a rapid assessment of the composition of macroinvertebrate assemblages that can be compared among sites, not an exhaustive inventory of biodiversity within sites. This area is inundated by water for most of the year, providing the most desirable habitat for macroinvertebrates. Evidence of an emergent zone includes the presence of cattails, sedges, bulrushes, reeds, and arrowheads. Submergent vegetation grows just underneath the water surface and includes species of hornwort, milfoil and pondweed. Pond lilies are not a good indicator of the emergent/submergent zone as they are floating-leaf plants and may grow in water that is beyond safe wading depth. Figure 2 below presents an example of a good sampling site, provided it is a safe wading depth. A canoe or small boat may be used for sampling if the conditions are not safe for wading.

It is important to carefully choose a vegetation zone according to the purpose or goal of your study. Each vegetation zone is characterized by distinct biophysical properties.

ECCC recommends that water and macroinvertebrates samples be collected in a similar vegetation zone. In addition, sampling must be carried out in the same type of plant stand. Based on hydrology and elevation, water chemistry and community composition often differs drastically but in a predictable manner among wetlands.

Figure 2 Example of a good sampling site with the regions of a wetland outlined above.



Site code, sampling crew, and date

The site code, sampling crew names, and the date are recorded at the top of every field sheet. This information ensures that the data on the sheet can be attributed to the proper sampling location and time, and that the field crew can be consulted if questions arise about the field data. This information also ensures data security, should the pages become separated.

Field Crew: _____ Site Code: _____

Sampling Date (DD/MM/YY): _____

Method

Record the field crew names, site code and date on the top of each field sheet. Be sure to record this information at the top of every page.

Site description

Description of the site includes notes and directions for getting to the site, a characterization of the surrounding land use, geographic coordinates, a map and photographs of the site. These observations describe the general characteristics of the site.

Important Note
Macroinvertebrate and water chemistry samples must be collected from an undisturbed area. Be careful not to disturb the sampling area.

If it is necessary to enter the wetland to complete the site description or wetland characteristics before the invertebrate and water chemistry samples are collected, exercise caution and remain a reasonable distance from the chosen sampling area.

Geographical description and notes

The geographical description and notes include directions to the site (roads, trails, landmarks and notable features of the sampling location. This information, along with the site map drawing (see below), can be used to return to the sampling location. Notes may be helpful for data interpretation.

GEOGRAPHICAL DESCRIPTION/NOTES:			
LAND USE POTENTIALLY INFLUENCING WETLAND (0–50 M) **LAND USE** *(CHECK THOSE PRESENT):*		**INFORMATION SOURCE:** _____	
☐ Forest	☐ Field/Pasture	☐ Agriculture	☐ Residential/Urban
☐ Logging	☐ Mining	☐ Commercial/Industrial	☐ Other _____
DOMINANT LAND USE *(CHECK ONE)*:		**INFORMATION SOURCE:** _____	
☐ Forest	☐ Field/Pasture	☐ Agriculture	☐ Residential/Urban
☐ Logging	☐ Mining	☐ Commercial/Industrial	☐ Other _____

Land use potentially influencing wetland and dominant land use

A general description of land use and human activity adjacent to the sampling site should be made to facilitate later consideration of potential influences on the macroinvertebrate community at the site.

Method

1. Check the appropriate box for all land use types that are upstream from the sampling site under the "Land Use Potentially Affecting Wetland" category.
2. Check the dominant land use type in "Dominant Land Use."
3. Indicate the information source, whether observed at the site, gathered from a map, aerial photos, local experts, or another source.

Location data

Obtain latitude and longitude coordinates and elevation for the sampling site from a handheld GPS device. The CABIN database contains the latitude and longitude coordinates in decimal degrees (DD) or degrees-minutes-seconds (DMS). Therefore, UTM coordinates must be converted before entry. Often the site coordinates are approximated using GIS or a topographic map before field sampling to aid in locating the site, and true coordinates are measured at the site using a handheld GPS unit.

Method

1. Measure latitude, longitude, and elevation with a handheld GPS unit.
2. Indicate whether the coordinates are reported in DD or DMS.
3. Indicate the GPS datum. The same datum should be used for all measurements.
4. If replicates are being collected at the same site, include GPS data for each one.

LOCATION DATA

Latitude: _____N Longitude: _____W (deg/min/sec or decimal deg

Elevation: _____(masl) GPS Datum: GRS80 (NAD83/WGS84) ☐ Other _____ ☐

Site map drawing

A hand-drawn map illustrates major wetland and landscape features, how the site was accessed, and relevant landmarks and delineates the sampling area. This map is used as a guide for later site visits, and provides additional information for potential interpretation of local influences on site observations (i.e., community composition, water quality). See Figure 3 for an example of a site drawing.

Method

1. Draw a map (plan view) of your sampling area on the field sheet in the appropriate space (Figure 3).
2. Indicate the macroinvertebrate sample location by sketching the sweep path taken during sample collection, the location of water quality samples, and meter readings.
3. Include wetland shape if it is small, habitats (i.e., fallen logs, vegetation connecting streams and any hazards), broad landscape features (i.e., cliffs, hills, vegetation), access point, roads and trails to the site, landmarks, and orientation. In addition, indicate the map's scale. This could be done using a range finder, or before sampling, with aerial photography from Google Earth.
4. Note any potential sources of disturbance.

Figure 3 Example of a completed site location map. NOTE: Limit the amount of time spent on the drawing. It is intended to be a simple sketch for future reference.

Site photographs

Site photographs capture broad features of the sample reach. Because returning to the site is often impractical, photos will serve as an important reference and can assist in addressing questions that arise during data entry or analysis. Photographs also illustrate how a site has changed, if it is revisited. The first photo taken is always that of the top of the completed field sheet (with site code, field crew and sampling date filled in) in order to reconcile site photographs with field sheets and other observations. See Figure 4 for examples of site photographs.

PHOTOS

☐ Field sheet _____ ☐ North _____ ☐ East_____ ☐ South _____ ☐ West _____

☐ Vegetation (in sampled area) _____ ☐ Exposed Substrate / Mud Flat _____

☐ Acces point _____ ☐ Aerial Photo _____ ☐ Other _____ (i.e., Sampling photos)

Method

1. A minimum of six photos should be taken of each site. Check the appropriate boxes once the photos are taken. Photos are taken from the site at which the invertebrate samples are collected.

2. Take photographs in the following order:

- Photograph 1 - Field Sheet with visible site code and sampling date
- Photograph 2 - Facing north across wetland
- Photograph 3 - Facing east across wetland
- Photograph 4 - Facing south across wetland
- Photograph 5 - Facing west across wetland
- Photograph 6 - Vegetation in sampling area

Photos of other notable features of interest, or hazards, the access point to the wetland, action shots of sampling, exposed substrate, aerial shots, and any other additional vegetation photos can be taken after Photograph 6.

3. If additional photos are taken, check the appropriate box and/or note each additional photo on the field sheet.

Figure 4 Examples of site photography. Clockwise from top left: Field Sheet, North, East, Vegetation, West, and South.

Wetland characteristics: whole wetland

HYDROLOGY				
WETLAND TYPE				
☐ Bog	☐ Fen	☐ Marsh	☐ Swamp	☐ Shallow water
WETLAND FORMS				
☐ Basin/Pond/Pool	☐ Lacustrine	☐ Riverine	☐ Peatland	
WETLAND WATER REGIME (CHECK ONE):				
☐ Temporary	☐ Seasonal	☐ Semi-permanent	☐ Intermittent	☐ Intermittent
☐ Permanent	☐ Unsure			
WETLAND WATER REGIME (CHECK ONE):				
☐ Dry	☐ Vestigial	☐ Recessional	☐ Intermediate	☐ Full
☐ Flooded	☐ Overflowing	Typical Depth_____(m)		

Wetland type

The hydrology of the wetland should be described, including the general hydrological type and the cycle phase. See Figure 5 for reference images.

If unsure of the wetland and hydrological type, it may be necessary to check more than one box and verify later with mapping tools or through additional research.

Figure 5 Various types of freshwater wetlands.
(Adapted from www.dem.ri.gov/programs/benviron/water/wetlands/)

Method

Several tools are available to identify wetland types. General maps of Canadian wetlands can be found on the Natural Resources Canada webpage: www.nrcan.gc.ca/earth-sciences/geography/atlas-canada/selected-thematic-maps/16888. For more refinement, consult the Canadian Wetland Inventory maintained by Ducks Unlimited Canada at http://maps.ducks.ca/cwi/.

This website provides a general description of wetlands, information contacts, and documentary sources. When in the field, use the Key to Canadian Wetland Classes provided below.

CLASSIFICATION KEY TO WETLAND CLASSES	
1.Terrain affected by water table at, near or above the land surface and which is saturated for sufficient time to promote wetland or aquatic processes	2. Wetland
2. Wetland ecosystems characterized by the accumulation ofpeat	3. Peatland
3. Peatland dominated by bryophytes and grainoids	4
4. Peatland receiving water exclusively from precipitation and not influenced by groundwater; Sphagnum-dominated vegetation	Bog
4. Peatland receiving water rich in dissolved minerals; vegetation cover composed dominantly of graminoid species and brown mosses	Fen
3. Peatland dominated by trees, shrubs, and forbs; waters are rich in dissolved minerals	Swamp
2. Wetland ecosystems characterized by minimal or no peat accumulation, although thin layers of muck and a mix of mineral and organic muck may be present	5. Mineral Wetland
5. Wetlands with free surface water persisting above the ground surface for variable periods or not at all. If surface water persists through the summer, water depths are sufficiently shallow to permit survival of woody or herbaceous vegetation which cover more than 25% of the surface area of the wetland	6
6. Periodically standing surface water and gently moving, nutrient-rich groundwater, with vegetation dominated by woody plants often more than 1 m high	Swamp
6. Periodic or persistent standing water or slow moving surface water which is circumneutral to alkaline and generally nutrient-rich. Vegetation is dominated by graminoids, shrubs, forbs or emergent plants	Marsh
5. Wetlands with free surface water up to 2 m deep, present for all or most of the year, with less than 25% of the surface water area occluded by standing emergent or woody plants. Submerged or floating aquatic plants usually dominate the vegetation	Shallow Water Wetland
1. Terrain not affected by high water table or excess surface water, or if affected, only for short periods such that hydrophytic vegetation or aquatic processes do not exist	Non-Wetland Upland

Adapted from: Warner and Rubec (eds), 1997

Delineating wetlands using geospatial information

Identifying the wetland catchment is important because it facilitates quantification of important information regarding the hydrology (e.g., connectivity, hydroperiod), surrounding vegetation, and surrounding land use of wetlands. The contributing area (catchment) for sites in rivers is normally delineated using topographic maps or from a digital elevation model (DEM) and GIS program. While the same approaches can be applied to some wetlands, the spatial resolution of topographic maps and DEMs (ca. ± 30 m) is usually too coarse; most wetlands are located in landscapes with very

little variation in elevation across a large area.

Remote sensing technologies such as airborne light radar (LiDar) can be used to construct fine resolution topographic maps, allowing wetland drainage points to be identified and variables such as watercourse connectivity to be accurately and precisely quantified. However, these products are not yet widely available, and may be costly to obtain for studies covering large areas.

Other sources of geospatial information may provide valuable information for the study, such as Google Earth 6 which allows users to view historical satellite and aerial photo imagery (www.google.ca/earth/explore/showcase/historical.html). If available, historical imagery can be used to identify the high/low water levels and coverage for a given wetland and provide other information on wetland hydrology or surrounding land use. Remote sensing imagery can also provide valuable information regarding the density of wetland habitat in a landscape (e.g., distance to nearest wetland, number of adjacent wetlands, wetland permanency classes of adjacent wetlands).

Wetland forms and water regime

Hydrology is a major environmental factor that influences the structure and composition of invertebrate communities in wetlands and ponds (Brazner et al., 2004; Collingson, 1995), rivers (Tall et al., 2008, 2015; Flinn et al., 2005), and lakes (Turner and Montgomery, 2009; Uzarski et al., 2009; White et al., 2011). Hydrology is also the most difficult factor to assess because it can vary on a daily, seasonal, and annual basis. Assessment of hydrologic conditions can therefore be complex and require long term follow-ups and significant investments (Bazoge et al., 2014).

According to Warner and Rubec (1997), wetland forms refer to "*subdivisions of each wetland class based on surface morphology, surface pattern, water type and morphology characteristics of underlying mineral soil*."

Where a hydrometric station exists, it is possible to obtain the hydrologic conditions for the time of sampling, as well as for the days, weeks, or months before the sampling visit.

The Water Survey of Canada (WSC) is responsible for more than 2,500 hydrometric stations (WSC; www.ec.gc.ca/rhc-wsc/). It is possible to determine whether a water body is served by a hydrometric station from the list of hydrometric stations or mapping tool. The federal network of hydrometric stations is comprised of several provincial networks. Links to these sites can be found in Appendix B.

Water-level stage at the time of sampling

The water-level stage at the time of sampling is recorded. It is measured in the location where the benthic sample is collected and can provide an estimate of water depth, along with observed depth (Figure 6). Since water levels often fluctuate significantly in wetlands, this water depth may be of limited value in data interpretation.

Figure 6 Measuring the water depth in the benthic habitat of a wetland

Wetland characteristics: vegetation cover

VEGETATION COVER

HIGHER PLANTS

Emergent

☐ 0% ☐ 1–5% ☐ 6–25% ☐ 26–50% ☐ 51–75% ☐ 76–100%

Floating

☐ 0% ☐ 1–5% ☐ 6–25% ☐ 26–50% ☐ 51–75% ☐ 76–100%

Submergent

☐ 0% ☐ 1–5% ☐ 6–25% ☐ 26–50% ☐ 51–75% ☐ 76–100%

ALGAE / CYANOBACTERIA

Algal bloom and/or cyanobacterial bloom

☐ Absent ☐ Present

FILAMENTOUS ALGAE

☐ Absent ☐ Present

BIOFILM PRESENT? ☐ Yes ☐ No

Comments (thickness, abundance, marl):_____

Higher plants

In lentic and lotic habitats, aquatic macrophytes affect processes such as water flow, temperature and clarity. They can increase the stability of the substrate, increase sediment deposition and provide a food source for benthic macroinvertebrates. Aquatic macrophytes support a greater diversity and higher densities of macroinvertebrates when compared to non-vegetated areas. Filter-feeding invertebrates attach to macrophytes and feed in the water column; invertebrates belonging to other orders (such as Odonata and Hemiptera) cling to macrophytes while hunting prey. Algae, bacteria, diatoms and periphyton grow on macrophytes and provide grazing opportunities for numerous species of macroinvertebrates. Macrophytes also serve as refuge from fish or amphibian predation (Peets and Miller, 1994).

For the purposes of this protocol, vegetation is considered as macroinvertebrate habitat, and does not represent an independent unit of assessment. If a precise description of a wetland's vegetation is needed for a specific study, see Appendix C for a detailed method for collecting and inventorying wetland vegetation.

Three broad classes of wetland vegetation are recognized: emergent, floating-leaf, and submergent. Emergent plants are rooted in sediment but have portions showing above the water surface, mostly their stems and leaves. Cattails, bulrushes, reeds, and arrowheads (Sagitaria) are examples of emergent plants. Floating plants, such as water lilies, duckweed and water lettuce, have leaves that float on the surface of the water; they may or may not be rooted in the sediment. Finally, submergent plants are those that grow completely underwater, often forming extensive stands on the bottom of water bodies. Among the best-known species are water milfoil, coontail, and riverweed. It should be noted that aquatic plants are distributed according to a depth gradient; emergent plants are found in the shallowest water, and submergent in the deepest.

Method

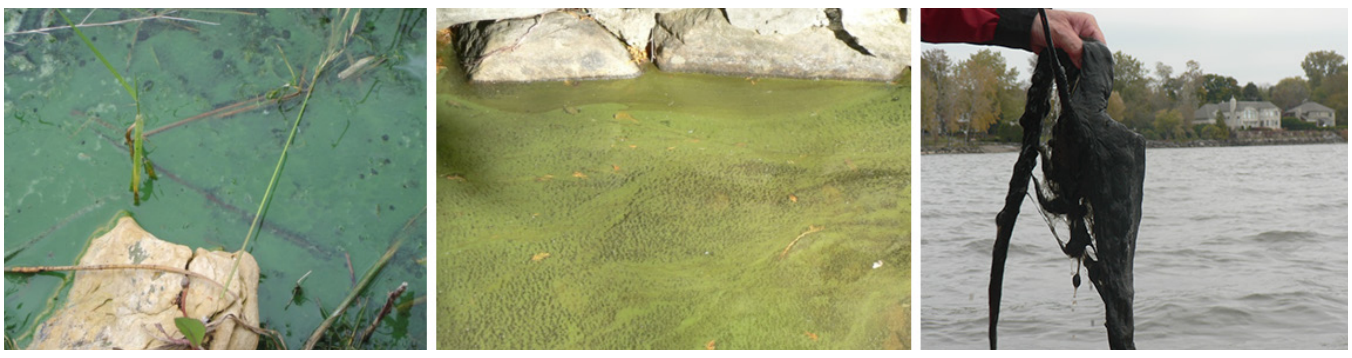
1. Observe stems of emergent and submerged plants at the sampling site.
2. Estimate the coverage of each category using the following scale: 0, 1–5%, 6–25%, 26–50%, 51–75%, 76–95%, and 96–100%.

If the study requires a more precise characterization of plant coverage, ECCC recommends using the procedure in Appendix C: Inventory of Wetland Vegetation.

Algae and cyanobacteria

The presence/absence of algae and cyanobacteria blooms, as well as of filamentous algae is noted. Examples of cyanobacteria blooms are shown in Figure 7.

Figure 7 Cyanobacteria bloom (left), algal bloom (middle) and filamentous algae (right). On the left, note the film resembling a coat of paint on the water surface. (Environment Canada, 2012)



Method

Observe the water around the sampling site. Note any algal or cyanobacteria blooms and the presence of filamentous algae.

1. Check the box on the field sheet for the appropriate category.

Biofilm

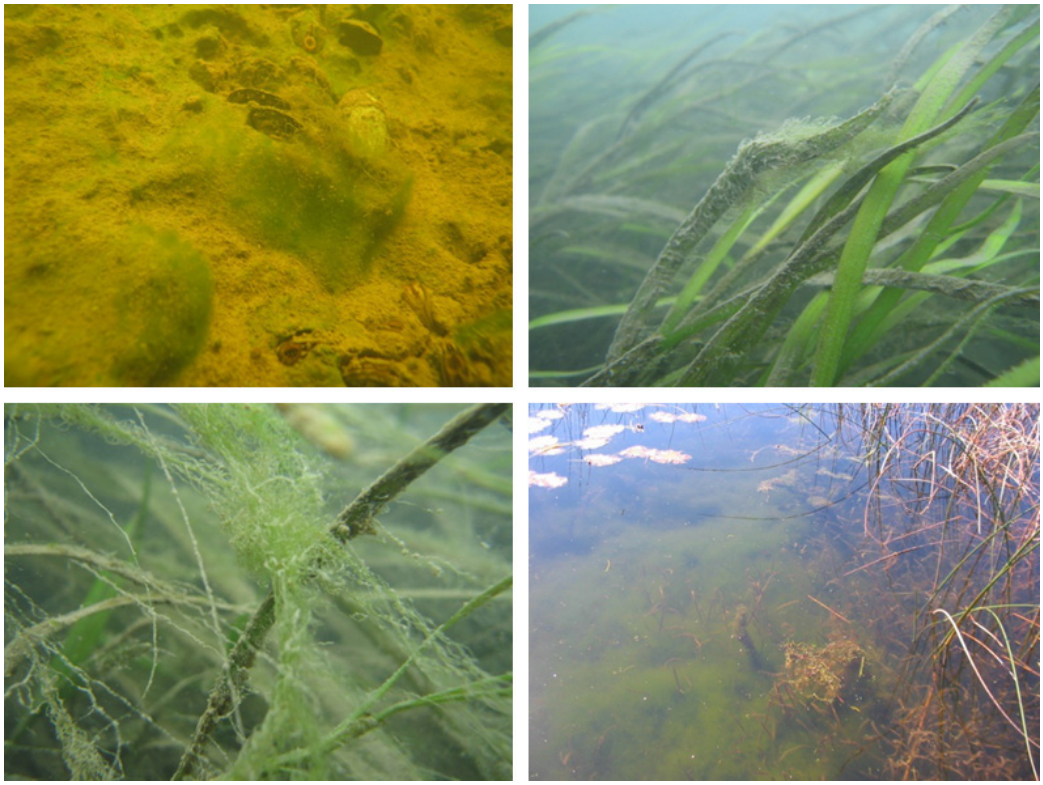
A biofilm is a layer composed of algae, fungi, cyanobacteria, bacteria, and detritus, which provides an important food source for many macroinvertebrates. Biofilms can be found on the surface of submerged leaves and stems of vegetation, on logs, and other submerged substrates.

Marl or marlstone is another type of film which may be present on submerged vegetation in wetlands. This calcium carbonate precipitate occurs in mineral rich freshwater; it is generally found in the substrate and as a milky white scale material coating vegetation.

Method

Examine the submerged leaves and stems of vegetation for biofilms and marl. Comments on thickness, coverage, and marl should be added to the field sheet. Figure 8 below shows examples of biofilms present in wetlands.

Figure 8 Biofilm and algae on aquatic vegetation. (Environment Canada, 2012)



Wetland characteristics: marginal vegetation

WETLAND MARGINAL VEGETATION 0–50 M AROUND WETLAND (CHECK THOSE PRESENT):				
☐ ferns/grasses/sedges	☐ shrubs	☐ deciduous trees	☐ coniferous trees	☐ agricultural crops
DOMINANT WETLAND MARGINAL VEGETATION (CHECK ONE):				
☐ ferns/grasses/sedges	☐ shrubs	☐ deciduous trees	☐ coniferous trees	☐ agricultural crops
CANOPY COVER / SHADING				
☐ Not shaded	☐ Partially shaded	☐ Fully shaded		
MANAGED BUFFER STRIP				
☐ Absent	☐ Present	☐ Natural setting, no buffer		

Marginal vegetation

The wetland margin is the zone extending from the edge of the wetland into the upland vegetation that is subject to flooding, especially in the spring and during rain events. In highly altered landscapes (i.e., urban areas), this area may extend only a few metres from the water's edge.

The delineation of a wetland is made through the examination of plants. This approach has the advantage of being simple, fast and efficient in the field. In addition, plants are ecological integrators, reflecting the hydrological history of a site regardless of the conditions observed during the field work. Many plants are adapted to flooding conditions or water-saturated soil. Some plants are adapted to wetlands and are rarely found in other conditions, whereas others are found only in well-drained terrestrial environments.

There is often a general vegetation gradient, ranging from obligate wetland species to terrestrial species requiring upland environments, and this can usually be observed along a transect perpendicular to the water. Care should be taken to identify the indicator plant species in wetland and upland environments (see Appendix D for regional wetland vegetation guides).

Assessments can also be conducted using other wetland indicators such as standing water, water-saturated soil in the upper 30 cm, presence of water marks (dock, rocks, trees, etc.), presence of shoreline debris from high water levels, sediment deposition, tree bark erosion, or sulfurous odour (rotten egg) (Bazoge et al., 2014).

Method

Prior to the field visit: Using an elevation map and aerial photos (or other sufficient tools), identify wetland boundaries near the selected sampling site.

In the field:

1. To confirm wetland boundaries, walk perpendicularly from the water to the adjacent upland.
2. Observe vegetation zonation and soil composition.
3. Check the box on the field sheet corresponding to the appropriate category.

Dominant wetland marginal vegetation

This section involves recording the most dominant vegetation present in the wetland.

Method

Observe vegetation during wetland boundary walk.

1. Check the box on the field sheet corresponding to the appropriate vegetation category. Confirm with sampling team. If in doubt, refrain from identifying a category.

Canopy cover

Many wetlands are open areas with very little canopy cover. However, tall emergent aquatic plants (i.e., cattails, sedges, duckweed) may create microhabitats and should be noted on the field sheet as present, absent or abundant. Vegetation is highly subject to both anthropogenic and natural change and is not considered a suitable variable for predictions in models at present. Therefore, minimal information and photos are all that is required at this stage for CABIN wetland samples.

Buffer strips

Buffer strips around wetland areas perform a variety of functions, including enhancing filtration of surface runoff, and sediment and nutrient retention in the soil. In general, buffer strips are between 20 and 200 metres wide. A well-developed buffer strip is often an indication of a wetland with relatively high integrity.

The buffer strip around a wetland can be delineated with the help of GIS and aerial photography prior to invertebrate sampling. In the field, a buffer strip can be visually estimated as an area of natural growth from the wetted edge of a wetland to an area of human development (i.e., agriculture or urban areas). Where human disturbance is not present, a buffer strip is considered non-applicable or natural.

Method

Prior to the field visit:

Using aerial photos in Google Earth, identify the presence of a buffer strip.

In the field:

Confirm the presence of a buffer strip and estimate its size.

1. Check the appropriate box on the field sheet and note the approximate size.

Wetland characteristics: water chemistry

WATER / SEDIMENT CHEMICAL AND PHYSICAL PARAMETERS			
Time (include time zone)			Check if samples collected:
Air Temp (°C)			☐ Nutrients & TSS ☐ Major Ions ☐ Sediment sample ☐ Other_____
Water Temp (°C)			
pH			
Specific Conductance (µs/cm)			
DO (mg/L)	1/3 Depth		
	5 cm above bottom		
DO (%)	1/3 Depth		
	5 cm above bottom		
Turbidity (NTU)			

Measurement of key water quality variables that can directly or indirectly affect the macroinvertebrate community can yield important information about the types of pollutants and their impact on wetlands. For CABIN wetlands, these parameters will aid interpretation of site assessment results from metrics and models.

Standard CABIN chemical and physical water quality parameters include temperature, pH, specific conductance, alkalinity, dissolved oxygen, turbidity, nutrients, metals, and ions. Project Managers may add other parameters such as polyaromatic hydrocarbons (PAHs) or pesticides. Ultimately, the data and samples that are collected will depend on local issues and management goals. Calibrated water quality probes can be used for in situ measurements while other parameters must be sent to a laboratory for analysis.

On-site water quality measurements

Basic water quality parameters are easily incorporated into any water quality monitoring program using individual field sensors or a multi-parameter field probe in situ. The table

below describes each of these commonly used parameters, their effect on the benthic macroinvertebrate community, and the natural and anthropogenic factors that may modify the parameter values (Table 4).

Table 4 Descriptions of common water parameters.

Temperature (°C)	Temperature is a key physical variable that directly affects many of the physical, biological and chemical factors influencing aquatic organisms. If organisms are exposed to temperatures outside their range of tolerance of for extended periods of time, they can become stressed and die. Temperature in wetlands is affected by various factors, including weather, removal of marginal vegetation, and turbidity. Temperature is measured in degrees Celsius for air and water. **NOTE:** Use an alcohol or mercury thermometer to measure air temperature.
Specific Conductance (µS/cm)	Conductivity is the ability of a solution to conduct an electrical current. This is dependent on the total concentration of ionized substances dissolved in the water and is measured in micro Siemens per centimetre (µS/cm). Deionized water has a conductivity approaching 0 µS/cm. Conductivity is affected by temperature, so ECCC recommends using specific conductance for CABIN. Specific conductance standardizes the measured conductivity to 25°C and many in situ probes now have this option. Standardization to 25°C allows for comparison of measurements that were taken from water with different temperatures. Conductivity provides a useful way to track point source discharges and it is a useful measure of salinity in wetlands. **NOTE:** Conductivity varies greatly across Canada, and conductivity meters cover different ranges. Be sure to choose the appropriate meter for the study area.
Turbidity (NTU)	Turbidity is a quantitative measure of the reduction in water clarity in the presence of suspended solids such as sediment, living organisms, and organic matter. It is the measure of the extent to which light penetration in water is reduced by suspended material. Generally, turbid waters are much lower in dissolved oxygen because suspended particles absorb heat from sunlight (warm water holds less oxygen than cold water), and because photosynthesis decreases with the reduced availability of light. Additionally, suspended solids in turbid water can clog fish gills, reduce growth rates, decrease resistance to disease, and prevent egg and larval development. Higher turbidity levels are also often associated with higher levels of disease-causing micro-organisms such as viruses, parasites, and some bacteria.

Dissolved Oxygen (DO mg/L) and % saturation (% DO) in the water column	Dissolved oxygen (DO) represents the concentration of oxygen dissolved in the water, and can be measured in both milligrams per liter (mg/L) and saturation (%DO). The amount of dissolved oxygen in water determines the types of organisms that may be present, and is dependent on temperature, barometric pressure, and, to a lesser degree, salinity. Water with a higher oxygen concentration is generally considered to be of higher quality and better able to support many types of animals. Areas with low oxygen concentrations (hypoxia) can occur naturally within aquatic ecosystems (i.e., at the bottom of productive lakes and some wetlands), and animals adapted to these conditions are often generally tolerant of anthropogenic pollutants. When dissolved oxygen decreases, organisms that require higher oxygen levels will emigrate or die, and organisms that can tolerate low oxygen (i.e., midge larvae, worms) will dominate macroinvertebrate assemblages. Temperature has a major influence on dissolved oxygen, as warm water holds less oxygen than cold water. Organic wastes such as agricultural runoff and sewage can cause a reduction in the dissolved oxygen level in receiving waters through bacterial decomposition of organic matter. Aquatic plants and algae produce oxygen during daylight but they also consume it at night, which means they may not replenish oxygen sufficiently in impacted systems. For CABIN wetlands, take at least two dissolved oxygen measurements for both concentration and saturation: one at 1/3 depth in the water column and one approximately 5 cm above the substrate.
pH	pH represents the hydrogen ion (H+) concentration in water, expressed as a unitless value between 0 and 14. The pH scale is logarithmic; thus, for every change of 1 unit there is a 10-fold change in acidity or alkalinity. A pH of 0 is strongly acidic, while 14 is strongly basic (alkaline). Pure water is neutral and has a pH of approximately 7. Water with a pH between 6.5 and 9 is suitable for a great diversity of aquatic organisms, and young fish and aquatic insects are especially sensitive to pH values outside this range. Acid rain, wastewater discharges, and drainage from coniferous forests (acidic) can contribute to a lower pH. Concentrations of dissolved metals are influenced by pH, which can in turn affect their toxicity to different aquatic species.

Method

The method for measuring the above parameters varies depending on the instrument being used. Follow the manufacturer's instructions for care, calibration, and proper handling. Most field probes will need to be calibrated before use.

General tips for collecting water quality data with in situ probes:

- Take water quality measurements away from areas that have been disturbed by the sampling crew.
- Be sure to place the probe in the water column, in an area where the water is free of large amounts of vegetation. This may prove difficult in very shallow conditions; however, a single reading from any point in the water column is sufficient. In such a case, only one location for oxygen will be recorded in the table.
- Always allow time for stabilization, especially for DO, turbidity, and temperature (this can take 10 to 15 minutes with some probes).
- For conductivity and pH only, a reading can be taken in water that has been collected in a clean container from an open water area in the wetland.
- Record DO results in concentration (mg/L) and saturation (%).
- Turbidity is typically measured from water samples sent to a laboratory for analysis; however, field probes are available for in situ measurements. A portable turbidimeter could also be used.
- Take care not to disturb the area where you are taking measurements.

Water quality samples for laboratory analysis

Although some water quality parameters can be measured directly in the field, others can only be quantified from water samples that are collected in the field and sent to an analytical laboratory. Communication with the lab is extremely important to ensure that the correct sample bottles are used (often provided by the lab prior to sampling) and the correct procedures followed for collection and preservation (if needed). Below are examples of parameters that can be analysed by a laboratory:

- Conductivity, pH and turbidity (if field probes are not available)
- Nutrients, including phosphorus (measured as total unfiltered phosphorus) and nitrogen
- Alkalinity
- Major ions (i.e., Ca, Mg, Na, K)
- Metals (optional)
- Total suspended solids (TSS)
- Chlorophyll-a (i.e., from water column, core sample of sediment, algae or biofilm)

Note: Be aware of specific restrictions related to the parameters that are sampled. Handling procedures for water quality samples vary depending on the analytical laboratory. The lab will indicate whether preservatives must be added to the nutrient bottle while in the field. Depending on the lab, some bottles may come with preservative already in the bottle, in which case sampling should be done carefully and while wearing gloves.

Method

1. Collect samples from open water free of large amounts of vegetation or flocculent debris. Do not touch the mouth of the bottles or the inside of the caps or lids. Let material settle in water before sampling, move slowly when proceeding to sampling spot.
2. To avoid getting surface particles in the bottle, submerge the bottle mouth down and then flip below the surface to fill it. This may also be difficult in very shallow conditions so look for deeper pockets to collect water. Confirm with the lab the head-space (small amount of air left at top of the sample bottle) required for water samples. Secure the lid on the bottle once filled.
3. Using a water- and solvent-proof marker, record the appropriate information on a suitable, legible label affixed to each bottle.

4. **Keep samples in a cool** (ideally 4 °C), **dark place** to prevent growth of bacteria and other organisms. A cooler with ice packs is sufficient until samples can be refrigerated.

5. Submit samples to the analytical laboratory as soon as possible. Confirm holding times with the analytical laboratory; most samples have a 72-hour maximum holding time and must be received by the laboratory within 24 hours.

Wetland characteristics: substrate

Wetlands are characterized by fine organic or mineral soft bottom substrates. The majority of sediment in wetlands falls into one of the following three categories: organic cover, sand/silt/clay, and coarse sand.

A more accurate description necessitates a grain-size analysis performed in a laboratory; this requires the collection of approximately 100 g of sediment. An analysis of the percentage of carbon and nitrogen can be added to the granulometric description of the sediment. The composition of the substrate material is important in identifying hydrological characteristics and the type of habitat available to aquatic organisms.

SUBSTRATE SIZE CLASSES	CLASS SIZE
Organic cover (> 50% of area)	0
< 0.1 cm (sand/silt/clay)	1
0.1–0.2 cm (coarse sand)	2
0.2-1.6cm (gravel)	3

Method

Take a pinch of sediment between fingers and thumb to appraise the texture and estimate the occurrence of sand, clay, and organic matter.

1. Circle the category of the substrate that corresponds to the sampling site.

Collection and preservation of sediment

To perform an in-depth grain-size analysis or heavy metals analysis:

1. Collect approximately 100 g of sediment into a Whirl-Pak Bag or other container.

2. Label the bag correctly and clearly with the site, date, name of the project, and any other information required by the analytical lab or Project Manager.

Check with the analytical laboratory prior to sampling to confirm proper procedures for handling and preservation of the sediment. For some chemical analyses of phthalates or mercury, the sediment sample should be frozen after returning from the site.

Sampling of wetland macroinvertebrates

To characterize changes in the macroinvertebrate community, sampling should be carried out in areas where species diversity is highest. In seasonal wetlands, species diversity is highest in the emergent and submergent vegetation zones (De Szalay and Resh, 2000), which are typically inundated for most of the year. It is recommended that sampling be focused in these vegetation zones because of the significant effects that macrophytes (rooted aquatic plants) have on wetland ecosystems.

In lentic and lotic habitats, aquatic plants affect aquatic processes such as flow, water temperature, and clarity. They can increase the stability of the substrate and increase sediment deposition. In addition, decaying macrophytes provide a food source to benthic macroinvertebrates. Aquatic plants also support high densities of macroinvertebrates, with a higher diversity of macroinvertebrates being present in vegetated areas than in non-vegetated areas along with a variety of functional groups. Filter-feeding invertebrates often attach to plant surfaces and feed in the water column (i.e., Daphnia); ambush predators such as dragonfly larvae use plants to hide from prey; and some macroinvertebrates feed directly on the plant itself. Algae, bacteria, diatoms, and periphyton grow on macrophytes, providing a food source for many macroinvertebrates. Aquatic plants also provide a refuge from fish predation (Peets and Miller, 1994). In prairie potholes, the abundance, biomass, and taxon richness of aquatic invertebrates are greatly influenced by the presence of insectivorous fish (Hanson and Riggs, 1995).

The CABIN wetlands protocol uses a **sweep method** standardized by sampling effort (time) to collect aquatic macroinvertebrates. The standard level of effort and integrated samples are important for comparisons among sites. Samples should be collected using a sweep net, consisting of a triangular metal frame supporting a net with 400-µm mesh size. A detachable collection cup is connected to the end of the net to facilitate rapid removal of the sample. A sturdy handle is connected to one end of the metal frame and the net is generally protected by a collar of canvas or rip-stop plastic tubing to withstand abrasion. Figure 9 illustrates the steps involved in collecting a wetland benthic sample.

To standardize the level of effort, ECCC recommends performing a 2-minute sweep in a zig-zag pattern through the emergent/submergent vegetation. A kick is not necessary in most wetlands as the action of walking through the area is enough to disturb the soft substrate. The sampler must walk in the wetland while sweeping the net up and down in the water column through the emergent and submergent vegetation. The challenge in wetlands is to strike a balance between sweeping vigour and duration in order to limit the amount of debris and vegetation collected in the net. This prevents clogging and ensures that representative numbers and densities of macroinvertebrates are collected.

Macroinvertebrate collection

Method

1. Select the area with emergent and submergent aquatic plants which is most representative of the wetland and has safe wading conditions.
2. Define the sweep area path before entering the wetland. Inform field team members so that this area is not disturbed.
3. To begin, dip the net into the water just above the substrate and start the timer.
4. Walk forwards in a zigzag pattern, moving the net up and down in the water column from just above the substrate to just below the surface of the water. A tapping motion on the substrate with the bottom of the net may be helpful to stir up benthic macroinvertebrates which would otherwise be missed.
5. Move carefully to avoid entraining too much sediment in the net.

6. Keep motion steady and consistently in one direction to ensure that most of the disturbed substrate and organisms are swept into the net.

7. Continue to zigzag through the emergent and submergent aquatic plants for 2 minutes.

8. If the sampler needs to stop to take a rest or adjust his/her footing, the timer pauses the stopwatch while the sampler lifts the mouth of the net from the water. Restart the stopwatch when the sampler is ready to continue sampling and submerges the mouth of the net.

Figure 9 Collection of macroinvertebrates using a sweep net and sieving the sample to remove fine particles before transfer (Environment Canada, 2010).

BENTHIC MACROINVERTEBRATE SAMPLES

Habitat(s) sampled: ☐ Emergent zone ☐ Submergent zone ☐ Other _____

400-MM MESH NET	SWEEP 1	QA/QC 1	QA/QC 2
Person sampling			
Sampling time (i.e., 2 min)			
No. of sample jars			
Typical depth (in sampling area)			

☐ Preservative used

_____

Sample transfer from net to sample jars

Method

1. Splash the outside of the net in the water to transfer material to the bottom of the net and the collection cup at the end of the net (ensure that the mouth of the net is out of the water).
2. If there is extensive debris in the net, it will likely require many sample jars or a rinsing strategy (see the section below on removal of vegetation section for further details on sample processing).
3. Remove the collection cup attached to the end of the net and empty the contents directly into 1-L wide-mouth plastic sample jar(s). Always work over a pail or tray in case of an accidental spill.
4. Samples with large amounts of sediment should be emptied into a pail or sieve to reduce volume before transferring to sample jar.
5. Wash any material remaining in the cup/net into the sample jar/pail/sieve using a squeeze bottle and forceps to remove any clinging organisms.
6. If large leaves or debris accumulate in your net, carefully rinse and discard.
7. Leave room in the sample jars for the preservative; use extra jars if needed.
8. Double check the net/cup/pail/sieve for remaining macroinvertebrates.
9. If the samples collected are for DNA extraction, remember to clean the net frame thoroughly with a DNA decontaminant and employ a clean net between samples.
10. Label the outside and top of jar with a waterproof pen (see Figure 10). An inside label should be written on waterproof paper marked with pencil. All labels must have the following information: site code, date, and sample jar number (i.e., 1 of 2, 2 of 2).
11. If the samples collected are for DNA extraction, remember to clean the net frame thoroughly with a DNA decontaminant and employ a clean net between samples.

NOTE: Seams and folds of the net should be checked carefully for hidden organisms.

Figure 10 The sample collected may require more than one jar, in which case it is critical to number and label the jars accordingly.

Removing vegetation

Macroinvertebrate samples from wetlands often contain large volumes of debris and vegetation which can be removed either in the field or in the lab.

Method

1. **In the field:** Vegetation may be rinsed and removed in the field to reduce the sample volumes to a manageable size between 500 mL and 1 L. All rinsing activities must be completed within the sweep net. Macroinvertebrates may also need to be picked from the vegetation using tweezers. Vegetation can be retained and examined in keeping with quality assurance guidelines to ensure that there is less than 5% loss. Time spent rinsing vegetation could result in loss of macroinvertebrates through predation if the sample is large and portions remain sitting in collection buckets.

2. **In the lab:** When vegetation is collected and preserved along with the macroinvertebrates, multiple sample jars will be needed. Vegetation must be removed, rinsed, and examined in the lab. Entire samples including debris and vegetation once preserved for 72 hours can then be processed in the lab (see Sample Processing below)

Sample Preservation

The sample is preserved with 10% buffered Formalin to fix the tissues of the organisms quickly, allowing the body to remain firm for identification and preventing decomposition. Be sure to refer to the MSDS for Formalin before handling the chemical.

Method

1. Wear protective gloves and goggles

2. Add Formalin into jar at a 1:3 ratio (1 part Formalin to three parts sample)

3. Optional: Wrap top of jar with parafilm and seal with the lid. Parafilm helps to prevent leaks and reduces Formalin fumes.

4. Cap jar, gently swirl the sample to distribute the Formalin. DO NOT shake the jar as large gravel and rocks in the sample will damage the organisms.

Samples are transferred to Ethanol after a minimum of 72 hrs in Formalin or upon arrival at the taxonomy laboratory, see CABIN Laboratory Methods manual for more information (available from CABIN website)

Wetland sample processing

The objectives of the CABIN Wetlands sample processing protocol are to provide:

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

Details of sample processing and associated QA/ QC procedures can be found in the CABIN Laboratory Methods, Processing, Taxonomy, and Quality Control of Benthic Macroinvertebrate Samples (http://ec.gc.ca/rcba-cabin/). This protocol outlines the steps for processing river and stream CABIN macroinvertebrate samples and can be used for wetland samples, with some additional steps outlined below.

Subsampling

All CABIN samples are subsampled to 300 organisms using a Marchant Box (Marchant 1989), following the protocol in the CABIN Laboratory Methods

manual. When collecting samples in a wetland, large macrophytes, organic debris and algal masses are usually collected with the sweep method outlined above and may pose an issue with regard to subsampling. Depending on the sampler, the vegetation may have been removed in the field prior to preservation and retained for QC purposes. The retained material MUST BE examined before subsampling. Any organisms found in the vegetation will be recorded (order or family level) and added back to the sample for subsampling. The debris is kept until all QA/QC steps are completed on the reported data.

If large vegetation and organic debris was preserved with the CABIN sample, the material must be thoroughly rinsed over a sieve and checked for clinging organisms. All organisms should then be added back to the original sample before subsampling with the Marchant Box. Retain the rinsed vegetation for any further QC measures.

Subsampling with the Marchant Box may prove difficult, even with all larger debris removed. Where a large mass cannot be subsampled effectively, see the protocol for subsampling by weight in the CABIN Laboratory Methods manual (Environment Canada, 2014).

Sorting protocol

Large zooplankton (Copepoda, Ostracoda, Cladocera) are an important and often abundant group in wetland communities. The CABIN Wadeable Streams Protocol does not count these taxa in the 300 organism count (Environment Canada, 2014). The taxonomist should note zooplankton presence and provide associated subsample counts and identifications. **At this stage, however, zooplankton will not be considered toward the 300 organism count for wetlands, but will be enumerated per cell and identified to the lowest practical taxonomic level**. See Table 3 below for other non-counted taxa rationale.

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

Table 5 Taxa not included in the 300 organism count for CABIN wetland samples.

TAXA GROUPS	RATIONALE
Porifera and Bryozoa	Colonial and cannot be quantified as number of individuals per sample like other taxa
Nematoda, Nematomorpha, Nemertea	Taxa are not adequately sampled using a 400-µm sweep
Ostracoda	Taxa can be found in extremely high numbers and bias a sample
Non-aquatic taxa	Terrestrial drop-ins such as earthworms, spiders and some beetles are not part of the benthic/pelagic community

Data entry

Taxonomy results should be entered in CABIN (www.ec.gc.ca/rcba-cabin) or a similar database.

Taxonomic identification with genomics application

The use of genomics and environmental DNA in biomonitoring is an emerging and exciting opportunity for project managers. Although the technology will not be available for all projects, the potential application of genomics in a macroinvertebrate bioassessment is outlined in Appendix E (Baird and Hajibabei, 2012).

If samples are to be used for DNA analysis, proper care must be taken during sample processing to sterilize utensils, containers and the Marchant Box with Eliminase or another DNA cleaning solvent to reduce contaminant DNA. De-ionized water must also be used for all rinsing activity.

References and further reading

Alberta Biodiversity Monitoring Institute. 2007. Wetland field data collection protocols (10035), Version 2007-12-13. Alberta Biodiversity Monitoring Institute, Alberta, Canada.

Baird, D. J. and Hajibabaei, M. (2012) Biomonitoring 2.0: a new paradigm in ecosystem assessment made possible by next-generation DNA sequencing. Molecular Ecology 21(8): 2039–2044.

Barbour, M. T., Graves, C. G., Plafkin, J. L. and Wisseman, R. W. (1992) Evaluation of EPA's rapid bioassessment benthic metrics: Metric redundancy and variability among reference stream sites. Environmental Toxicology and Chemistry 11(4): 437-449

Bradley, B. P.Batzer, D. P., Shurtleff, A. S. and Rader, R. B. (2001) Sampling Macroinvertebrates in Wetlands. P. 339-354 in Rader, R. B., Batzer, D. P. and Wissinger, S. A. (eds.) Bioassessment and Management of North American Freshwater Wetlands. John Wiley and Sons, Inc. New York.

Batzer, D. P., Palik, B. J. and Buech, R. (2004) Relationships between environmental characteristics and macroinvertebrate communities in seasonal woodland ponds of Minnesota. Journal of the North American Benthological Society 23(1): 50–68.

Batzer, D. P. (2013) The seemingly intractable ecological responses of invertebrates in North American Wetland: A Review. Wetlands 33:1–15.

Batzer, D. P. and D. Boix (2016) An introduction to freshwater wetlands and their invertebrates. Chap. 1: 1–23 in Batzer, D. P., D. Boix (eds.) Invertebrates in Freshwater Wetlands. Springer International Publishing.

Bazoge, A., Lachance, D. and Villeneuve, C. (2014) Identification et délimitation des milieux humides du Québec méridional. Ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques. Direction de l'écologie et de la conservation et Direction des politiques de l'eau. 64 pages + annexes.

Burton, T. M., Uzarski, D., Gathman, J. P., Genet, J. A. and Keas, B. E. (1999) Development of a preliminary invertebrates index of biotic integrity for Lake Huron coastal wetlands. Wetlands 19(4): 869–882.

Background on Water Quality parameters http://waterontheweb.org/under/waterquality/index.html

CABIN website: http://cabin-rcba.ec.gc.ca/

Cheruvelil, K. S., Soranno, P. A. and Madsen, J. D. (2001) Epiphytic Macroinvertebrates Along a Gradient of Eurasian Watermilfoil Cover. Journal of Aquatic Plant Management. 39: 67–72.

Collinson, N. H., Biggs, J., Corfield, A., Hodson, M. J., Walker, D., Whitfield, M. and Williams, P. J. (1995) Temporary and permanent ponds: an assessment of the effects of drying out on the conservation value of aquatic macroinvertebrate communities. Biological Conservation 74: 125–133.

Cooper, M. J., Uzarski, D. G., Burton, T. M. and Rediske, R. R. (2006) Macroinvertebrate community composition relative to chemical/physical variables, land use and cover, and vegetation types within a Lake Michigan drowned river mouth wetland. Aquatic Ecosystem Health & Management 9(4): 463–479.

Cooper, M. J. and D. G. Uzarski (2016) Invertebrates in Great Lakes Marshes. Chap. 9: 287-319 Batzer, D. P., D. Boix (Eds) Invertebrates in Freshwater Wetlands. Springer International Publishing.

Cyr, H. and Downing, J. (1988) The abundance of phytophilous invertebrates on different species of submerges macrophytes. Freshwater Biology 20: 365–374.

Davis, J., Horwitz, P., Norris, R., Chessman, B., McGuire, M. and Sommer, B. (2006) Are river bioassessment methods using macroinvertebrates applicable to wetlands? Hydrobiologia 572: 115–128.

De Szalay, F. A. and Resh, V. H. (2000) Factors influencing macroinvertebrate colonization of seasonal wetlands: responses to emergent plant cover. Freshwater Biology 45(3): 295–308.

Drinan, T. J., Foster, G. N., Nelson, B. H., O'Halloran, J. and Harrison, S. S. C. (2013) Macroinvertebrate assemblages of peatland lakes: Assessment of conservation value with respect to anthropogenic land-cover change. Biological Conservation 158: 175–187.

Ecological Stratification Working Group (1995) (http://sis.agr.gc.ca/cansis/nsdb/ecostrat/intro.html).

Euliss, N. H., LaBaugh, J. W., Fredrickson, L. H., Mushet, D. M., Laubhan, M. K., Swanson, G. A., Winter, T. C., Rosenberry, D. O. and Nelson, R. D. (2004) The wetland continuum: A conceptual framework for interpreting biological studies. Wetlands 24(2): 448–458.

Flinn, M. B., Whiles, M. R., Adams, S. R. and Garvey, J. E. (2005) Macroinvertebrate and zooplankton responses to emergent plant production in upper Mississippi River floodplain wetlands. Archiv für Hydrobiologie 162(2): 187–210.

Gathman, J. P. and Burton, T. M. (2011) A Great Lakes Coastal wetland invertebrate community

gradient: Relative influence of flooding regime and vegetation zonation. Wetlands 31(2): 329–341.

Government of Alberta (2016) Guide for Assessing Permanence of Wetland Basins. 28 pp.

Government of Canada (1991) The Federal Policy on Wetland Conservation. Environment Canada, Ottawa. 13 pp.

Government of Canada (2014) Laboratory Methods, Processing, Taxonomy, and Quality Control of Benthic Macroinvertebrate Samples. Environment Canada, Ottawa. 36 pp.

Hajibabaei, M., Shokralla, S., Zhou, X., Singer, G. A. and Baird, D. J. (2011) Environmental barcoding: a next-generation sequencing approach for biomonitoring applications using river benthos. PLoS One 6(4): e17497. (available free online).

Hanson, M. A. and Riggs, M. R. (1995) Potential effects of fish predation on Wetland invertebrates: A comparison of wetlands with and without fathead minnows. Wetlands 15(2): 167–175.

Hanson, A., Swanson, L., Ewing, D., Grabas, G. Meyer, S., Ross, L, Watmough, M.D. and Kirby, J. (2008) Wetland Ecological Functions Assessment: An Overview of Approaches. Environment Canada, Canadian Wildlife Service Technical Report Series, No. 497, Atlantic Region, 59 pp.

Helgen, J. C. and Gernes, M. C. (2001) Monitoring the Condition of Wetlands: Indexes of Biological Integrity using Invertebrates and Vegetation. In Rader, R. B., Batzer, D. P. and Wissinger, S. A. (Eds). Bioassessment and Management of North American Freshwater Wetlands. John Wiley & Sons, Inc.

Hentges, V. A. and Stewart, T. W. (2010) Macroinvertebrate assemblages in Iowa prairie pothole wetlands and relation to environmental features. Wetlands 30(3): 501-511.

Hinojosa-Garro, D., Mason, C. F. and Underwood, G. J. C. (2010) Influence of macrophyte spatial architecture on periphyton and macroinvertebrate community structure in shallow water bodies under contrasting land management. Fundamental and Applied Limnology 177(1): 19-37.

Iwasaki, Y., Kagaya, T., Miyamoto, K. and Matsuda, H. (2009) Effects of heavy metals on riverine benthic macroinvertebrate assemblages with reference to potential food availability for drift-feeding fishes. Environmental Toxicology and Chemistry 28(2): 354–363.

Jude, D. J. and Pappas, J. (1992) Fish utilization of Great Lakes coastal wetlands. Journal of Great Lakes Research 18(4): 651–672.

Jurado, G. B., Masterson, M., Harrington, R. and Kelly-Quinn, M. (2008) Evaluation of sampling methods for macroinvertebrate biodiversity estimation in heavily vegetated ponds. Hydrobiologia 597: 97–107.

Kenney, M. E., Sutton-Grier, A. E., Smith, R. F. and Gresens, S. E. (2009) Benthic macroinvertebrates as indicators of water quality: The intersection of science and policy. Terrestrial Arthropod Reviews 2: 99–128.

Liston, S. E., Newman, S., Trexler and J. C. (2008). Macroinvertebrate community response to eutrophication in an oligotrophic wetland: An in situ mesocosm experiment. Wetlands 28(3): 686-694.Mellina, E., and S. G. Hinch, (1995) "Overview of Large-Scale Ecological Experimental Designs and Recommendations for the British Columbia Watershed Restoration Program," Ministry of Environment, Lands and Parks and Ministry of Forestry, Vancouver.

Neff, M. R. and Jackson, D. A. (2011) Effects of broad-scale geological changes on patterns in macroinvertebrate assemblages. Journal of the North American Benthological Society 30(2): 459–473.

Peets, R. and Miller, A. C. (1994) Effects of three species of aquatic plants on macroinvertebrates in Lake Seminole, Georgia. Technical Report A-94-5 U.S. Army Corps of Engineers Washington, DC. 65 pp.

Porst, G., Naughton, O., Gill, L., Johnston, P. and Irvine, K. (2012) Adaptation, phenology and disturbance of macroinvertebrates in temporary water bodies. Hydrobiologia 696(1): 47–62.

Rosenberg, D. M., Davies, I. J., Cobb, D. G. and Wiens, A. P. (1997) Protocols for measuring biodiversity: benthic macroinvertebrates in fresh waters. Department of Fisheries and Oceans, Freshwater Institute. Winnipeg, Manitoba. 46 pp.

Rosenberg, D. M. and Resh, V. H. (1996) Use of aquatic insects in Biomonitoring, In Merritt, R. W. and Cumming, B. F. (Eds), An Introduction to the Aquatic Insects of North America. 3rd Edition. Kendall/Hunt Publishing Company.

Rosenberg, D. M. and Resh, V. H. (Eds.) (1993) Freshwater biomonitoring and benthic macroinvertebrates, Chapman and Hall, New York. ix + 488 pp. Aquatic Conservation: Marine and Freshwater Ecosystems 4: 92.

Ruhí, A., Winfield Fairchild, G., Spieles, D. J., Becerra-Jurado, G. and Moreno-Mateos, D. (2016) Invertebrates in created and restored wetlands. Chap. 15: 525-564 in Batzer, D. P., D. Boix (Eds) Invertebrates in Freshwater Wetlands. Springer International Publishing.

Sarma, S. S. and Nandini, S. (2006) Review of recent ecotoxicological studies on cladocerans. Journal of Environmental Science and Health, Part B 41(8):1417–1430.

Stricker, C. A. and Burns, D. A. (2005) Macroinvertebrate response to land cover, habitat, and water chemistry in a mining-impacted river ecosystem: A GIS watershed analysis. Aquatic Sciences 67(4): 403–423.

Suter, G. W. and Cormier, S. M. (2015) Why care about aquatic insects: Uses, benefits, and services. Integrated Environmental Assessment and Management, 11: 188–194. doi: 10.1002/ieam.1600.

Suhett, A. L, Santangelo, J. M., Bozelli, R. L., Eugen, C., Steinberg, W. and Farjalla, V. F. (2015) An overview of the contribution of studies with cladocerans to environmental stress research. Acta Limnologica Brasiliensia. 27(2): 145–159.

Tall, L., Méthot, G., Armellin, A. and Pinel-Alloul, B. (2008) Bioassessment of benthic macroinvertebrates in wetland habitats of Lake Saint-Pierre (St. Lawrence River). Journal of Great Lakes Research 34: 599–614.

Tall, L., Armellin, A., Pinel-Alloul., B., Méthot, G. and Hudon, C. (2015) Effects of hydrological regime, landscape features, and environment on macroinvertebrates in St. Lawrence River wetlands. Hydrobiologia 778: 221–241. DOI 10.1007/s10750-015-2531-7.

Tarr, T. L., Baber, M. J. and Babbitt, K. J. (2005) Macroinvertebrate community structure across a wetland hydroperiod gradient in southern New Hampshire, USA. Wetlands Ecology and Management 13: 321–334.

Tessier, C., Cattaneo, A., Pinel-Alloul, B., Hudon, C. and Borcard, D. (2007) Invertebrate communities and epiphytic biomass associated with metaphyton and emergent and submerged macrophytes in a large river. Aquatic Sciences 10(1): 10–20.

Tiner, R. W. (1998) In Search of Swampland: A Wetland Sourcebook and Field Guide. Rutgers University Press, P.O. Box 5062, New Brunswick, NJ 08903.

Turner, A. M. and Montgomery, S. L. (2009) Hydroperiod, predators and the distribution of physid snails across the freshwater habitat gradient. Freshwater Biology 54: 1189–1201.

U.S. EPA. (2002a) Methods for Evaluating Wetland Condition: Introduction to Wetland Biological Assessment. EPA-822-R-02-014. Office of Water, U.S. Environmental Protection Agency, Washington DC, 35 pp.

U.S. EPA. (2002b). Methods for Evaluating Wetland Condition: Developing an Invertebrate Index of Biological Integrity for Wetlands. Office of Water, U.S. Environmental Protection Agency, Washington, DC. EPA-822-R-02-019

Uzarski, D. G., V. J. Brady, M. J. Cooper, D. A. Wilcox, D. A. Albert, R. P. Axler, P. Bostwick, T. N. Brown, J. J. H. Ciborowski, N. P. Danz, J. P. Gathman, T. M. Gehring, G. P. Grabas, A. Garwood, R. W. Howe, L. B. Johnson, G. A. Lamberti, A. H. Moerke, B. A. Murry, G. J. Niemi, C. J. Norment, C. R. Ruetz III, A. D. Steinman, D. C. Tozer, R. Wheeler, T. K. O'Donnell and J. P. Schneider (2017) Standardized Measures of Coastal Wetland Condition: Implementation at a Laurentian Great Lakes Basin-Wide Scale. Wetlands 37:15–32

Uzarski DG, Burton TM, Kolar RE, Cooper MJ (2009) The ecological impacts of fragmentation and vegetation removal in Lake Huron coastal wetlands. Aquatic Ecosystem Health and Management 12:45–62

Uzarski, D. G., Burton, T. M. and Genet, J. A. (2004) Validation and performance of an invertebrate index of biotic integrity for Lakes Huron and Michigan fringing wetlands during a period of lake level decline. Aquatic Ecosystem Health & Management 7(2): 269–288.

Vymazal, J. (2007) Removal of nutrients in various types of constructed wetlands. Science of the Total Environment 380 (1–3): 48–65.

Warner, B. G. and Rubec, C. D. A. (Eds., 1997). The Canadian Wetland Classification System. Second Edition. National Wetlands Working Group. 76 pp.

White, M. S., Xenopoulos, M. A., Metcalfe, R. A. and Somers, K. M. (2011) Water level thresholds of benthic macroinvertebrate richness, structure, and function of boreal lake stony littoral habitats. Canadian Journal of Fisheries and Aquatic Sciences 68(10): 1695–1704.

Wissinger, S. A. (1999) Ecology of wetland invertebrates: Synthesis and applications for conservation and management p. 1043-1086 in Batzer, D. P., Rader, R. B. and Wissinger, S. A. (Eds) Invertebrates in Freshwater Wetlands of North America: Ecology and Management. John Wiley & Sons.

Appendix A: CABIN Wetland Field Sheet and Site Inspection Sheet

Field Crew: _____ Site Code: _____

Sampling Date (DD/MM/YY): _____

OHS: Site Inspection Sheet Completed ☐

PRIMARY SITE DATA

CABIN Study Name: _____ Wetland Name: _____

Local Basin Name: _____ Ecoregion: _____

Arrival time: _____ Departure time: _____

GEOGRAPHICAL DESCRIPTION/NOTES:

LAND USE POTENTIALLY INFLUENCING WETLAND (0–50 M)
LAND USE *(CHECK THOSE PRESENT)*: **INFORMATION SOURCE:** _____

☐ Forest ☐ Field/Pasture ☐ Agriculture ☐ Residential/Urban

☐ Logging ☐ Mining ☐ Commercial/Industrial ☐ Other_____

DOMINANT LAND USE *(CHECK ONE)*: **INFORMATION SOURCE:** _____

☐ Forest ☐ Field/Pasture ☐ Agriculture ☐ Residential/Urban

☐ Logging ☐ Mining ☐ Commercial/Industrial ☐ Other_____

Field Crew: _____ Site Code: _____

Sampling Date (DD/MM/YY): _____

LOCATION DATA

Latitude: _____N Longitude: _____W (deg/min/sec or decimal deg

Elevation: _____(masl) GPS Datum: GRS80 (NAD83/WGS84) ☐ Other _____ ☐

SITE LOCATION MAP DRAWING

Field Crew: _____ Site Code: _____

Sampling Date (DD/MM/YY): _____

PHOTOS

☐ Field sheet _____ ☐ North _____ ☐ East _____ ☐ South _____ ☐ West _____

☐ Vegetation (in sampled area) _____ ☐ Exposed Substrate / Mud Flat _____

☐ Acces point _____ ☐ Aerial Photo _____ ☐ Other _____ (i.e., Sampling photos)

WETLAND CHARACTERISTICS
WHOLE WETLAND

HYDROLOGICAL TYPE

☐ Basin/Pond/Pool ☐ Lacustrine ☐ Riverine

WATER-LEVEL STAGE AT THE TIME OF SAMPLING *(CHECK ONE):*

☐ Dry ☐ Vestigial ☐ Recessional ☐ coniferous trees ☐ Intermediate s

☐ Full ☐ Flooded ☐ Overflowing Typical Depth _____(m)

Field Crew: _____ Site Code: _____

Sampling Date (DD/MM/YY): _____

VEGETATION COVER

HIGHER PLANTS

Emergent

☐ 0% ☐ 1–5% ☐ 6–25% ☐ 26–50% ☐ 51–75% ☐ 76–100%

Floating

☐ 0% ☐ 1–5% ☐ 6–25% ☐ 26–50% ☐ 51–75% ☐ 76–100%

Submergent

☐ 0% ☐ 1–5% ☐ 6–25% ☐ 26–50% ☐ 51–75% ☐ 76–100%

ALGAE / CYANOBACTERIA

Algal bloom and/or cyanobacterial bloom

☐ Absent ☐ Present

FILAMENTOUS ALGAE

☐ Absent ☐ Present

BIOFILM PRESENT

☐ Yes ☐ No

Comments (thickness, abundance, marl): _____

Field Crew: _____ Site Code: _____

Sampling Date (DD/MM/YY): _____

MARGINAL WETLAND

WETLAND MARGINAL VEGETATION 0–50 M AROUND WETLAND (CHECK THOSE PRESENT):

☐ Ferns/grasses/sedges ☐ Shrubs ☐ Deciduous trees ☐ Coniferous trees ☐ Agricultural crops

DOMINANT WETLAND MARGINAL VEGETATION *(CHECK ONE)*:

☐ Ferns/grasses/sedges ☐ Shrubs ☐ Deciduous trees ☐ Coniferous trees ☐ Agricultural crops

CANOPY COVER / SHADING

☐ Not shaded ☐ Partially shaded ☐ Fully shaded

MANAGED BUFFER STRIP

☐ Absent ☐ Present ☐ Natural setting, no buffer

Field Crew: _____ Site Code: _____

Sampling Date (DD/MM/YY): _____

WATER / SEDIMENT CHEMICAL AND PHYSICAL PARAMETERS			
Time (include time zone)			Check if samples collected:
Air Temp (°C)			☐ Nutrients & TSS ☐ Major Ions ☐ Sediment sample ☐ Other _____
Water Temp (°C)			
pH			
Specific Conductance (µs/cm)			
DO (mg/L)	1/3 Depth		
	5 cm above bottom		
DO (%)	1/3 Depth		
	5 cm above bottom		
Turbidity (NTU)			

SUBSTRATE SIZE CLASSES	CLASS SIZE
Organic cover (> 50% of area)	0
< 0.1 cm (sand/silt/clay)	1
0.1–0.2 cm (coarse sand)	2
0.2-1.6cm (gravel)	3

Field Crew: _____ Site Code: _____

Sampling Date (DD/MM/YY): _____

BENTHIC MACROINVERTEBRATE SAMPLES

Habitat(s) sampled: ☐ Emergent zone ☐ Submergent zone ☐ Other_____

400-MM MESH NET	SWEEP 1	QA/QC 1	QA/QC 2
Person sampling			
Sampling time (i.e., 2 min)			
No. of sample jars			
Typical depth (in sampling area)			

☐ Preservative used

_____

Field Crew: _____ Site Code: _____

Sampling Date (DD/MM/YY): _____

SITE SAFETY INSPECTION

Notes to Safety Officer:
Remind crew to keep eyes open for anything unusual
Communicate any hazards continually
Pay attention to minor safety concerns: dehydration, hunger, fatigue, illness
Promote open communication among crew members

Site Inspected by _____:

- ☐ Itinerary left with contact person
- ☐ Call-in procedure activated

THA/SWP read and signed by staff for

- ☐ Wading
- ☐ Driving
- ☐ Wading
- ☐ West Nile / Hanta Virus / Lyme disease
- ☐ Helicopter Safety (if applicable)
- ☐ All staff trained in boat safety, swiftwater, first aid, helicopter egress, wilderness survival.
  If not, specify who is not trained: _____

Boat Safety

- ☐ Safety equipment (first aid, fire extinguisher, blanket, emergency kit, flares, safety survival bag)
- ☐ Equipment and chemicals safely secured for transport
- ☐ Boat moored safely, two ropes for securing

Shore & Site Hazards and Wading Safety

- ☐ Wetland hazards identified (i.e., logs, deep pools)
- ☐ First Aid kit
- ☐ Bear Spray / Bear Bangers / Air Horns on site
- ☐ Poor weather (i.e., too hot or lightning)
- ☐ Signs of wildlife (bears, cougars, moose)
- ☐ Wetland hazards identified (i.e., logs, deep pools, slippery rocks, holes, muskrat / beaver run, foot entrapment in substrate) and listed

☐ PFDs worn; ☐ appropriate footwear, waders, wading belt; ☐ wading staff; ☐ Remote survival gear bag appropriate for sampling area
Notes:

Appendix B: Provincial and Territorial Hydrologic Stations

DEPARTMENT / AGENCY	WEB PAGE
Aboriginal Affairs and Northern Development Canada	www.aadnc-aandc.gc.ca/eng/1100100034879/1100100034883
Alberta – Environment and Water	http://environment.alberta.ca/
British Columbia – Ministry of Environment	www.gov.bc.ca/env/index.html
Prince Edward Island – Environment, Energy and Forestry	www.gov.pe.ca/envengfor/
Manitoba – Water Stewardship Division	www.gov.mb.ca/waterstewardship/
New Brunswick – Department of Environment	www.gnb.ca/0009/index-e.asp
Nova Scotia Environment	www.gov.ns.ca/nse/
Nunavut – Department of Environment	http://env.gov.nu.ca/
Ontario Ministry of NaturalResources	www.mnr.gov.on.ca/en/index.html
Quebec – MDDEP Centre d'expertise hydrique du Québec	www.cehq.gouv.qc.ca/suivihydro/index.asp [French only]
Saskatchewan Watershed Authority	www.swa.ca/
Newfoundland and Labrador – Water Resources Management	www.env.gov.nl.ca/env/waterres/index.html
Yukon Territory – Environment Yukon	www.env.gov.yk.ca/
Northwest Territories – Department of Environment and Natural Resources	www.enr.gov.nt.ca/_live/pages/wpPages/home.aspx

Appendix C: Inventory of Wetland Vegetation

The type of vegetation that is found in wetlands often depends on wetland class, hydrology and water chemistry. Wetland vegetation may be diverse and heterogeneous with numerous species, or simple and homogenous with one or two dominant species. Vegetation, on its own, can serve as an indicator for biomonitoring. The difficulty with a vegetation-based indicator is that sampling can be time-consuming; it may take many hours to carry out sampling in a large wetland. Plant identification is most efficient when carried out in the field by an expert in plant taxonomy. If plants cannot be identified in situ, they may be collected with roots and flowering parts intact, to be preserved and identified in the laboratory; however, this is a much more time-consuming process. Large-scale GIS monitoring is another option for vegetation-based monitoring, although the time frame for observing changes at this scale would be much greater than with other indicator species, such as macroinvertebrates.

Even though vegetation was not chosen as an indicator for this protocol, its importance in wetland data collection cannot be ignored. Plants provide habitat and food for wetland macroinvertebrates. The form and structure of aquatic macrophytes, such as the number of stems, branches and leaves, influence macroinvertebrate colonization of vegetated areas. Plants in the emergent zone are rooted in sediment and have underwater portions which provide a growing surface for periphyton. Periphyton provides food for many macroinvertebrates, such as scrapers and grazers, many of which are preyed on by predatory macroinvertebrates. Macrophytes create a microclimate with less variation in water temperature and less physical disturbance from wave action or wind. These factors contribute to a high abundance and diversity of macroinvertebrates in vegetated zones of wetlands (Cheruvelil et al., 2001).

Currently, the three most dominant species in the emergent zone where macroinvertebrate sampling is carried out are identified to lowest taxonomic level in the field and photos are taken of each species. The abundance of each species is estimated using the following cover classes: 0, 1–5%, 6–25%, 26–50%, 51–75%, 76–95%, and 96–100% (REF for vegetation description; Braun-Blanquet).

Method for estimating plant coverage if greater precision is required:

Use flags to mark off the 5 x 5 m quadrat to be used for sampling. The size of the quadrat allows users to validate aerial photos and describe wetlands. Smaller quadrats (1 x 1 m) can also be used. On the sketch, draw the various plants found at the site.

1. Assess percent coverage for each dominant plant species according to the following classes:

    a. 0%
    b. 1–5%
    c. 6–25%
    d. 26–50%
    e. 51–75%
    f. 76–95%
    g. 96–100%

2. Assess each vegetation stratum: record emergent plants, submerged plants and floating plants. Do not forget to estimate the percentage of open water.

3. Note that due to the vertical stratification of aquatic plants, the total coverage (i.e., the sum of the coverage of each species in a given quadrat) can be greater than 100%.

4. Do not hesitate to validate your assessment with a third person because estimation of vegetation coverage varies from one individual to the next.

Appendix D: Identification of Wetlands in Canada

British Columbia

MacKenzie, W. H. and Moran, J. R. (2004). Wetlands of British Columbia: a guide to identification (No. 52). Victoria, B.C.: Res. Br., B.C. Min. For. Retrieved from http://best-practices.ltabc.ca/media/resources/baseline-documentation/BC_GOV_Wetlands_of_BC_A_Guide_to_Identification.pdf

Beaudry, L., Coupé, R., Delong, C. and Pojar, J. (1999). Plant Indicator Guide for Northern British Columbia: Boreal, Sub-Boreal, and Subalpine Biogeoclimatic Zones: BWBS, SBS, SBPS, and northern ESSF (46). Victoria, BC: British Columbia Ministry of Forests Research Program. Retrieved from https://www.for.gov.bc.ca/hfd/pubs/Docs/Lmh/Lmh46.htm

Alberta

Alberta Wetland Classification System (http://aep.alberta.ca/water/programs-and-services/wetlands/documents/ClassificationSystem-Jun01-2015.pdf)

Saskatchewan

McLaughlan, M. S., Wright, R. A. and Jiricka, R. D. (2010). Field Guide of the Ecosites of Saskatchewan's Provincial Forests. Prince Albert, Saskatchewan: Saskatchewan Ministry of Environment, Forest Service. Retrieved from www.publications.gov.sk.ca/details.cfm?p=31663

Ontario

Ontario Ministry of Natural Resources. (2014). Ontario Wetland Evaluation System — Northern Manual., 280. Retrieved from http://files.ontario.ca/environment-and-energy/parks-and-protected-areas/ontario-wetland-evaluation-system-northern-manual-2014.pdf

Ontario Ministry of Natural Resources. (2014). Ontario Wetland Evaluation System — Southern Manual., 284. Retrieved from http://files.ontario.ca/environment-and-energy/parks-and-protected-areas/ontario-wetland-evaluation-system-southen-manual-2014.pdf

Quebec

Bazoge, A., Lachance, D. and Villeneuve, C. (2014). Identification et délimitation des milieux humides du Québec méridional. Québec: Ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques, Direction de l'écologie et de la conservation et Direction des politiques de l'eau. Retrieved from www.fil-information.gouv.qc.ca/Pages/Article.

Nova Scotia

Nova Scotia Wetland Indicator Plant List http://www.novascotia.ca/nse/wetland/indicator.plant.list.asp

Appendix E: Genomics Methods and Applications in Freshwater Invertebrate Bioassessment

The basic structure, function and metabolism or all living organisms is regulated by deoxyribonucleic acid (DNA). DNA is a polymer composed of four basic nucleic acids arranged in base pairs that in different combinations compose different genes. Genes provide instructions for making a variety of structural and functional proteins. The complete DNA sequence of most multicellular organisms, its genome, is millions of base pairs in length and encodes thousands of different genes. DNA is inherited by offspring from their parent(s). For certain genes, the base-pair sequence is rarely inherited with a mutation. This characteristic is useful for species identification. Within a species, these genes will show very little sequence variation, while between species greater differences will be observed. These gene regions, known as DNA barcodes, are typically several hundred base pairs long and can be sequenced and compared to a sequenced reference library to identify an organism.

Although other barcode regions exist, an important region for invertebrates is cytochrome oxidase c subunit 1, or CO1. All invertebrates possess this gene, and numerous studies have shown it has a strong ability to discriminate between genera and species. Extensive CO1 reference libraries exist for most invertebrate groups, and are constantly being expanded and updated.

The advent of high-throughput DNA sequencing techniques (HTS) combined with increased computing and bioinformatics power has led to the development of DNA-based tools for organism identification, with clear applications to biodiversity measurement and biomonitoring. Large amounts of DNA can now be extracted from a bulk sample of organisms or a water sample, which is referred to as environmental DNA, or eDNA, as it consists of DNA fragments shed by organisms into the environment in the form of faeces, mucous, cells, etc. This approach offers several advantages over morphological techniques: faster turnaround, higher taxonomic resolution and often reduced costs. Moreover, DNA-based identification using HTS can potentially identify all of the taxa present in a sample, as opposed to the subsample usually identified with traditional tools. When multiple barcode markers are used, genomic techniques can provide a comprehensive picture of biodiversity, from bacteria to plants, to invertebrates, to vertebrates.

Application of genomic tools in CABIN site assessments

Any groups interested in using genomic tools should contact their CABIN regional lead. However, relatively few adjustments are required to incorporate genomic approaches. The most important consideration is to minimize DNA contamination among samples. Several steps can be taken to prevent DNA contamination:

- Use a new net for each invertebrate sample, or thoroughly clean collecting equipment with Eliminase® between samples;
- Ensure that all equipment and gear (i.e., waders) is free of obvious biological material (i.e., mud, plant matter, attached invertebrates) before moving between sites;
- All field personnel should wear a new pair of nitrile gloves at each site; nitrile gloves should always be worn when handling samples;
- Sample containers should be left unopened until needed;
- If collecting a water sample for eDNA analysis, the same considerations apply

Bulk organismal samples are collected by following the same procedures as outlined in the Sampling Strategy for Wetland Macroinvertebrates section. However, sample preservation should always be done using 95% ethanol. After preservation,

samples should immediately be placed on ice in a cooler or in a portable freezer. Samples should be promptly placed in a freezer until they are shipped to a morphology lab or sequencing facility. If the ethanol is replaced in the lab, the old ethanol should be saved in a clean container and labelled, as DNA is often leached into solution.

Standard CABIN methods do not exist for the collection of eDNA water samples. The total volume of sample collected and the number of subsamples taken will vary depending on the system and study. Samples should be frozen as soon as possible and kept cold during shipment to the sequencing facility.

Additional considerations: Excess plant material can often interfere with obtaining CO1 sequences from bulk samples of invertebrates. This can be a major issue in wetlands. As much vegetation as possible should be removed from the sample in the field before preservation (see procedures in the Vegetation Removal section). Alternatively, excess plant material may be visually inspected and removed in the lab.

Laboratory and bioinformatics procedures

If a sample is to be morphologically identified prior to HTS, it is crucial that cross-sample contamination be minimized. This means that any surface that comes into contact with multiple samples (i.e., Marchant Box, forceps, sorting trays) must be cleaned with Eliminase® between samples. All subsampled material should be returned to the original sample following sorting and identification, although voucher specimens should be noted and retained according to normal procedures. The sample should be re-preserved in 95% ethanol and stored in the freezer before shipment to a sequencing facility.

Bulk samples will be homogenized before extraction and sequencing. Sequencing procedures are optimized to ensure that a single gene (the barcode marker, i.e., CO1) is targeted. Another major advantage of HTS is that it permits the processing of multiple samples in a single sequencing run by giving each sample a unique tag. The raw sequence data from the sequencer is "cleaned" to remove any short fragments or non-target sequences, and then sorted into samples based on their unique tags. These sequences are then compared to publicly available barcode reference libraries (i.e., GenBank, Barcode of Life Datasystems, BOLD) to identify the taxa present. If a sequence is not associated with any species sequence in the reference library, it may still be associated with a higher taxonomic level (genus or family).

Data outputs

The data output from a metagenomics analysis consists of a list of taxa from a sample and the number of sequences associated with each taxon, as well as the number of discarded and unassociated sequences. This information can be used to calculate a large number of biodiversity measures (i.e., taxa richness, evenness, dominance, Shannon diversity). Research is currently underway to understand how to incorporate metagenomics data into bioassessment for both wetlands and rivers. However, the higher resolution taxonomic information obtained from HTS should permit greater discrimination among sites and a more complete picture of biodiversity at a given site.