January 2007

Ontario Benthos Biomonitoring Network: Protocol Manual

Protecting our environment. Ontario

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ONTARIO BENTHOS BIOMONITORING NETWORK

PROTOCOL MANUAL

January 2007

1 Executive Summary

The main purpose of the Ontario Benthos Biomonitoring Network (OBBN) is to enable assessment of aquatic ecosystem condition using benthos as indicators of water and habitat quality. This manual is a companion to the OBBN Terms of Reference, which detail the network's objectives, deliverables, development schedule, and implementation plan. Herein we outline recommended sampling, sample processing, and analytical procedures for the OBBN.

The reference condition approach (RCA), which compares the benthic community at test sites (where biological condition is in question) to that of multiple minimally-impacted reference sites, is a commonly used bioassessment study design. Because benthic community composition is determined in large part by environmental attributes (e.g., catchment size, substrate type), a combination of catchment- and site-scale habitat measures are used to select appropriate reference sites that are used to develop bioassessment criteria. A variety of minimally impacted sites must be sampled in order to permit evaluation of the wide range of potential test sites in Ontario.

We detail sampling and sample processing methods for lakes, streams, and wetlands. Recommended sampling methods define sampling units, and specify sampling effort, replication, collection procedures (e.g., Travelling-Kick-and-Sweep), mesh size, and sampling periods throughout the year. Sample-processing recommendations cover sub-sampling methods, picking methods, detail (taxonomic level) of benthos identification, and sample archiving. Protocols are consistent with a rapid-bioassessment approach. We have tried to strike a balance between protocol standardization and flexibility. Standardization is important to allow comparisons between sites and times, to facilitate data sharing among network participants, and to permit development of quality control procedures. Flexibility is important to allow participation by partners having different amounts of expertise, time, and money, and to allow protocols to be used in studies aimed at answering different questions.

In our analysis section, we discuss the need to select appropriate reference sites using suitable predictors of biological community composition. We then describe methods for testing our bioassessment null hypothesis, that the test site is normal (or in reference condition). The biological condition of both the test site and reference sites are first summarized using a set of indices (e.g., percent mayflies, site-score from 1st axis extracted in a correspondence analysis). Using calculations easily performed in Microsoft Excel, we then apply Test Site Analysis (TSA) to convert the values of the selected indices into a single number (a multivariate distance measure). We statistically compare this distance measure to the distances among reference sites to determine how unusual the test site is.

Several appendices provide supplementary information: a list of diagnostic characters for the benthos groups comprising our minimum acceptable level of taxonomic resolution, a checklist of families, blank field and taxa enumeration sheets, a list of catchment-scale habitat variables for characterizing sites, OBBN research priorities, a paper explaining how to do TSA in Microsoft Excel, and an equipment checklist. All protocols are subject to testing and refinement in subsequent editions.

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5 Disclaimer

The procedures described in this manual are subject to field-testing and may be revised in subsequent editions; wetland protocols in particular should be considered experimental. The protocols described herein should work in most situations; however, unforeseen conditions, requiring modification of techniques, may arise.

When sampling benthos, aim for minimal disturbance of habitat. In particular, do not disturb spawning fish and amphibians, and avoid sensitive habitats for fish and wildlife. Contact the Ontario Ministry of Natural Resources for guidance on the timing of sample collection and for information on sensitive habitats.

Always get landowner permission prior to sampling on private property.

Contact Chris Jones for technical guidance on the use of this manual (phone: 705 766-1724, fax: 705 766-2254, e-mail: f.chris.jones@ontario.ca).

6 Acknowledgements

This document integrates the procedures of Environment Canada's Canadian Aquatic Biomonitoring Network (Reynoldson *et al*. 2003) and the Ontario Ministry of Environment's Rapid Bioassessment Protocol (David *et al*. 1998). Stream protocols are also described in the Ontario Stream Assessment Protocol (Stanfield 2005). Our methods are based in part on benthos biomonitoring methods used elsewhere in North America, the United Kingdom, and Australia (e.g., Bailey *et al*. 2004, Wright *et al*. 2000, Barbour *et al*. 1999).

We wish to thank the other members of the Ontario Benthos Biomonitoring Network's Technical Advisory Committee (TAC), who guided the development of this manual:

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7 Introduction

Ontario Benthos Biomonitoring Network (OBBN) participants comprise our intended audience. The OBBN Terms of Reference describe the network's purpose, deliverables, schedule for development, and implementation model. In this companion to the Terms of Reference, we describe the standard operating procedures for the OBBN, including basic aquatic bioassessment, sampling, sample processing, and analytical methods.

The reference condition approach (RCA) is a useful study design for environmental studies (Bowman and Somers 2005), and is a central concept in the OBBN. For this reason, we used the steps in the RCA (Figure 1) to structure this manual: sections dealing with the concept of reference sites, sample collection, processing, and analysis are ordered chronologically, as if part of an RCA study.

Throughout this manual, terms included in the glossary are highlighted in **bold.**

7.1 Benthos Study Designs

For **biomonitoring** to be successful, sampling and analytical methods must be carefully designed so that **bioassessment** questions can be answered with confidence (e.g., Bowman and Somers 2005). In the OBBN, we are mainly concerned with the question, is the benthos community at a **test site** normal? Clearly, a suitable experimental design for **bioassessment**s is needed.

Four standard guides on experimental design and biostatistics for benthos studies are Green (1979), Underwood (1997), Zar (1999), and Manly (2001). Green outlines five basic study designs: baseline or monitoring studies, designs in which impact is inferred from temporal change alone, designs in which impact is inferred from spatial pattern alone, designs aimed at determining when and where an impact occurred, and optimal impact study designs. There are several variations on Green's original "optimal design" (Table 1). Underwood built on Green's work by describing these designs in detail, by identifying pitfalls, and by suggesting some alternate approaches. Zar's text is a general introduction to biostatistics. Manly discusses principles of environmental sampling and statistical inference. Bailey *et al.* (2004) summarized the basic concepts of the RCA using examples from Canada and Australia.

Table 1: Some experimental designs used in benthic invertebrate assessments (Green 1979, Bailey *et al.* 2004).

Design Type	Experimental Control	Experimental Treatment
Before/After/Control/ Impact (BACI)	Single reference area, located upstream of the stressor discharge, sampled before and after the discharge begins	Test area downstream of the stressor discharge
Control/Impact (C/I)	Single reference area, upstream of stressor discharge, established after discharge initiated	Test area downstream of the stressor discharge
Multiple Control/Impact (MCI)	Multiple reference areas (unaffected by stressor of interest) in the same or environmentally similar adjacent watersheds as test area	One or more test areas receiving similar stressor discharges

Design Type	Experimental Control	Experimental Treatment
Simple Gradient (SG)	A series of reference stations with no or low impact (i.e., representing the distal end of a declining stressor gradient)	Test areas at various locations across an anticipated impact gradient
Radial or Multiple Gradient (RMG)	A series of reference stations situated along an impact gradient from a **test site**. Sites farthest from the discharge are used as reference sites.	Multiple **test site**s at increasing distances from the discharge
Reference Condition Approach (RCA)	Multiple reference sites that are grouped by like biological condition and then matched to the **test site** of interest	Single **test site** compared against the most appropriate group of reference sites

7.2 The RCA Experimental Design

As Green (1979) proposed, the optimal experimental design is the before/after/control/impact (BACI) design. Unfortunately, the information needed for a BACI design is seldom available. Consequently, the Reference Condition Approach (RCA) is growing in popularity (e.g., Norris and Georges 1993, Wright *et al*. 2000, Reynoldson *et al*. 2003, Bailey *et al*. 2004, Bowman and Somers 2005). The RCA uses a set of minimally **impacted** reference sites to evaluate **test site**s. By recommending the RCA, we assume that biological data from test areas can be compared to data from minimally **impacted** reference areas to assess impairment (e.g., Yoder 1991), diagnose stressors (e.g., Fletcher *et al*. 2001), evaluate temporal and spatial trends (e.g., Yoder 1989, Yan *et al*. 1996), and provide data for water management (e.g., Ohio Environmental Protection Agency 1990).

As described by Bailey *et al.* (2004), the RCA has six steps (Figure 1):

1. Minimally **impacted** reference sites spanning a range of physiographic conditions are selected (ideally this is done randomly) and sampled.
2. Biological condition is summarized, and reference sites are grouped according to the similarity of their biological communities.
3. **Niche variables** are identified.
4. A model that predicts reference group membership is built using **niche variables**.
5. Biological, habitat, and physiographic data associated with a **test site** are gathered.
6. The **test site** is matched with its predicted reference-site group.
7. A statistical test is used to determine if the **test site** falls within the **normal range** of biological condition exhibited by reference sites.

Different authors approach key decisions in the RCA differently. As a result, there is some variation in authors' definitions of minimal impact, in reference site classification methods, and in how the typical RCA null hypothesis (H_0: test site is in reference condition) is tested (e.g., Wright *et al*. 2000, Bowman and Somers 2005, Linke *et al*. 2005).

7.3 Site Selection Guidelines for Biomonitoring Surveys

We typically provide guidance to OBBN partners on reference site selection, but **test site**s can be selected by any partner to support their own interests. Practitioners frequently request guidance on site selection for **biomonitoring** surveys when their question is, what is the biological condition of aquatic ecosystems in my area? We give some site selection guidance below:

1. Clearly define study boundaries (e.g., a municipality, region, lake, stream, or **drainage basin**).
2. Map the surface water network (i.e., lakes, streams, and wetlands), and identify features of interest.
3. Consult local agencies (e.g., municipality, conservation authority) to determine if monitoring information is already available.
4. Determine the amount of sampling, sample processing, and analyses that you can realistically afford given available money, time, and expertise.
5. For general questions about aquatic ecosystem condition in unfamiliar areas, spread **test site**s across the study area to screen for problem areas. If you are interested in streams, stratify sampling sites so that small, medium and large streams are sampled. Also ensure adequate spatial coverage across the area. Where resources are limiting, locate sampling sites near important nodes (e.g., near confluences of major **catchment**s). If there are discernable gradients across your study area (e.g., a transition from forest to agriculture and rural development) consider stratifying by land-use zone.
6. If your question relates to effects of a specific stressor, locate **test site**s across an anticipated gradient of stressor impact (e.g., at increasing distances from a contaminant source).
7. Select and sample reference sites that match the habitat types of the **test site**s. Generally reference sites should be minimally **impacted,** but if your study is investigating a specific stressor, reference sites that are **impacted** may be used as long as they are not exposed to the stressor of interest.
8. Sample all mandatory sites (e.g., required by a regulatory agency, or control sites for a specific study)

Detailed texts on **biomonitoring** study design and site selection are available (e.g., Green 1979, Rosenberg and Resh 1993, Underwood 1997, Barbour *et al*. 1999, Ontario Ministry of Natural Resources 2003, Bailey *et al.* 2004).

7.4 The Reference Site

Sampling "minimally **impacted**" reference sites is the first step in the RCA. Reference sites are used to define the **normal range** of biological condition for a given habitat type. In this section we describe the concept of minimally **impacted** reference sites and provide a set of criteria for their identification.

For bioassessments using the RCA, reference sites are experimental controls and thus should be minimally impacted; however, they are not expected to represent "pristine" (i.e., pre-European settlement) conditions. Rather, reference sites are expected to reflect biological conditions in areas where impacts from human disturbance are minimal (Simon 1991). In cases where we are attempting to assess cumulative impacts, impacted sites can be used as reference sites as long as

they are minimally exposed to the stressor of interest. For example, in the Environmental Effects Monitoring Program for metal mines, several urban reference sites may be used as controls for urban sites that are **impacted** by mines. In this case, minimally impacted sites are still required in the assessment to indicate relative condition (i.e., the position of test, urban and reference sites along an impairment gradient).

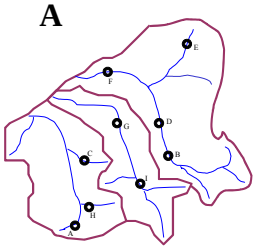
No objective, quantitative criteria for "minimally **impacted**" exist. Any site should be considered a candidate reference site if it is not obviously **impacted** by human activity. A number of factors should be considered when screening potential reference sites:

- Point-source contamination
- Regulation of water level (e.g., effects from dams and impoundments)
- Loss of natural **riparian vegetation**
- **Catchment** deforestation
- Aquatic habitat disruption (e.g., lake dredging, stream channel alteration)
- Development or urban land-use in **catchment**
- Agricultural land-use in **catchment**
- Imperviousness and artificial drainage in **catchment**
- Anthropogenic acidification
- Water chemistry

Ultimately, quantitative criteria for minimally **impacted** (i.e., thresholds for the factors listed above) will be developed for various regions of Ontario.

To reduce confusion over the terms *criteria for minimally impacted* and ***niche variable**s,* we emphasize that criteria for minimally **impacted** are simply rules used to determine if a given site qualifies as a reference site. **Niche variable**s measure natural, usually physiographic, features that are known to influence biological communities; if reference sites are grouped or classified according to similarity of biological composition, **niche variables** distinguish reference-site groups and are useful for building models that help select reference sites for comparison against a given test site.

A



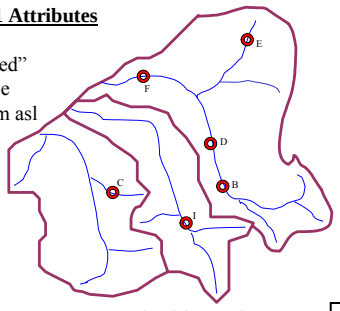
Watershed boundary
River
Minimally impacted reference sites

B

	Reference Site		
	A	B	C
Richness	12	16	17
% EPT	45	53	60
% Diptera	19	14	11
% Worms	3	2	1
% Predators	10	6	8
Hilsenhoff HBI	3.01	3.70	4.02
BioMAP WQId	22.02	32.33	24.94
Shannon-Weiner Diversity	0.897	1.234	1.091
$CA_{abundance}$ Axis 1	-1.2	3.2	4.1

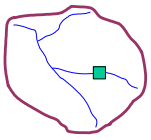
Reference Group 1 Attributes
5-320 km^2
40-60% "well drained"
2.1-4.1% mean slope
Elevation 320-431 m asl

C



Watershed boundary
River
Group 1 Reference Sites
Test Site

Test Site Attributes
7 km^2
58% "well drained"
4 % mean slope
Elevation 330 m asl



D

WITHIN NORMAL RANGE	Reference Group 1		Test Site
	$\bar{x}_{ref}$	SD_{ref}	$\bar{x}$
Richness	15.2	2.1	18.2
% EPT	56.4	6.8	62.5
% Diptera	13.1	4.5	14.3
% Worms	1.5	0.9	2.4
% Predators	8.2	1.3	10.3
Hilsenhoff HBI	3.83	0.40	3.62
BioMAP WQId	25.62	3.2	24.86
Shannon-Weiner Diversity	1.04	0.11	1.12
$CA_{abundance}$ Axis 1	0	0.96	1.1

Select minimally impacted reference sites that span the range of catchment physiographies encompassed by test sites (ideally this is done randomly); sample benthos and characterize habitat

Summarize biological characteristics of all reference sites

Build model that selects appropriate reference sites for a specified test site

Sample the test site's biological community and characterize habitat using niche variables

Use model to select appropriate reference sites for comparison with test site

no appropriate reference group available

Appropriate reference group available

Determine if the test site community is in reference condition (e.g., test the null hypothesis, H_0: $|D_{test} - D_{reference\ mean}| \leq$ critical effect size; see Appendix 9)

Test site falls within normal range

Test site falls outside normal range

Site likely unimpaired; resample periodically and confirm reference group selection

Site may be impaired; confirm reference group selection and resample; if same result, investigate cause of impairment

Figure 1: Recommended application of the reference condition approach in the OBBN: (A) hypothetical study area showing minimally impacted reference sites in three watersheds; (B) summarized biological condition of selected reference sites (hypothetical data); (C) natural habitat features of a reference site group selected for comparison with a test site; (D) calculation of summary indices (hypothetical data).

7.4.1 Reference Site Sampling Strategy

To permit the **normal range** to be defined for as many test sites as possible, partners should sample many different minimally **impacted** lake, stream, and wetland habitats in the first few years of OBBN implementation. Under-representation of minimally **impacted** sites for certain habitat types (e.g., large rivers and low gradient, low base flow streams) may be problematic. As the OBBN evolves, we will do our best to expand reference site coverage. Where insufficient reference sites exist, the **normal range** can be estimated using a combination of best available sites, historical or paleo-ecological data, experimental laboratory data, modeling, and best professional judgment (Gerritsen *et al.* 2000, Chessman and Royal 2004).

Periodic re-sampling should be done to determine if any widespread community shifts are occurring. Partners should reserve at least 10% of their annual sampling effort for reference site re-sampling (e.g., Barbour et al. 1999). Re-sampling can be done at the same sites each year, or at different sites in different years: using the same sites permits precise characterization of temporal variation, but gives little information about how widespread the observed pattern is; selecting a different set each year allows less precise characterization of temporal variation, but gives more information about the spatial extent of observed patterns.

Because the RCA has not been applied routinely throughout Ontario, a number of research questions pertaining to reference sites are listed in Appendix 8. These questions are presented to identify uncertainties, stimulate discussion, and identify opportunities for collaborative research.

7.5 Sampling Benthos and Characterizing Habitat

We describe benthos collection, sample processing, and habitat characterization methods for reference- and **test site**s in this section. As indicated in the OBBN Terms of Reference (Jones *et al*. 2004) these methods balance flexibility and standardization (Table 2). Flexibility is important because it ensures that all partners can participate in the network, regardless of their financial resources and expertise. Standardization is important to ensure that data collected by different partners are comparable. New methods may be added and existing methods may be refined following future assessments of repeatability, cost, and required effort and expertise.

Table 2: Summary of preferred and optional methods for the Ontario Benthos Biomonitoring Network.

Biomonitoring Component	Recommendation
Study Design	Reference Condition Approach
Benthos Collection Method	Travelling-Kick-and-Sweep (where possible); replication in lakes and wetlands, sub-sampling in streams
Mesh Size	500 μm
Time of Year	Any season; assessment comparisons use data from the same season
Picking	In lab (preferred) or in field (optional); preserved (preferred) or live (optional), microscope (preferred) or visually unaided (optional); random

Biomonitoring Component	Recommendation
	sub-sampling using Marchant Box (preferred) or Bucket Method (optional) to provide a minimum 100-animal count per sample
Taxonomic Level	Mix of 27 Phyla, Classes, Orders and Families (minimum); Family (preferred); Genus/Species (optional, recommended for reference sites)[1]
Analysis (**Bioassessment** Hypothesis Testing)	Test Site Analysis (TSA; see Appendix 9): Mahalanobis distance (e.g., Legendre and Legendre 1998) calculated across selected summary metrics; non-central significance test to determine if biological distance between test-site and reference-site-group mean is larger than a specified effect size; if the null hypothesis (H_0: $\left\| D_{test} - D_{reference\ mean} \right\| \leq$ critical effect size) is rejected, use discriminant function analysis to identify metrics contributing most to the separation between the test site and reference condition

[1]**Picked reference site samples and associated field sheets can be sent to the OBBN coordinator. We will try to provide identifications at lowest practical taxonomic level.**

Collection Method

All collection methods yield relative benthos abundance information for a sampling unit. The Travelling-Kick-and-Sweep is the standard sampling method; it is typically applied by wading along **transect**s through the habitat of interest, kicking the **substrate** to dislodge benthos, and collecting dislodged benthos by "sweeping" a hand-held net through the water. Other optional methods can be used in atypical habitats and special studies (Table 3).

Collection methods are detailed in sections 6.5.1-6.5.3.

Table 3: Benthos collection methods by waterbody type; ✓= preferred, O=optional.

Collection Method	Streams	Lakes	Wetlands
Traveling kick and sweep; standard method for wadeable habitats	✓	✓	✓
Grab samples (Ekman Dredge, Ponar Grab, or similar); option for deep water sites	O	O	
Jab and Sweep; option for wadeable, sparsely vegetated, soft sediments			O
Coring; option for deep or very shallow water (especially in shallow wetland soils)		O	O
Artificial substrate; option for atypical habitats or special studies	O	O	O

Mesh Size

The standard OBBN net mesh opening size is 500 μm. A number of factors were considered in making this recommendation:

- Most benthos **biomonitoring** surveys use a net mesh size between 250 micron and 1 mm.

- Fine mesh sizes retain more animals than large mesh sizes; however, many of the retained animals may be too small to identify to genus/species level with confidence. Conversely, coarser mesh retains larger animals that are easier to identify, but biases collections in favor of larger animals by excluding worms, young insects, and other small animals.
- Fine mesh tends to clog with organic matter and sand faster than coarser mesh.

Being in the middle of the range of mesh sizes commonly used, we believe that standardizing to 500 μm mesh represents a reasonable compromise for OBBN participants.

Time of year

Partners may sample in any season (e.g., Barbour 1999), preferrably during specified sampling periods (Table 4). **Bioassessment** comparisons must be made using reference and **test site** data from the same season (e.g., Linke *et al.* 1999).

We have defined sampling periods using calendar dates. As described in Appendix 8, we are planning studies to investigate seasonal changes in benthos composition, and may revise sampling periods depending on the outcomes of these studies. In addition, if alternate variables (e.g., stage of spring leaf-out, water temperature, degree of fall leaf-colour development) are more strongly correlated with the onset of periods of rapid benthic community change, sampling periods may be redefined using these variables.

Although our protocol permits sampling during any season, partners in neighboring jurisdictions may benefit from coordinated sampling to ensure that adequate reference site information is available. We will be meeting with groups of partners from different parts of the province to discuss opportunities for regional coordination of sampling activities.

There are pros and cons associated with collecting benthos at any time of the year (e.g., Barton 1996, Griffiths 1998 and 1999, Barbour *et al*. 1999, Linke *et al*. 1999, Reynoldson *et al*. 2003):

- Benthic community composition changes seasonally, and some animals are more abundant and easily identified at certain times of the year (Griffiths 1998, 1999).
- Winter and spring run-off events make sampling difficult and unsafe (Barbour 1999).
- Sampling at different times of the year may affect estimates of the degree of impairment because many stressors are seasonal and because communities may be naturally more variable at certain times of the year (Barton 1996, Parsons and Norris 1996).

Table 4: Pros and cons of benthos sampling at different times of year.

Season (sampling window in parenthesis)	Pros	Cons
Winter (January, February)	High **richness**; benthos often large and easily identified	Difficult or unsafe conditions prevail; community composition may not reflect water quality (Barton 1996) because benthos tend to expand their ranges in winter (perhaps because of low temperature and high oxygen saturation) by colonizing areas that

Season (sampling window in parenthesis)	Pros	Cons
		are not accessible at other times of the year
Spring (May)	High **richness**; animals large and easily identified (Griffiths 1998, 1999); significant amount of spring data exists	Short sampling period between spring freshet or ice-out (e.g., Barbour et al. 1999) and peak times for insect emergence
Summer (July, August)	The most stressful season for biota because of high water temperature and low oxygen level; invertebrates are most likely to show a response to impacts (Barton 1996); many partners have additional staff for summer sampling	Variable (often low) **richness**; conflicts with other fieldwork; drought conditions (no flow)
Fall (October)	High **richness**; composition may reflect summer impacts; significant amount of fall data exists	Prevalence of small juveniles (difficult to identify); community composition may not reflect water quality (Barton 1996) because benthos tend to expand their ranges in cooler months (perhaps because of low temperature and high oxygen saturation) by colonizing areas that are not accessible at other times of the year
Recommendation: Sampling may be done during any season, but **bioassessment**s must compare data collected in the same season.		

Seasonal variation in benthos community composition generally relates to life history patterns. Specifically, many benthic macroinvertebrates have non-aquatic life stages (i.e., many aquatic insects moult to winged adults that emerge from the water to reproduce, disperse and deposit eggs). Consequently, samples collected from the same body of water at different times of the year may have markedly different relative densities of taxa, even in the absence of human influence.

Water temperature and photoperiod are common triggers for emergence and mating, but thresholds vary among taxa. To illustrate the effects of seasonality on shallow-water invertebrate communities in small lakes in south-central Ontario, Reid *et al*. (1995) collected samples from 5 sites in Blue Chalk Lake every 6th week, from May until November (Figure 2). The average number of individuals collected at a site ranged from approximately 1200 in May through September, to about 1800 in November. The percent amphipods peaked in September when the percent Chironomidae was lowest. The proportion of the sample represented by Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) was highest in May and

November, but lowest during the summer months when emergence peaked. This example indicates that: (1) summary index values will change over the year, and (2) **bioassessment**s should be based on samples collected at the same time of year (e.g., Barbour et al. 1999).

Sample Picking

The OBBN protocol is flexible with respect to sample picking (the process of separating animals from gravel, sand, organic matter, and other material contained in the sample). The preferred picking method is in-lab, with preserved samples, using a Marchant Box to randomly sub-sample, and microscope assisted picking. Methods that reduce equipment costs and processing time (i.e., field picking, processing live samples, "Bucket" sub-sampling methods (see David et al. [1998] for details), and visually unaided picking) are optional (Table 5). Our flexibility on picking methods reflects the assumption that picking can be done with acceptable accuracy on live or preserved samples, with differing gear, and with different sub-sampling methods, as long as care is used (e.g., Reynoldson *et al.* 2003, Barbour *et al.* 1999).

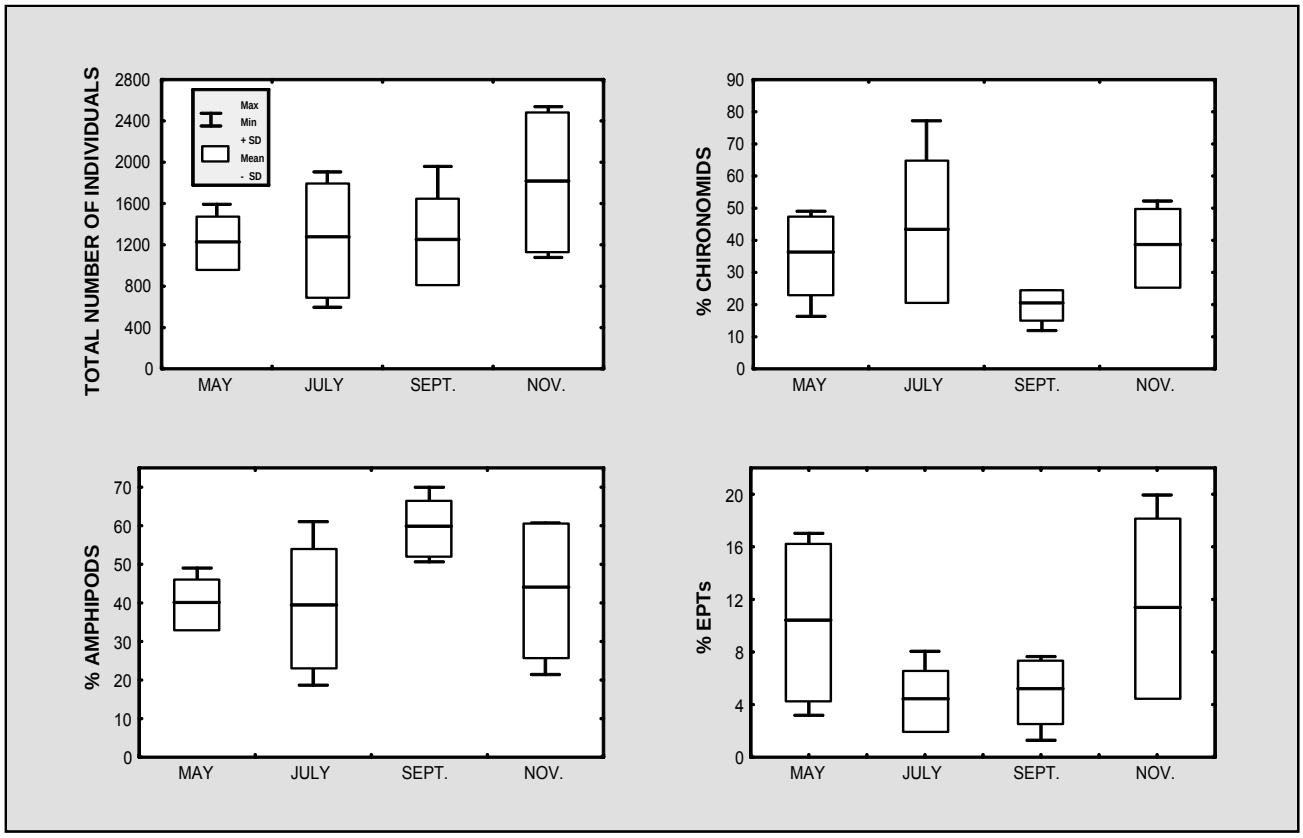


Figure 2: Seasonal changes in Blue Chalk Lake benthic-community composition.

Table 5: Preferred and optional sample-picking methods

Consideration	Preferred Methods	Optional Methods
Picking location (e.g., Barbour *et al.* 1999, Reynoldson *et al.* 2003)	Lab • Fewer distractions • Generally simplifies process	Field • Conditions may make picking difficult.
Preservation (alcohol or formalin; e.g., Griffiths 1998 and 1999, Barbour *et al.* 1999, Reynoldson *et al*. 2003)	Preserved • Required if samples will be stored for more than ~24 hr. prior to picking • Alcohol (at least 70% concentration in sample) or formalin (~5% in sample) may be used. • Preservation removes identification cues related to movement and requires attention to safety and disposal, but reduces transportation costs because more samples can be collected at one time. • Formalin should be buffered to prevent calcareous shells from dissolving.	Live • Samples must be protected from heating or animals will die and decompose. • Predation occurring in the sample can alter composition (Reynoldson et al. 2003)
Sub-sampling (e.g., Reynoldson *et al*. 2003)	Marchant Box • Gives more reliable estimates of sample abundance • Easily randomized • More costly and time consuming	"Bucket Method" • Fast • Minimal equipment • Inadequate randomization can bias results
Magnification	Microscope • May be more time consuming • More small animals found (e.g., Griffiths 1998, 1999) • Requires expensive equipment	Visually unaided • May be faster • No special equipment • May bias in favour of large specimens
Recommendation: The preferred picking method is in-lab with preserved samples using Marchant Box sub-sampling and microscope assisted picking. Optional methods that reduce equipment costs and processing time may be used.		

Taxonomic Level

The minimum level of benthos identification is a coarse mix of 27 Phyla, Classes, Orders, and Families (Table 9, Appendix 3). OBBN partners with sufficient expertise and financial resources are encouraged to identify benthos to lower taxonomic levels. We particularly encourage detailed (genus/species) identification for reference sites; this will ensure that the taxonomic resolution of bioassessments is not limited by the detail of reference site data, and will make OBBN data more useful for biodiversity studies.

As indicated in Table 6, the main issues related to benthos identification level are: time and expertise requirements (which increase with the detail of identifications), sensitivity and **diagnostic** power (more detailed identifications are assumed to provide more sensitivity to detect impairment and better ability to diagnose type of impairment), and probability of errors (which increase with taxonomic detail).

Table 6: Taxonomic-level considerations for benthos identification.

Issue	Coarse (27 group OBBN mix)	Family	Genus/species
Sensitivity[1]	Screening level	Intermediate	Most sensitive
Diagnostic Power[1]	Limited	Better	Best
Cost	Low	Moderate	High
Probability of Errors[2]	Low	Medium	High
Recommendation: Benthos should be identified to at least the coarse (27 group) level for test sites, and to lowest practical level for reference sites. Family-level identification is generally recommended for bioassessment of test sites.			

[1] Subject of scientific debate
[2] Decreases with increasing training

Identification to the coarse 27-group level is sufficient for OBBN participation. More detailed identification may provide additional sensitivity and allow better characterization of response, but this additional information may be offset by longer sample processing time and more errors (Resh and Unzicker 1975, Rahel 1990, Marchant *et al.* 1995, Barton 1996, Bournaud *et al.* 1996, Bowman and Bailey 1997, Roux *et al*. 1999, Guerold 2000, Hawkins *et al*. 2000, Bailey *et al.* 2001, Lenat and Resh 2001, Culp *et al.* 2003, Landis 2003, Orr *et al*. 2003, Gayraud 2003).

Replication

Collection of three **replicate** samples is recommended for the following reasons:

- Understanding variability among **replicate**s is important for **bioassessment** because many hypothesis testing procedures compare within-group variance against between-group variance (as in ANOVA); without replication, variance cannot be estimated.
- If you only have one sample, you have little confidence that it is representative of the relative densities occurring in your sampling unit. Two samples are better, but if they are markedly different, you have no idea which one is a better representation of the community. Three samples is a reasonable minimum because it provides more confidence in estimates of means.

Although replication is generally desirable, it is not always practical. To reduce sampling time and reduce the need to access large (often privately owned) stretches of river, true replication (which is optional) will typically not be done in riverine habitats for OBBN assessments; rather,

three 100-animal **sub-sample**s are collected in one sampling unit. Not only is a sub-sampling approach more practical, but it is also more consistent with the methods of the Canadian Aquatic BIomonitoring Network (CABIN) since the three 100-animal **sub-sample**s can be pooled for a CABIN-like analysis (e.g. Reynoldson *et al.* 2003) of one 300-count sample. In any case, if replication or the collection of multiple **sub-sample**s is not possible, partners should collect what samples they can: some information is better than none.

7.5.1 General Sampling Concepts for Lakes, Streams, and Wetlands

Before using OBBN sampling methods, partners should understand our definitions of lake, stream, and wetland sampling units; replication and sub-sampling procedures, and the purpose of our sampling effort guidelines. These concepts are explained below.

Lake, Stream, and Wetland Sampling Units

We refer to lake sampling units as *Lake Segments;* stream sampling units are called *Sampling Reaches*; and wetland sampling units are called *Wetland Segments*. Lake- and Wetland Segments are shallow, near-shore areas that are generally wadeable and support the majority of benthic taxa present in the waterbody (e.g., Johnson 1998). Stream **Sampling Reach**es typically comprise a meander sequence, containing both **pool** and **riffle** areas where transect samples are collected.

We are interested in characterizing the biological condition of the specific lake, stream, or wetland area where samples are collected. Presumably, the entire upstream **catchment** affects the biological condition of any sampled area within a given waterbody; however, evaluating the health of an entire **catchment,** lake, stream, or wetland (for all but the smallest of these) generally requires corroborating information from numerous sampling units.

Some guidance can be given on the selection of sampling areas in lakes and wetlands when the goal is to produce a whole-waterbody assessment. In general, near-shore sampling can be done, especially near areas contributing potentially contaminated over-land flow, to evaluate non-point source impacts. Sampling areas may be concentrated around lake inflows (i.e., near-shore areas adjacent to river mouths) to evaluate point-source impacts. Sampling areas may be clustered around lake outflows to evaluate overall lake condition. In the case of lakes and wetlands, assessment of biological condition in the near-shore zone should provide early warning of main basin impacts.

Replication

Lake and wetland sampling methods use replication: 100-animal samples are collected from each of three separate Lake- or Wetland Segments. Replication is typically *not* done in streams: instead, three **sub-sample**s (i.e., two **riffle** and 1 **pool** transect samples) are collected. Sub-samples are processed separately, resulting in three 100-animal collections for the **Sampling Reach,** allowing within-sampling-unit variance in community composition to be estimated.

Sampling Effort

Sampling effort guidelines are provided to ensure that at least 100 animals are collected per **replicate** or **sub-sample**, even in sparse habitats. If experience shows that benthos are abundant at a given location, sampling effort (e.g., time spent sampling, distance covered, number of pooled grabs) can be reduced accordingly. Conversely, if benthos have very low abundance, sampling effort may need to be increased.

7.5.2 Near-Shore Lake Sampling Methods

The near-shore Travelling-Kick-and-Sweep sampling method for lakes is given below (and see Figure 3):

1. Apply appropriate safety measures.
2. Choose a set of 3 representative Lake Segments (ideally this is done by randomly selecting 3 from a set of possible locations on a lake), in which a series of **transect**s (running from the water's edge to the 1 m depth; see Figure 3) will be sampled. These Lake Segments should be enclosed in the area where aquatic ecosystem condition is questioned.
3. Fill out a field data sheet (Appendix 4)
4. Use a 500 µm mesh net and a Travelling-Kick-and-Sweep along **transect**s to collect the sample. Vigorously kick the **substrate** to disturb it to a depth of ~5 cm. To collect dislodged materials, sweep the net back and forth and up and down as you move along the transect. Sample for about 10 minutes per **replicate**, or until you are sure that at least 100 animals have been collected. At least one complete **transect** (from shore to 1 m depth) must be sampled. Sieve the collected sample in the net. Rinse off and remove large rocks, plant material, etc. Release any non-benthic animals collected. Transfer net contents to a bucket. To prevent the net from clogging, material may need to be transferred several times as you collect each **replicate**.
5. Record sampling time (active sampling time only, time spent transferring net contents to bucket not included), distance, and all other information required on the field sheet.
6. Repeat steps 2-5 until three **replicate**s are collected.

Artificial substrates may be used in lakes when a Travelling-Kick-and-Sweep method is not appropriate. Design specifications and minimum colonization times for artificial substrates have not yet been established for the OBBN, but information is available (e.g., Buikema and Voshell 1993, Casey and Kendall 1996, Benoit *et al*. 1998, Carbone *et al.* 1998, Carter and Resh 2001, Muzaffar and Colbo 2002).

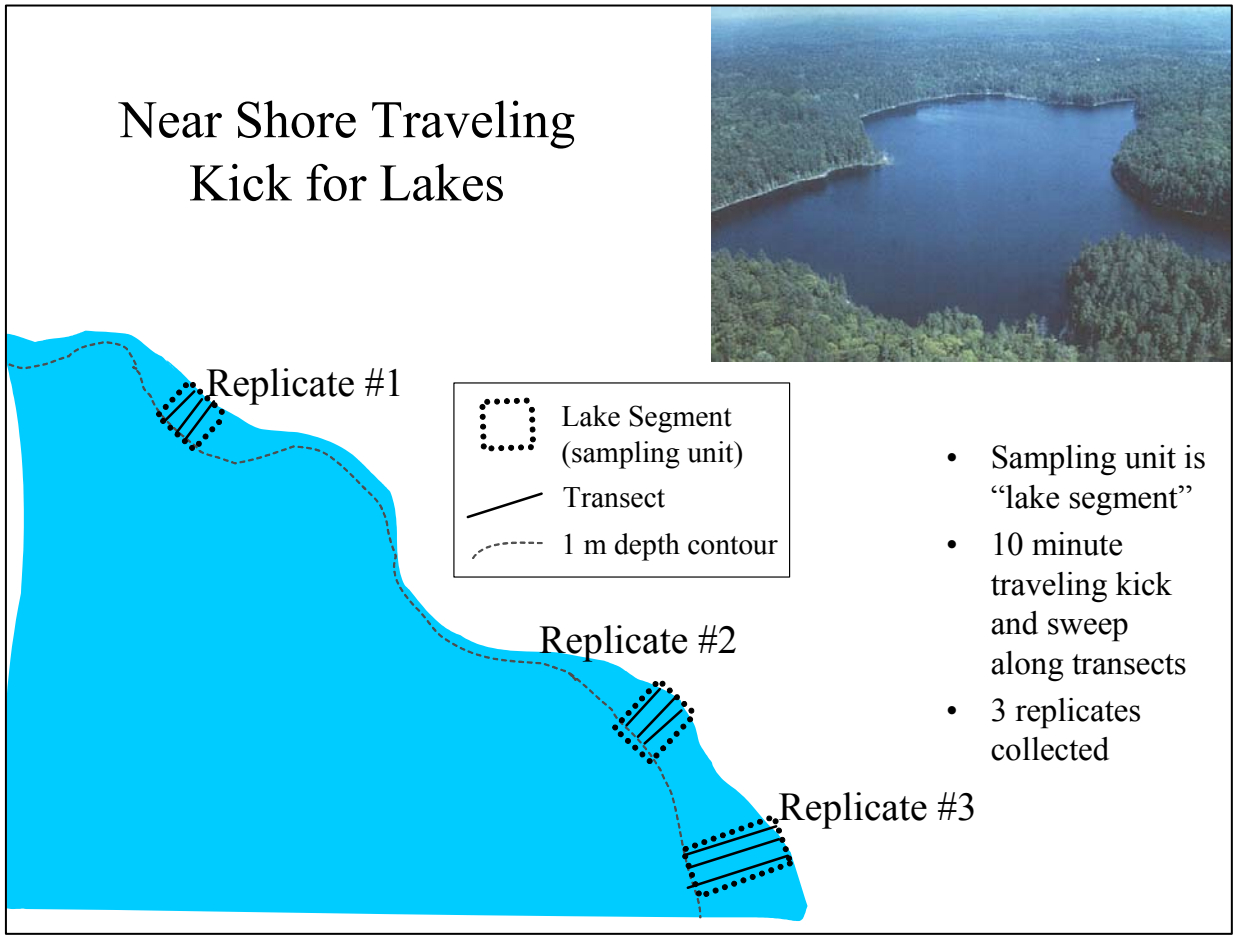


Figure 3: Travelling-Kick-and-Sweep method for lakes.

7.5.3 Stream Sampling Methods

The preferred sampling method for streams is the Travelling-Kick-and-Sweep-Transect Method (Transect Kick); however, alternate methods are available for use in special studies or atypical habitats (Table 7).

The **Sampling Reach** should be a long enough channel segment to encompass 2 **riffle**s and 1 **pool**. In alluvial systems, typical of southern Ontario, a **Sampling Reach** can usually be defined as 1 meander wavelength (Figure 4). In the simplest case, three **cross-over**s define the **Sampling Reach** (they occur at the beginning, in the middle, and at the end of each **Sampling Reach**). A sampling unit thus defined would offer a choice of 3 **riffle** sampling locations (i.e., at **cross-over** points) and two **pool**s (i.e., at outside bends of meanders). This morphological definition of **pool**s and **riffle**s is difficult to apply in rivers where channel features are shaped more by rocky features than by alluvial processes. In such systems, **pool**s and **riffle**s do not occur in regular patterns, and sampling partners should define **Sampling Reach**es to encompass functionally defined **pool**s and **riffle**s (i.e., slow/deep and fast/shallow areas, respectively), regardless of where they occur in the meander sequence. Where **pool**s and **riffle**s cannot be distinguished,

such as in municipal drains and other straightened channels, the **Sampling Reach** can be defined as 14-20 times the **bank-full width**, which corresponds to the normal meander wavelength of similar sized streams with natural channels (Reynoldson *et al*. 2003, Stanfield 2005). In this case, mark the start of the **Sampling Reach** and set the end at about 20 times the **bank-full width**. When using the Transect Kick method (described below) **transect**s can then be located at the start, mid point, and end of the defined **Sampling Reach,** giving **transect** spacing that is typical of most natural streams with similar **bank-full width**.

A summary of recommended stream sampling procedures is given in Figure 5.

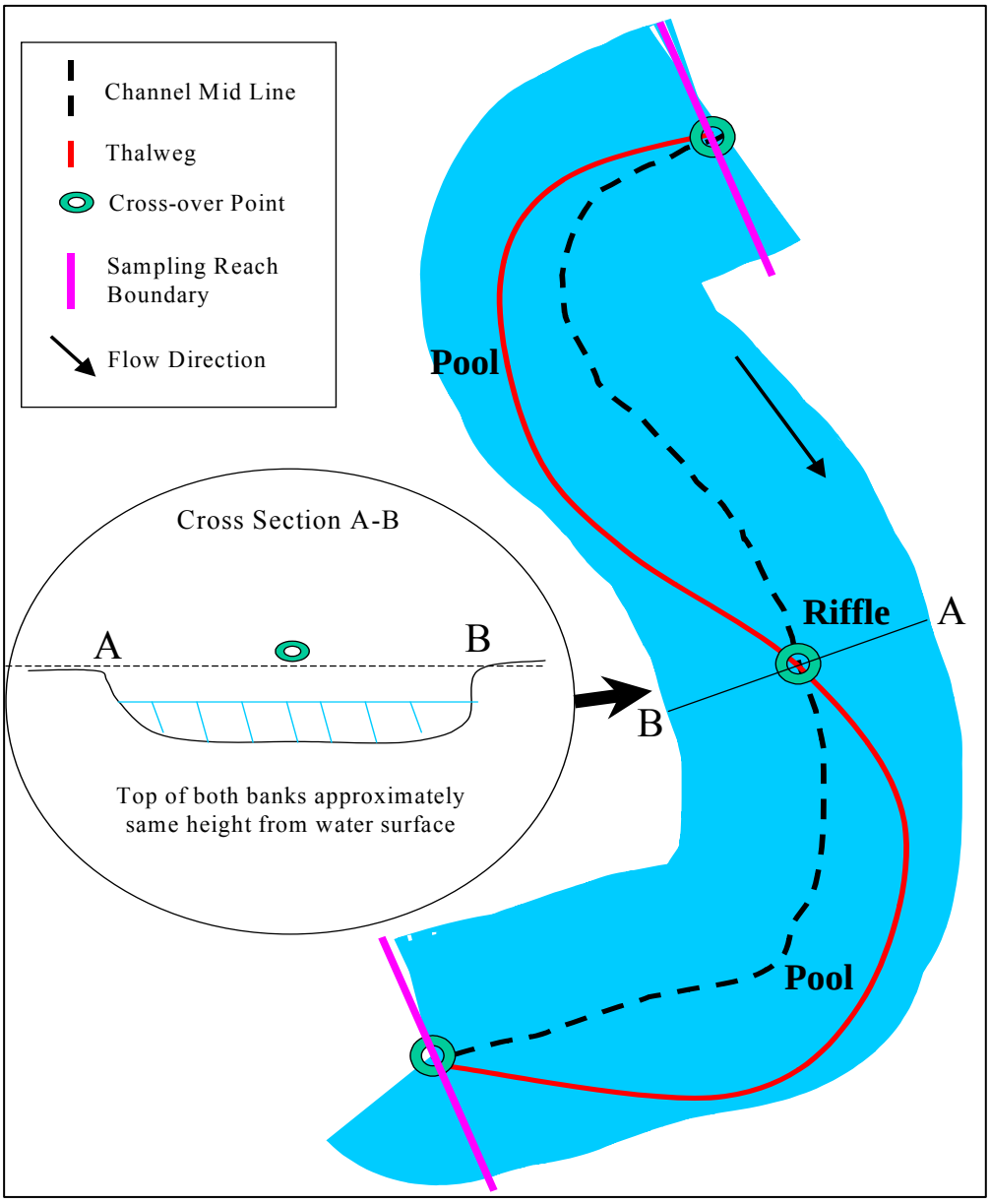


Figure 4: A stream meander wavelength typical of streams shaped by alluvial processes. The key morphological attributes used to define a Sampling Reach (i.e., cross-overs and pools) are shown (adapted from Stanfield 2005).

Table 7: Strengths and weaknesses of several commonly used stream benthos collection techniques.

Collection Method	Gear Type Examples	Pros	Cons
Travelling Kick	D-net, kick net, poled seine net, or similar	Can be used in a variety of channel types, with varying bottom materials and water depths; can be used to sample multiple habitats within the channel	Travelling kick may traverse a number of heterogeneous habitats, making habitat characterization and determination of appropriate reference group challenging; variability associated with sampling different habitats may reduce sensitivity (e.g., Parsons and Norris 1996); stream must be wadeable
Stationary Kick (e.g., 1 m^2 quadrat)	D-net, kick net, poled seine net, or similar	Samples a relatively homogeneous unit of habitat, thereby simplifying habitat characterization and selection of reference sites.	Stream must be wadeable
Fixed Area Collection (grabs or kick and sweeps)	Hess Sampler, Surber Sampler, T-sampler, Ponar Grab, Ekman Dredge	Permits good control of sampling effort and yields per-area densities; grabs can be used from a boat in deep, non-wadeable **reach**es	Each type of gear has a specific range of substrates in which it can be used efficiently; sampling gear is more expensive than hand-held nets
Artificial substrate	Rock baskets, Hester-Dendy (plate-type samplers)	Suitable for studies in which other sampling methods are inappropriate	No established guidelines for construction.
Recommendation: Travelling-Kick-and-Sweep sampling for wadeable streams and wadeable margins of partially wadeable streams. Grab samples for non-wadeable streams. Artificial substrates for situations when other techniques will not work			

Detailed stream benthos collection methods are given below:

1. Apply appropriate safety measures.
2. Fill out a field data sheet (Appendix 5).
3. Where possible, identify a **Sampling Reach** that contains 2 **riffle**s and 1 **pool**.
4. Select a collection method. For wadeable or partially wadeable streams, use the Travelling-Kick-and-Sweep-Transect Method (Transect Kick; Figure 6). For non-wadeable streams (e.g., having excessive depth, strong current, or unstable bottom), use a grab sampling method (Figure 9). If none of the above methods are suitable, artificial substrates can be used.

(Continue to step 5 on following page, under heading of selected sampling method: either Travelling-Kick-and-Sweep or grab sampling)

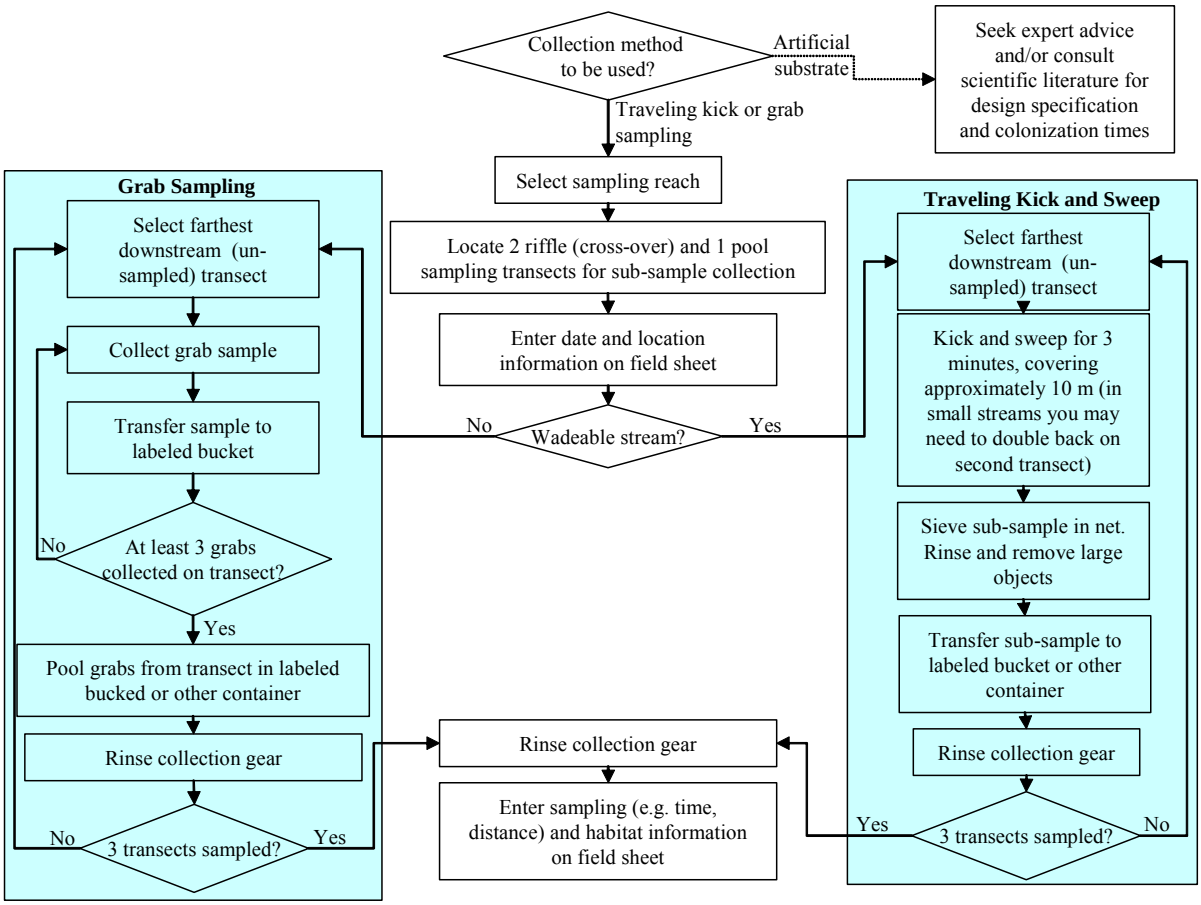


Figure 5: Stream sampling protocols. Optional procedures shown as dotted lines.

Travelling-Kick-and-Sweep-Transect-Method (Transect Kick; Figure 6)

The Transect Kick is a modification of the bank-to-bank, zigzagging, Travelling-Kick method (e.g., Reynoldson et al. 2003); it standardizes sampling effort in both **riffle** and **pool** habitats within the **Sampling Reach**. Compared to a zigzag method, the Transect Kick also simplifies habitat characterization and estimation of distance and area covered during benthos collection.

5. Identify a **Sampling Reach** and locate 2 **riffle** and 1 **pool transect**s. In **Sampling Reach**es containing multiple **riffle**s or **pool**s, **transect**s should ideally be located randomly; however, safety and ease of access must be considered.
6. Sample the farthest downstream **transect** in the **Sampling Reach**. Place a 500-µm-mesh net downstream of you (usually the net is held close to the stream bottom). Start your timer. Beginning at either the right or left bank, walk along the **transect** to the opposite bank, vigorously kicking the **substrate** to disturb it to a depth of ~5 cm. Sweep the net back and forth (both vertically and horizontally through the water column) and keep it downstream of, and close to, the area being disturbed so that dislodged invertebrates will be carried into the net. A good sweeping motion is particularly important in areas of slow current to ensure animals are collected in the net (the sweeping motion is less important when sampling in strong current). Kick-and-sweep about 10 m of the **transect** in about 3 minutes (this

sampling effort may be reduced if benthos are known to be abundant). To stick to the approximately 3 minute/10 m guideline in large rivers, sample short segments along the **transect** (essentially a point-**transect** approach), in a way that covers the range of current velocities exhibited across the channel cross-section (Figure 7). On the other hand, sticking to the 3 min./10 m guideline in small streams requires that several **transect**s be positioned in the same **riffle** or **pool** (Figure 8).

7. Sieve the collected sample in the net. Rinse off and remove from the sample large material like rocks and wood. Release any non-benthic animals collected. Transfer net contents to a bucket. To prevent the net from clogging, material may need to be transferred several times as you sample each **transect**. Placing your bucket on the side of the stream where you start sampling allows frequent trips to the bucket without disturbing **transect** sections not yet sampled.
8. Record sampling time (active sampling time only, time spent transferring net contents to bucket not included), distance, and all other information required on field sheet.
9. Move to the next upstream **transect** and repeat steps 6-8. Repeat these steps until all **transect**s have been sampled. If you encounter non-wadeable portions of the channel cross section as you progress along any **transect**, sample only the safely wadeable portion.
10. Record the number of **transect**s used, total distance traveled on each **transect**, total time spent collecting invertebrates, and **wetted width** at each **transect**, as well as all other information on the field sheet.
11. Rinse the net and retain any recovered benthos with the sample
12. Repeat steps 3-10 until 3 **sub-sample**s are collected.

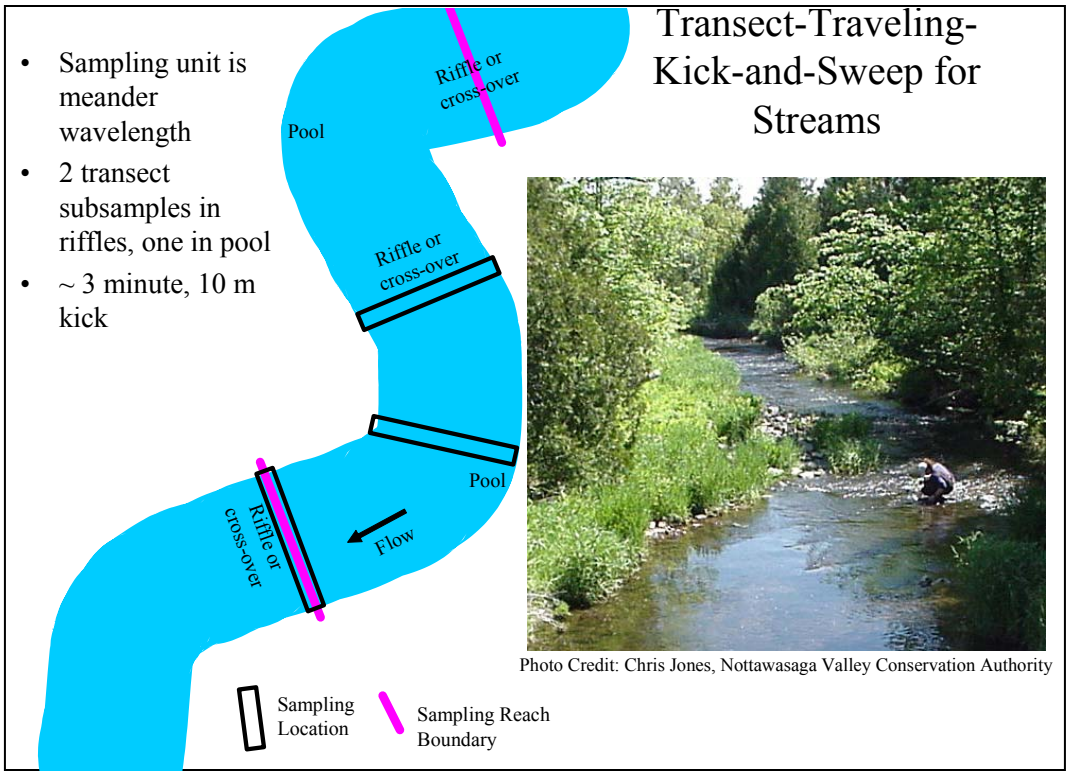


Figure 6: Travelling-Kick-and-Sweep-Transect Method for wadeable or partially wadeable streams.

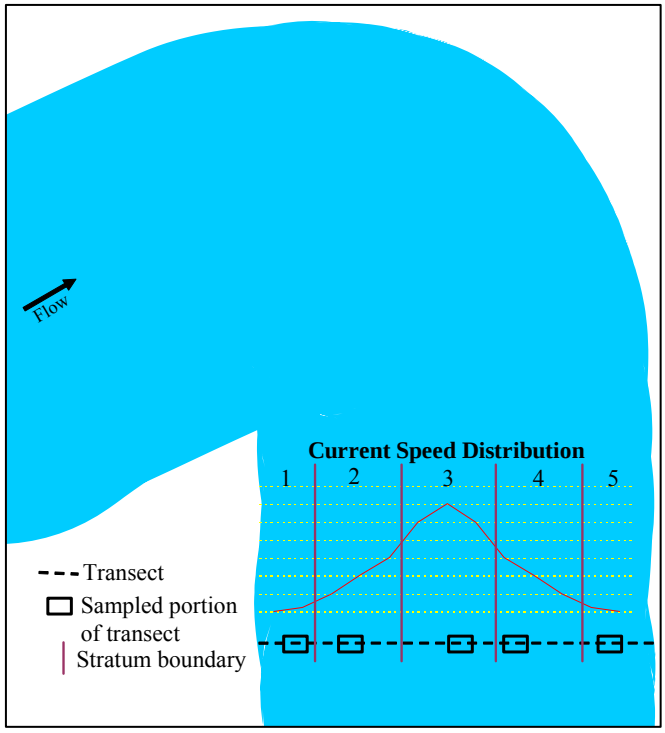


Figure 7: Large river Transect Kick Method. Portions of the transect are selected randomly within each current speed stratum (labeled 1-5) to give an approximate 10 m and 3 minute composite sample for the transect.

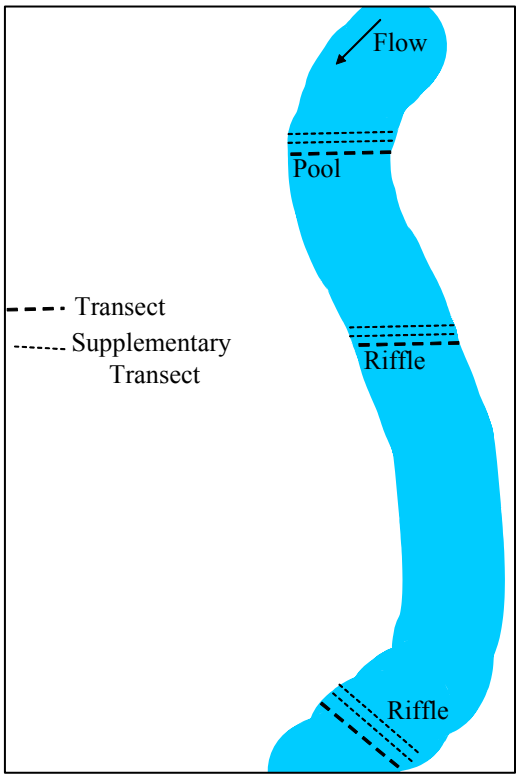


Figure 8: Small river Transect Kick Method. Additional supplementary transects are located immediately upstream of each pool and riffle transect to provide sufficient sampling distance (i.e., approximately 10 m).

Grab Sampling (e.g., Resh and Jackson 1993)

The recommended grab sampling method for the OBBN was designed to yield a composite Transect-Kick-like sample for each **pool** and **riffle** transect in the **Sampling Reach** (Figure 9). Grab sampling is most often done in slow, deep, non-wadeable streams.

5. Select 2 **riffle** and 1 **pool transect**s
6. Sampling from a boat, bridge or similar vantage point that allows access across the stream cross section, collect and **pool** at least three grab (e.g., ekman dredge or ponar grab) samples per **transect** to ensure 100 animals are collected. Use an ekman dredge or ponar grab in fine sediments where jaw closure is not a problem. A Ponar Grab can be used in most other cases. Other types of equipment can be used where appropriate. Record sampling device on the field sheet and provide device specifications.
7. Rinse the sampling gear into the sample collection bucket. Rinse and discard any large **substrate** features collected with the sample. Release any non-benthos animals collected.
8. Record the number of grabs pooled per **transect**, and all other information required on the field sheet.

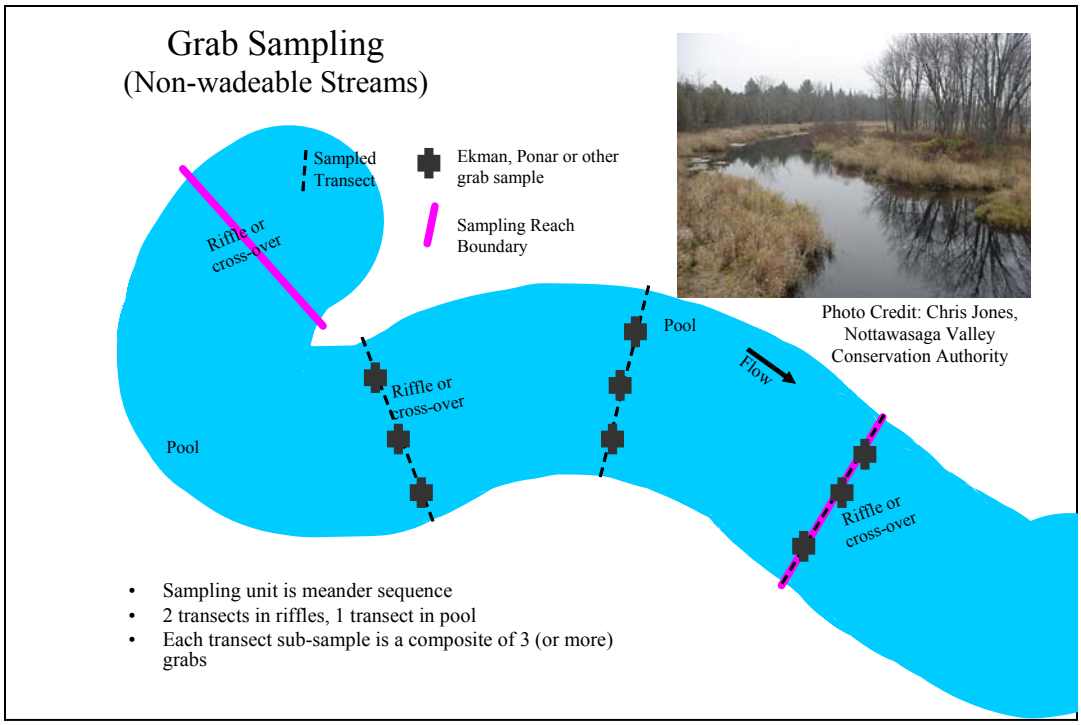


Figure 9: Grab sampling method for non-wadeable streams.

Artificial Substrates

Artificial substrates may be used in streams when Travelling-Kick and grab sampling is not appropriate. Design specifications and minimum colonization times for artificial substrates have not yet been established for the OBBN, but information is available (e.g., Buikema and Voshell 1993, Paller 1996, Swift *et al*. 1996, Casey and Kendall 1997, Humphries *et al*. 1998, Mason 1998, Carter and Resh 2001).

7.5.4 Wetland Sampling Methods

Wetland sampling procedures are described below (and see Figure 10):

1. Apply appropriate safety measures.
2. Choose 3 Wetland Segments
3. Fill out a field sheet (Appendix 6)
4. Choose appropriate sampling gear and apply collection technique based on wetland habitat type, water depth, and the questions to be answered by your study (Table 8).

Table 8: Selection criteria for wetland sampling techniques.

Water Depth	Substrate Type	Plant Density	Recommended Gear	Recommended Technique
0.15-1 m	Granular/mineral (e.g., sand/gravel)	Low	D-net	Travelling-Kick-and-Sweep
0.05-1 m	Soft (e.g., organic, muck)	moderate	D-net	Jab and Sweep
<0.05 m or saturated soils	Soft to moderately stable	Any	Stovepipe Corer	Core

Wetland Travelling-Kick-and-Sweep (e.g., Nelson *et al*. 2000, David *et al*. 1998, Reynoldson *et al*. 2003, Schneider and Frost 1996)

5. Plan a set of **transect**s within a Wetland Segment.
6. Use a 500 µm mesh net. Walk along wadeable **transect**s, vigorously kicking the **substrate** to dislodge benthos and bottom materials. Sweep the net through the water column to catch dislodged material. Transfer net contents to a bucket frequently to prevent the net from clogging.
7. Continue to sample **transect**s for 10 minutes or until 100 animals have been collected. At least one **transect** that spans the length of the Wetland Segment must be sampled.
8. Record time spent sampling, distance covered, and all other information required on the field sheet.
9. Thoroughly rinse net contents into a bucket.
10. Repeat steps 6-8 for each **replicate**.

Jab and Sweep (e.g., King and Richardson 2002)

5. Select locations for jab and sweep sampling within a Wetland Segment
6. Jab a 500 µm mesh D-net into the **substrate** to a depth of 5 cm and sweep it forward until the net fills with disturbed material. Pool 3 or more jab and sweeps per **replicate** to ensure that at least 100 animals are collected.
7. Be sure to record the number of jab and sweep samples pooled per **replicate** as well as other information required on field sheet.
8. Thoroughly rinse net contents into a bucket.
9. Repeat steps 6-8 for each **replicate.**

Coring (e.g., Reinhardt *et al*. 2000, Findlay *et al*. 1989, Clements 1994, Kiffney and Clements 1994)

A standard corer has not been selected for the Ontario Benthos Biomonitoring Network, but stove pipe-type corers are easily made (e.g., Davis *et al*. 1999). Record corer specifications on the field sheet.

7. Continue sampling until 3 **replicate** samples are collected.
8. Be sure to record the number of cores pooled per **replicate**, corer specifications, and other information required on field sheet.

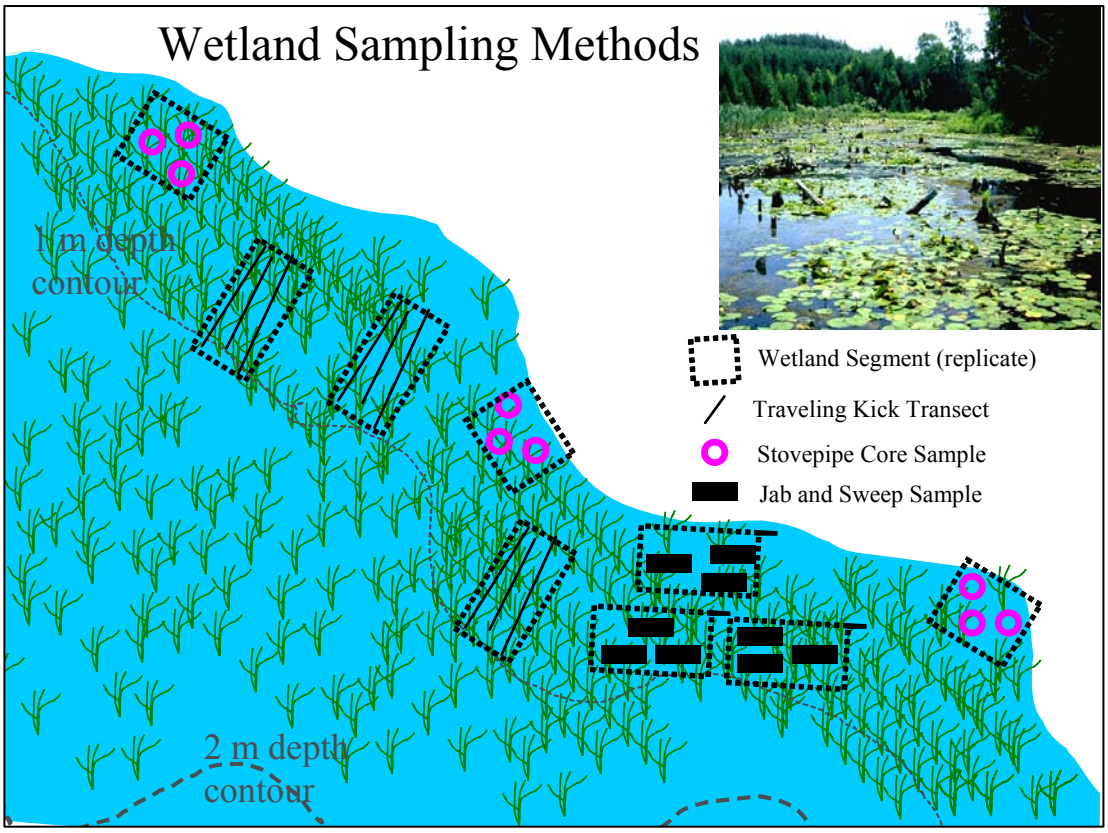


Figure 10: Wetland benthos collection methods (Photo credit: Chris Jones, Nottawasaga Valley Conservation Authority).

7.5.5 Sample Processing

Once samples have been collected, sample picking, invertebrate identification, enumeration, and sample preservation (optional) should be carried out (Figure 11).

Preparing Samples for Transportation to the Laboratory

If necessary, pool samples to generate each **replicate** (e.g., samples from each **transect** in a sampling unit in lakes or several cores from a wetland) or **sub-sample** (e.g., when more than 1 grab sample was used per **transect** in streams). Samples should be sieved in the net in the field. At this time, rocks, wood, leaves and other large items found in the sample may be discarded after removing all attached benthos. Also check for and release any non-benthos animals collected (e.g., fish). If samples are to be picked live, they should be kept cool and should be processed within 48 hours. For transportation to the lab, we recommend decanting bucket contents into a wide-mouth plastic jar to avoid spills during transportation and to conserve

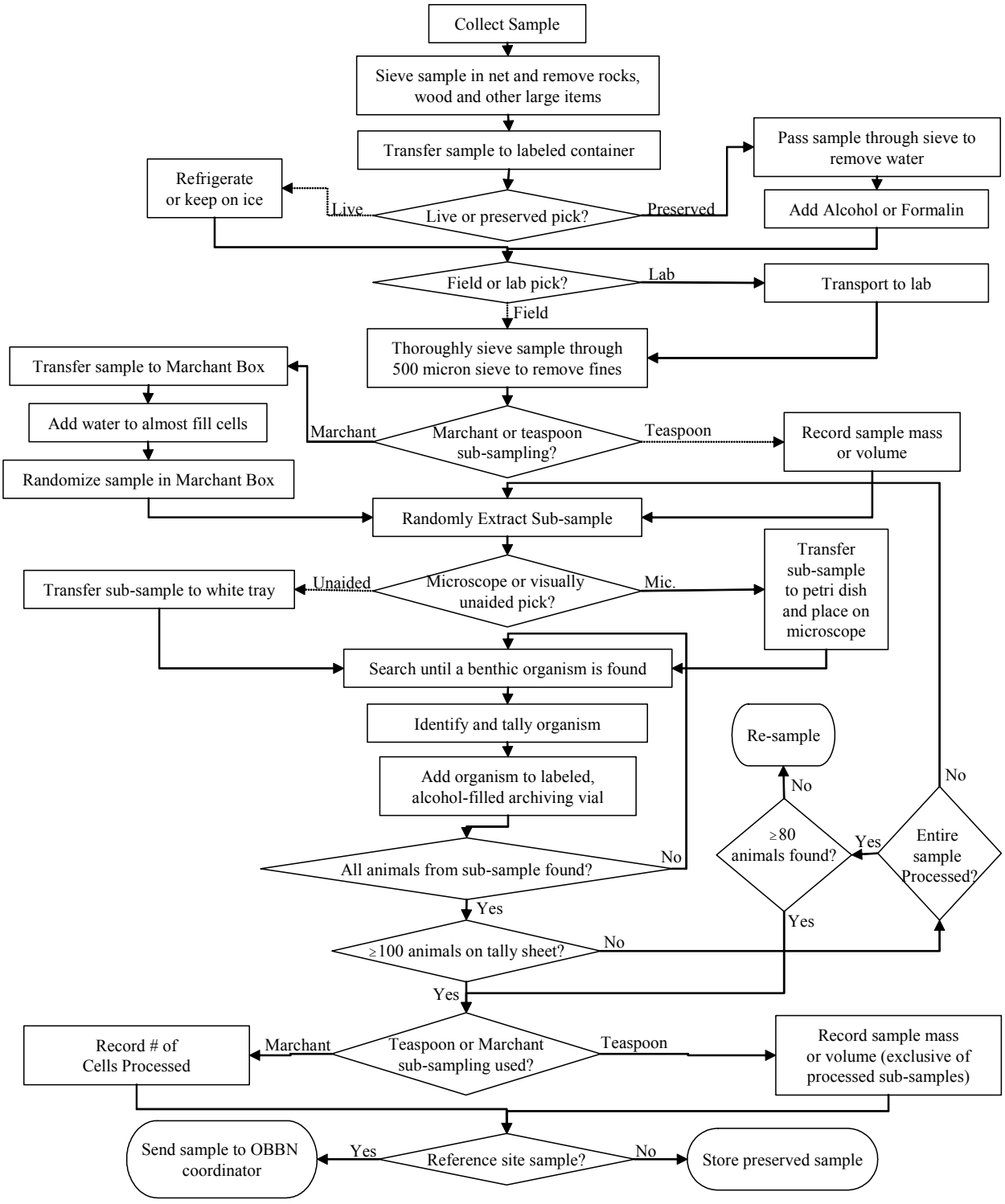


Figure 11: Sample processing procedures. Optional procedures indicated as a dotted line.

refrigerator space (a consideration when samples will be picked live). Label samples with lake, stream, or wetland name (or code), date and sample number. We recommend inserting labels in the vessel containing the sample in case labels get washed off the container; regular paper and pencil work fine for this purpose.

Samples may be preserved with 10% buffered formalin (a good fixative) as long as safety precautions are observed (e.g., Puget Sound Water Quality Authority 1987). Alcohol (e.g., ethanol, methanol, isopropanol) can also be used to preserve samples. If buffered formalin is used for initial fixation, replace it with alcohol after a couple of days to prevent hard body parts (e.g., clam and snail shells) from dissolving. When using alcohol for preservation in the field, a good method is to first sieve the sample to remove much of the water, transfer to a suitable container, and then add a generous amount of alcohol.

Sieving the Sample

It is important to remove fine particulate matter and preservative from the sample prior to picking benthos. Fines cloud the water in sorting trays, making the task of finding animals much more difficult.

Thoroughly sieve the sample:

1. Transfer the sample to a 500 µm sieve (500 µm D-net can be used as a sieve) and rinse well with water to remove preservative (if used) and fine suspended particles.
2. Thoroughly rinse and discard large items, such as pieces of wood, rocks, and leaves.

Rinsate from preserved samples will be sufficiently dilute and of low enough volume to permit disposal via a septic system or municipal sewage system. When disposing sample preservative to a septic system, keep daily 10% formalin discharge to 10 L or less.

Obtaining Benthos Sub-samples

Sub-sampling is a method of removing manageable portions of the sample so that invertebrates can be more easily separated from debris in the sample.

Optional sub-sampling methods are given below:

1. Marchant Box Method (Marchant 1989; preferred): The standard sub-sampling box is a modified Marchant design consisting of an approximately 27 x 27 x 15 cm box that is divided into 100 cells and has a water tight lid. Wash the sample from the sieve into the Marchant Box and fill with water to a depth just below the height of the walls dividing the cells. *Water depth is important. In the case of live samples, water deeper than the dividing walls will allow animals to swim between the cells once the contents have been randomized. Less water will make it difficult to distribute the sample among the 100 cells*. Close and fasten the lid. Invert and gently mix the sample with side-to-side rocking motions. Right the box quickly and set on a level surface to let contents settle into cells. Using random numbers for the 10 columns and 10 rows, randomly select one or more cells and transfer contents to a

suitable container or petri dish using a pipette (or turkey baster), vacuum pump or aspirator and suction flask, or similar method.

The cell-extraction method used for Marchant sub-sampling strongly influences sample-processing time. Consider the costs of more sophisticated equipment such as aspirators, pumps, suction flasks, and tubing in relation to the improved efficiency resulting from their use. Using an aspirator and suction flask may be the best balance between minimal cost and extraction efficiency.

2. Bucket Method (optional): Wash the sample from the sieve back into a large container (a bucket works well). Gently swirl the bucket contents to randomly distribute the sample. Randomly remove a small quantity of the sample (using a spoon, ladle or similar gadget) and transfer it to a suitable container.

Picking, Identifying, Enumerating, and Preserving Benthos

Sub-samples should be sequentially removed and picked until at least 100 animals are retained from each sample. 100-animal fixed counts yield reliable estimates of relative abundance and allow samples to be processed relatively quickly (as opposed to full enumerations; Somers *et al*. 1998). In sparse samples (i.e., containing fewer than 100 animals), the entire sample is processed. If fewer than 80 animals are collected, re-sample (Figure 11).

To be counted, a specimen must have enough intact body parts to permit its identification to the targeted level, and it must have a head (this prevents double counting). Larval exuviae and empty shells (e.g., snails and clams) and cases (e.g., of caddisflies) are not counted.

It is generally most efficient to identify, tally and preserve benthos as they are picked from the sample as follows:

1. Transfer a **sub-sample** into a suitable picking container. If you are picking under a microscope, a petri dish works well. For typical bench-top picking, we suggest a white tray. Add additional water to the tray to aid sorting. Sort through the sample, removing all benthos. When working at coarse taxonomic levels, it is most efficient to identify and tally animals as you remove them; however, when picking live, speed is important so it may be best to identify animals after all samples are picked. Specimens that require detailed observation to identify should be set aside for later identification. The minimum detail for identification is a coarse 27 group mix of Phyla, Orders, Classes and Families (Table 9, Appendices 1 and 3).
2. Place animals into a labeled container with alcohol preservative after they are identified and tallied. Glass jars with lids that give a good seal are commonly used, but there are other options, for example animals can be preserved in snap-cap microcentrifuge vials, which are themselves stored in ethanol in a larger vessel (microcentrifuge vials are inexpensive and don't break when dropped like glass does). Animals that cannot be identified should be archived with the rest of the sample; their presence should be recorded on the tally sheet (as unknown), but their count is not considered part of the 100-animal **sub-sample**.

3. Continue picking the **sub-sample** until all benthos have been removed. A **sub-sample** is generally considered to be fully picked when no more animals are found during a reasonable period of searching.
4. Return to step 1 and continue to sort and identify animals until at least 100 invertebrates have been tallied. The entire **sub-sample** that contains the 100th animal must be picked in its entirety to allow abundance estimation.

In most cases, 1 container is used for each sample. For special studies or to build voucher or reference collections, multiple containers may be used (i.e., different containers for different taxonomic groups).

Additional pointers for sample picking

When picking live samples, watch for movement. Moving animals are easily seen and the type of movement is an important clue to coarse identification. Any animals that swim or are too small to be picked out easily using tweezers can be scooped from the water using a small piece of screen or eye dropper. Be sure to search for elusive animals: snails, small clams, and flat worms are cryptic, tend to stick to the bottom and sides of sorting trays, and are easily missed. Caddisflies have cases that resemble rocks or organic junk in the sample; faster moving animals often try to hide under **detritus**, twigs and pebbles in sorting trays; and many animals (e.g., small mayflies, stoneflies and various dipterans) are often caught in the surface tension of the water. If you are picking directly into alcohol, wipe the tips of your forceps before dipping back into the tray, because the alcohol temporarily breaks down the surface tension of the water and can send animals spinning off in every direction. Adding a few drops of a weak soap solution (i.e., 1 part dish soap to 6 parts water) breaks down the surface tension and causes most floaters to sink.

One difficulty associated with preservation in alcohol is maintaining an adequate concentration. This is generally not an issue with 100-count samples unless unusually small vials are used or animals tend to be large. To be sure that samples will be adequately preserved, decant the fluid from the vial and replace with fresh alcohol when you are finished picking.

Preserved reference-site samples should be sent to the OBBN Coordinator, so that genus/species level benthos identification can be done. Send samples to:
Ontario Ministry of Environment
Dorset Environmental Science Centre
1026 Bellwood Acres Road
Dorset Ontario
P0A 1E0
Attention: OBBN Coordinator

Until a central archive for benthos samples is established, all samples should be archived by their collector for future reference. Clearly label containers and store them in a cool place to reduce evaporative loss of alcohol preservative. Plain paper labels that are either hand-written in pencil or laser printed can be taped to the outside of containers. Acid resistant paper labels (similarly printed) work well inside containers. As a minimum, labels should specify organization, date, sampling location (e.g., Rocky Brook), sample number or code (e.g., RB1), **replicate** or **sub-**

sample number (e.g., sub-sample 1, **riffle**), and number of archival vials (e.g., vial 1 of 1). If many samples are to be collected, an indexing system to track the location of samples in storage is helpful.

Table 9: 27 groups forming the minimum taxonomic detail for benthos identification.

Coelenterata (Hydras)	Gastropoda (Snails, Limpets)	Ceratopogonidae (No-see-ums, Biting Midges)
Trombidiformes-Hydracarina (Mites)	Pelecypoda (Clams)	Chironomidae (Midges)
Turbellaria (Flatworms)	Anisoptera (Dragonflies)	Culicidae (Mosquitoes)
Nematoda (Roundworms)	Coleoptera (Beetles)	Simuliidae (Black Flies)
Oligochaeta (Aquatic Earthworms)	Ephemeroptera (Mayflies)	Tabanidae (Horse Flies, Deer Flies)
Hirudinea (Leeches)	Hemiptera (True Bugs)	Tipulidae (Crane Flies
Amphipoda (Scuds, Side-swimmers)	Lepidoptera (Moths)	Plecoptera (Stoneflies)
Isopoda (Sow Bugs)	Megaloptera (Fishflies, Alderflies)	Trichoptera (Caddisflies)
Decapoda (Crayfish)	Miscellaneous Diptera (Misc. True Flies)	Zygoptera (Damselflies)

Abundance Estimation

Knowing the portion of the sample processed to give 100 animals allows us to estimate sample abundance. In the case of the Marchant Box, the portion of the sample processed can be easily calculated as the portion of the 100 cells picked. Estimation is often less precise with the Bucket method, and can be done by weight or volume (i.e., by recording the sample's weight or volume prior to picking and after all **sub-sample**s have been removed).

7.5.6 Habitat Characterization

In an RCA **bioassessment** approach, the primary reason for characterizing habitat is to predict a **test site** to a set of reference sites; in other words, to build a model that predicts which reference sites a **test site** should be compared to (e.g., Corkum 1989, Corkum 1992, Heino *et al*. 2003, Bailey *et al.* 2004). Such models are built using **niche variable**s (e.g., Table 10). Secondarily, in the case of **impaired** sites, habitat information can help to identify the cause of biological responses (i.e., **diagnostic** variables, see Table 10). In this section we propose a set of habitat measures for these purposes.

At present we don't know which site-level and **catchment**-level habitat measures will have the greatest predictive power as **niche variable**s. In addition, it is likely that these important predictive variables will be different for different regions of Ontario. We recommend a set of field measures in Table 10. Habitat measures for each sample are recorded on the appropriate lake, stream, or wetland field sheets (see Appendix 4, 5 or 6 respectively). Samples of completed field sheets (hypothetical data) are provided in Figures 12-17. Our list of habitat measures may be refined in subsequent editions once we have a better understanding of Ontario reference conditions and the environmental factors that determine benthos-assemblage types.

Table 10: Habitat measures recorded on field sheets. Optional measures are shown in shaded rows. NV=candidate niche variable; D=May be diagnostic.

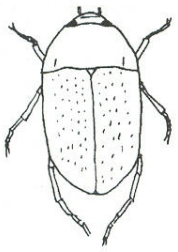
Feature	Application	Method	Use
Location (latitude & longitude)	All samples	GPS or map; **Sampling Reach** centroid for streams; centroid of each **replicate** for lakes and wetlands (Stanfield 2005: S.1.M.2.)	NV
Elevation (m above sea level)	All samples	Interpolate from topographic map	Surrogate for thermal regime (NV)
Water Temperature	All samples	Calibrated thermometer or data logger (Stanfield 2005: S.2.M.4.); should be measured at location of sample collection, at mid-depth (or note alternate location in comments section on field sheet)	NV, D
Dissolved oxygen (mg/L), pH, Conductivity (µS/cm), Alkalinity (mg/L as $CaCO_3$)	All samples	Calibrated field instruments (e.g., multi-probe) or laboratory analysis of water samples; should be measured at location of sample collection, at mid-depth (or note alternate location in comments section on field sheet)	NV, D
Maximum Depth (m)	All samples	Ruler or tape; typically measured at the **thalweg-transect** intersection in streams (Stanfield 2005: S.4.M.3.)	Correlated with stream size and flow (NV, D)
Maximum **hydraulic head** (mm)	Streams	Metre stick held vertically in current at **thalweg-transect** intersection (Stanfield 2005: S.4.M.2.)	Surrogate for current speed (NV, D)
Wetted width (m)	Streams	Measuring tape (Stanfield 2005: S.3.M.1.)	Correlated with stream size and flow (NV, D)
Dominant **substrate** class	All samples	Classification (e.g., abundant, present, or absent) according to estimated areal coverage; visual estimation or pebble count (Stanfield 2005: S.4.M.2.)	NV, D
Organic matter, areal coverage	All samples	Classification by relative areal coverage (e.g., abundant, present, or absent); visual (Stanfield 2005: S.4.M.1.) or point **transect** estimate (Stanfield 2005: S.4.M.2.)	NV, D
Riparian	All samples	Classification by dominant	NV, D

Feature	Application	Method	Use
Vegetation		community type (e.g., no plants, **cultivated**, **meadow**, **scrub land**, or forest) in three bands: 1.5-10 m, 10-30 m, 30-100 m (Stanfield 2005: S.1.M.3.)	
Canopy cover (%)	Streams	Estimate percent tree canopy shading over wetted area of stream **Sampling Reach**; optional visual estimate or use of instrument such as densiometer (e.g., Barbour *et al*. 1999)	Related to in-stream habitat type, thermal regime, erosion, and food sources for aquatic biota; typically recorded only for stream habitats; NV, D
Aquatic macrophytes and algae	All samples	Classification by relative areal coverage (e.g., abundant, present, or absent) plus indicate dominant type (Stanfield 2005: S.4.M.2.)	NV, D
Bank-full width (m)	Streams	Measuring tape (Stanfield 2005: S.4.M.3.)	Related to stream size and flow regime; NV, D
Instantaneous Discharge (m^3/s)	Streams	Speed-area-**transect** (using current meter), weir calculation, direct volumetric, or other method (Stanfield 2005: S.4.M.3. and S.4.M.4.)	Related to stream size and flow regime; D
Flow permanence	Streams and wetlands	Indicate permanence of flow (if known)	Related to flow regime and groundwater inputs; D

In addition to characterizing habitat with metrics, we recommend taking several photos of each site. Although the completed field sheet should provide the information needed to characterize a site, photographs can help to solve problems that arise during sample processing and data analysis. Photographing the first field sheet page is an easy way to identify a series of photos associated with a particular site. In the case of stream sites, photos should include upstream, downstream and cross-sectional views.

Several **catchment**-related habitat variables (Appendix 7) are also calculated for submitted reference sites by the OBBN coordinator, using Ontario Flow Assessment Techniques [OFAT v. 1.0] (Chang *et al*. 2002).

Ontario Benthos Biomonitoring Network Field Sheet-LAKES

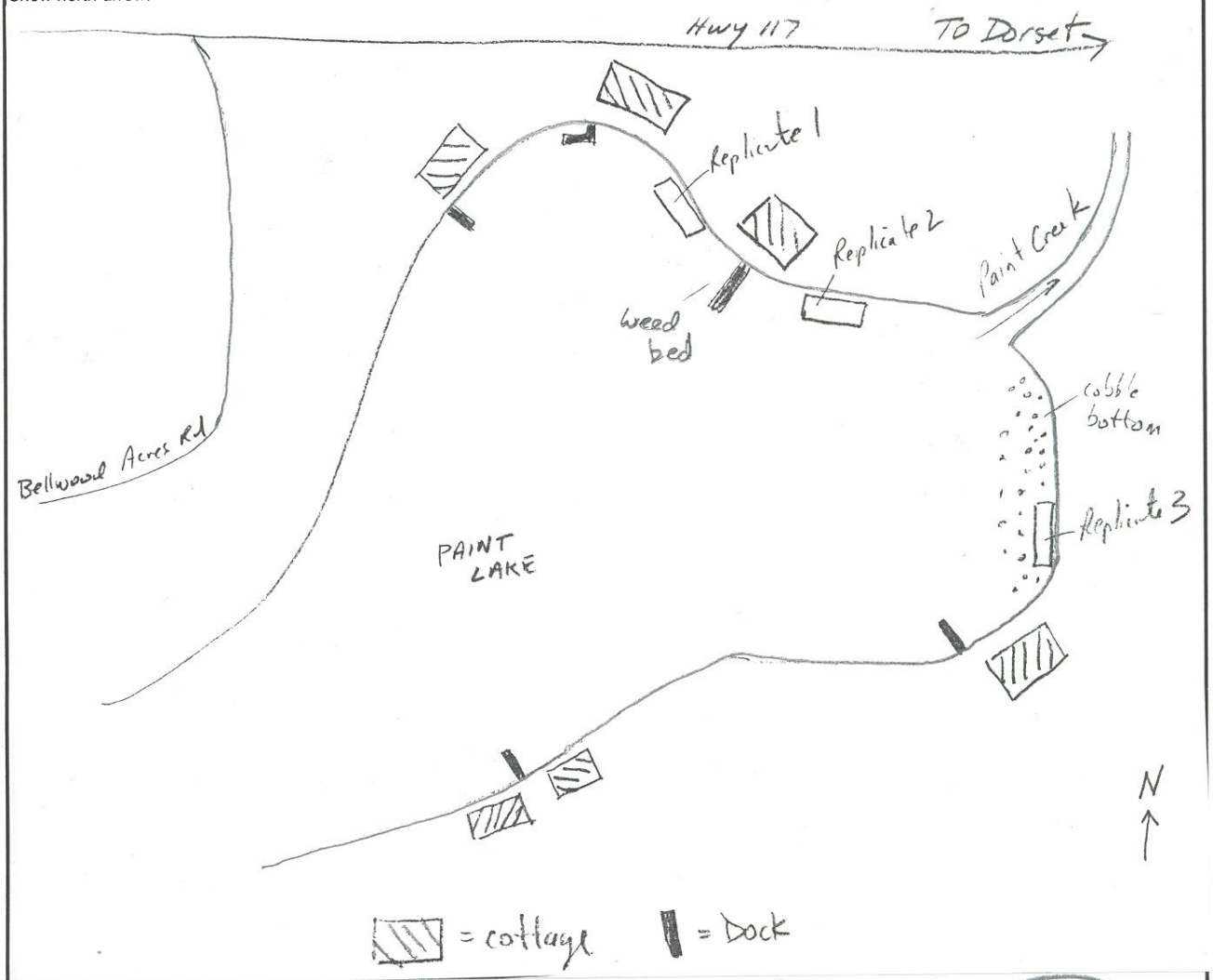


Date: 3 May 2004
Time 15:130
Agency: Ministry of Environment
Investigators: Jones, Reid

Lake Name: Paint Lake
Site #: PL1
Location: centroid of 3 replicates; Lat/Long or UTM
45° 13' 31.4" N
78° 55' 25.4" W
Elevation (m asl): 315
Datum & Zone: NAD83

Water Quality
Water Temperature (°C): 11°C
DO (mg/l): 10.77
Conductivity (uS/cm): 50
pH: 6.5
Alkalinity (mg/l as $CaCO_3$): 2

Site Description and Map
Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)
Show north arrow.



Benthos Collection Method (circle one):
• Traveling Kick & Sweep • Other (specify):

Gear Type (circle one)
• D-net • Other (*specify*):

Mesh Size: 500 micron (or specify)

Replicates	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	Replicate locations (Degrees/Minutes/Decimal seconds or specify): Latitude (N)	Longitude (W)
Sample 1	8	2.5	1	45°13'32.29"	78°55'1.31"
Sample 2	7	2.5	1		
Sample 3	15	3.5	1		

Figure 12: Completed lake field sheet example (page 1, Hypothetical data).

Substrate

Enter dominant substrate class and second dominant class for each sub-sample

	Sample 1	Sample 2	Sample 3
Dominant	3	3	5
2nd Dominant	4	4	4

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock

Substrate Notes: Plant matter and detritus quickly clogged net - contents transferred to bucket frequently

Organic Matter-Areal Coverage
Use 1: Abundant, 2: Present, 3: Absent

	Sample 1	Sample 2	Sample 3
Woody Debris	3	3	3
Detritis	1	1	2

Riparian Vegetative Community
Use: 1 (None), 2 (cultivated), 3 (meadow), 4 (scrubland), 5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (dist. From water's edge)	Sample 1	Sample 2	Sample 3
1.5-10 m	2	2	4
10-30 m	2	2	5
30-100 m	4	4	5

Aquatic Macrophytes and Algae (Use: 1 (Abundant), 2 (Present), 3 (Absent). Circle dominant type.

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent	2	2	3
Rooted Floating	3	3	3
Submergent	1	1	3
Free Floating	3	3	3

Algae	Sample 1	Sample 2	Sample 3
Floating Algae	3	3	3
Filaments	3	3	3
Attached Algae	3	3	2
Slimes or Crusts	3	3	2

Lake Morphometry (optional, will be calculated by OBBN Coordinator using OFAT)

Perimeter (m): — Volume (m^3): — Fetch (m): — Surface area (m^2): — Order: —

Notes (esp. related to land-use, habitat, obvious stressors)

Replicates 1 and 2 possibly impacted by cottage development and shoreline armouring

Candidate reference Site - Minimally Impacted? (circle one) Yes (No)

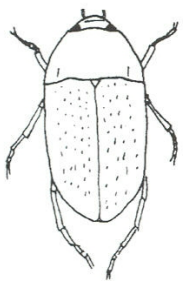
General Comments

- Several smallmouth bass observed beside rep. 2 location on gravel area
- Cottage owner requested copy of results.

Lake Sheet-Pg. 2

Figure 13: Completed lake field sheet example (page 2, hypothetical data).

Ontario Benthos Biomonitoring Network Field Sheet: STREAMS



Date: 3 May 2004
Time 13:00
Agency: Ministry of Environment
Investigators: Jones/Reid

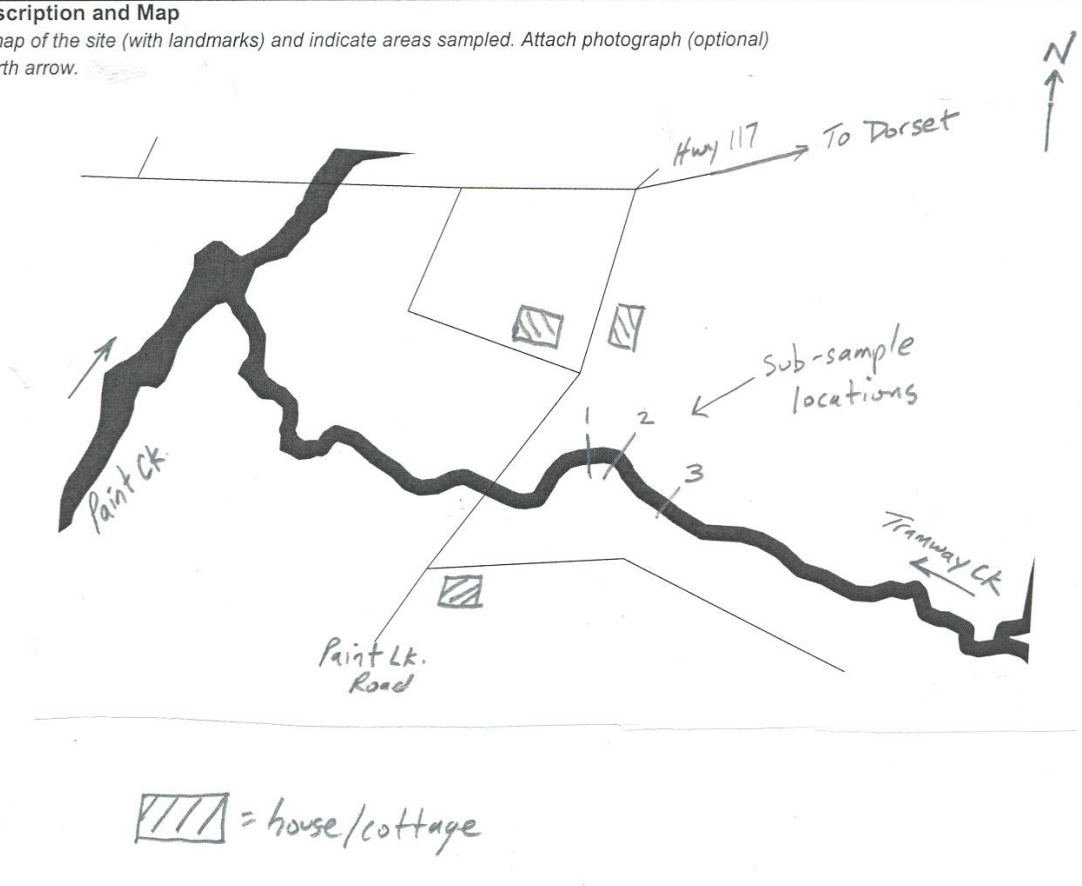
Stream name: Tramway Creek
Site #: TC1
Location: centroid of 3 replicates; Lat/Long or UTM
45°13' 32.2" N
78° 55' 11.3" W
Elevation (m asl): 317
Datum/zone: NAD83

Water Quality

Water Temperature (°C): 10°C
DO (mg/l): 10.77
Conductivity (uS/cm): 72
pH: 6.4
Alkalinity (mg/l as $CaCO_3$): 10

Site Description and Map

Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)
Show north arrow.



Benthos Collection Method (circle one):
• Traveling Kick & Sweep • Grab Sample
• Other (specify):

Gear Type (circle one)
• D-net • Ponar • Other (*specify*):
• Ekman • Rock Baskets
Mesh Size: 500 micron (or specify)

Sub-samples	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	Wetted Width (m)	Max. Hydraulic Head (mm)	# Grabs pooled per sample
Sample 1: Riffle (cross-over)	11	3	0.15	3.2	11	—
Sample 2: Pool	10	3	0.34	4.3	2	—
Sample 3: Riffle (cross-over)	10	3	0.18	3.6	14	—

Figure 14: Completed stream field sheet example (page 1, hypothetical data)

Substrate

Enter dominant substrate class and second dominant class for each sub-sample

	Sample 1	Sample 2	Sample 3
Dominant	4	4	4
2nd Dominant	5	5	5

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock

Substrate Notes

Very uniform cobble and gravel substrate throughout the reach

Organic Matter-Areal Coverage

Use 1: Abundant, 2: Present, 3: Absent

	Sample 1	Sample 2	Sample 3
Woody Debris	3	2	3
Detritis	2	1	2

Riparian Vegetative Community

Use: 1 (None), 2 (cultivated), 3 (meadow), 4 (scrubland), 5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (dist. From water's edge)	Left Bank	Right Bank (facing downstream)
1.5-10 m	6	6
10-30 m	6	6
30-100 m	6	6

% Canopy Cover (circle one)

0-24 25-49 50-74 (75-100)

If instrument used, record type: —

Aquatic Macrophytes and Algae (Use: 1 (Abundant), 2 (Present), 3 (Absent). Circle dominant type.

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent	3	3	3
Rooted Floating	3	3	3
Submergent	3	3	3
Free Floating	3	3	3

Algae	Sample 1	Sample 2	Sample 3
Floating Algae	3	3	3
Filaments	2	2	2
Attached Algae	2	2	2
Slimes or Crusts	3	3	3

Stream Size/Flow

Bank Full Width (m): ~ 6.5m Discharge (m^3/s, optional, indicate method): —

River Characterisation (circle one) (Perennial) Intermittent Unknown

Notes (esp. related to land-use, habitat, obvious stressors)

No obvious stressors

Candidate reference Site - Minimally Impacted? (circle one) (Yes) No

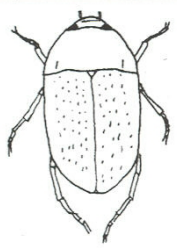
General Comments

This stream did not have a regular pool-riffle sequence as is typical of a channel defined by alluvial processes. "Riffle" transects were located in fast/shallow areas, "Pool" transect was located in a deeper, slower flowing area

Stream Sheet-Pg. 2

Figure 15: Completed stream field sheet example (page 2, hypothetical data)

Ontario Benthos Biomonitoring Network Field Sheet-WETLANDS



Date: 4 May 2004
Time 09:35
Agency: Min. of Environment
Investigators: Jones, Reid

Wetland Name: Turtle Swamp
Site #: TS1
Location (centroid of 3 replicates, use deg./min./sec. or specify other)
Latitude: 45°9'2.8" N Elevation (m asl): 328
Longitude: 78°58'25.7" W (From OBM 1:10,000)

Water Quality
Water Temperature: 12°C Conductivity (uS/cm): 30 pH: 6.4
DO (mg/l): 10.53 Alkalinity (mg/l as $CaCO_3$): 14

Site Description and Map
Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)
Show north arrow.

Benthos Collection
Method (circle one):
- Traveling Kick & Sweep
- Jab & Sweep
- Coring
- Artificial Substrate
- Other (specify):

Gear Type (circle one)
- D-net
- Corer
- Rock Baskets
- Other

Corer/Artificial Substrate specifications:

Mesh Size: 500 micron (or specify)

Replicates	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	# Pooled per replicate	location (Deg./Min./Dec.sec. Use centroid for pooled samples) Latitude (N)	Longitude (W)
Sample 1	2	—	0.35	2	45°9'6.4"	78°58'26.8"
Sample 2	2	—	0.29	2	45°9'3.7"	78°58'24.5"
Sample 3	2	—	0.45	2	45°8'59.7"	78°58'26.4"

TRAVELING KICK AND JABS ONLY

(All jab samples covered ~1m of substrate)

Figure 16: Completed wetland field sheet example (page 1, hypothetical data).

Substrate

Enter dominant substrate class and second dominant class for each sub-sample

	Sample 1	Sample 2	Sample 3
Dominant	8	8	8
2nd Dominant	3	3	3

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock
8	Organic

Organic Matter-Areal Coverage

Use 1: Abundant, 2: Present, 3: Absent and circle dominant type

	Sample 1	Sample 2	Sample 3
Woody Debris	2	1	2
Detritus	1	1	1

Substrate Notes

Riparian Vegetative Community

Use: 1 (None), 2 (cultivated), 3 (meadow), 4 (scrubland), 5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (dist. From water's edge)	Sample 1	Sample 2	Sample 3
1.5-10 m	6	6	6
10-30 m	6	6	5
30-100 m	6	6	5

Aquatic Macrophytes and Algae (Use 1: abundant, 2: Present, 3: Absent. Circle dominant type)

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent	1	1	1 (Treed)
Rooted Floating	3	3	3
Submergent	3	3	3
Free Floating	3	3	3

Algae	Sample 1	Sample 2	Sample 3
Floating Algae	3	3	3
Filaments	3	3	3
Attached Algae	3	3	3
Slimes or Crusts	3	3	3

Wetland Description (Circle)

• Marsh • Fen • Other

• Swamp • bog forested swamp

Physiographic location

• Riverine, floodplain • Coastal (lakeshore)

• Riverine, headwater • Inland

Presence of Standing Water:

• Seasonal • Unknown

• Permanent

Wetland Morphometry (optional, will be calculated by OBBN Coordinator using OFAT)

Surface area (m^2): — Perimeter (m): —

Notes (esp. related to land-use, habitat, obvious stressors)

Some habitat damage from ATV use

Candidate reference Site - Minimally Impacted? (circle one) Yes No

General Comments

ATV damage concentrated in NE corner of swamp. No evidence of impact where samples were collected. Possible reference site.

Wetland Sheet-Pg. 2

Figure 17: Completed wetland field sheet example (page 2, hypothetical example).

7.6 Assessing Biological Condition

The process of assessing biological condition is simplified in Figure 1. In reality, assessing the biological condition of a **test site** is a 4 step process:

1. Summarize biological condition using a set of metrics (7.6.1; we use the term *metric* interchangeably with the term *index*)
2. Predict a **test site** to a set of reference sites using **niche variable**s
3. Use the reference sites to establish the **normal range** of biological condition to be expected for a **test site** (7.6.2)
4. Test the hypothesis that the **test site** falls within the **normal range** (7.6.3)

7.6.1 Summarizing Biological Condition with Indices

The counts of animals in various taxonomic groups are the raw data used to characterize the biological condition of a body of water. After samples are processed and raw data are collated, a series of biological indices or metrics is calculated to summarize biological condition. In this section, we describe commonly used indices that are used to summarize biological condition.

Many different types of biological indices or metrics have been used by aquatic ecologists (e.g., Washington 1984, Plafkin *et al*. 1989, Barton and Metcalfe-Smith 1992, Gibbons *et al*. 1993, Norris and Georges 1993, Resh and Jackson 1993, Lenat and Barbour 1994, Metcalfe-Smith 1994, Barbour *et al*. 1999). Each index summarizes and emphasizes particular attributes of the raw data (Table 11). The simplest summaries are counts, such as the total number of taxonomic groups (**richness** measures) or the total number of individual organisms in a sample (abundance measures). Compositional indices are abundance measures that are calculated as portions of total counts (often percentages) and therefore emphasize relative, rather than absolute, differences between samples. Compositional indices can be recast into a parallel series of metrics by replacing taxonomic categories with functional feeding group, reproductive guild, or other ecological categories (Cummins and Wilzbach 1985): the individual taxonomic categories are thus re-grouped, and community information is summarized on a functional rather than taxonomic basis (e.g., all predators, filterers, and shredders are grouped separately). Rearranging raw data tables allows any index to be calculated for any dataset (e.g., Resh and Jackson 1993, Lenat and Barbour 1994).

Richness and abundance are key pieces of information for summarizing community composition. **Richness** and abundance information can be combined to produce diversity indices, which summarize the evenness of the abundances of collected taxa (e.g., Shannon 1948, Washington 1984, Norris and Georges 1986). Biotic indices are a family of weighted summaries that combine the known pollution tolerances of taxa with **richness** or abundance information (Washington 1984, Hilsenhoff 1987, Resh and Jackson 1993, Jones *et al.* 2002); they are typically based on empirical evidence about the tolerances of individual taxa (within some geographical area) to a specific stressor. Biotic and diversity indices have been used extensively, partly because they evaluate biological condition based on ecological theories such as the diversity/stability hypothesis (e.g., Goodman 1975, Hurlbert 1984), information theory, the river continuum concept (Vannote *et al*. 1980, Griffiths 1993, 1998, and 1999), and competitive

interaction (e.g., Shannon 1948, Washington 1984, Norris and Georges 1986). On the other hand, their use has often been criticized because underlying theories do not necessarily account for much of the variability among sites (e.g., Barton and Kilgour 1998).

Another reason for the widespread use of biotic and diversity indices is their attractiveness to lay personnel; such indices allow minimally-trained practitioners to manipulate complex community data using simple mathematical calculations; therefore, assessment can be as simple as comparing an index value against established thresholds or standards (Norris and Georges 1986, Wilhm 1972, Hellawel 1986). Remember that in an RCA, **bioassessment** is done using expected values of indices from an appropriate set of reference sites. Biotic and diversity indices are also valued because they enable spatial or temporal comparisons of biological data that are collected using different sample sizes and collection methods (Norris and Georges 1986). Of course, widespread use of any one index is limited by the need to use different tolerance values for different stressors (Klemm *et al*. 1990), and different taxa-tolerance lists for different geographic areas.

Biological community information can also be summarized with multivariate metrics, such as distance or similarity measures and ordination scores. Multivariate metrics illustrate the biological similarities or differences (distances) among a series of samples, considering all taxa and all counts simultaneously. The traditional multivariate summary is a graphical cluster analysis or ordination (e.g., Jackson 1993, Norris and Georges 1993).

Table 11: Selected indices used to characterize aquatic biological condition using benthos.

Type of Index	Example	Explanation
Simple Summaries		
Counts (Richness Measures)	Taxonomic Richness	Number of taxonomic groups found
	Number of Insect Groups	Number of insect taxonomic groups found
Enumerations (Abundance Measures)	Total Number of Individuals	Total number of invertebrates in sample
	Total Number of EPTs	Total number of mayflies, stoneflies and caddisflies
Compositional Indices	Percent Amphipods	Ratio of number of amphipods to total number of individuals
	Percent Diptera	Ratio of number of flies to total number of individuals
Diversity Indices	Shannon-Wiener Diversity	Evenness of the counts among the taxonomic groups
	Percent Dominants	Ratio of most abundant taxon to total number of individuals
Weighted Summaries		
Pollution-Tolerance Indices	Trent Biotic Index	Pollution-tolerance weighted richness
	Hilsenhoff Biotic Index	Pollution-tolerance weighted abundance
Multivariate Summaries - may be used alone or in cluster analyses or ordinations		
Pairwise Similarity Indices	Jaccard's Coefficient of Community Similarity	Degree of taxonomic similarity between two samples
	Percent Similarity	Degree of compositional similarity between two samples
Pairwise Distance Indices	Euclidean Distance	Absolute difference between two samples
	Bray-Curtis Distance	Distance complement of percent similarity (1 - PS)
Comparison to a Standard	Index of Biotic Integrity	Tolerance-weighted sum across a selection of indices
	Percent Model Affinity	Relative diffference between a sample and a target
Other Summaries - may include some or all of the above by replacing taxonomic categories		
Trophic or Functional Feeding Groups	Number of Predators	Total number of predatory taxa found
	Percent Shredders	Ratio of shredders to total number of individuals
Groups Based on Reproductive Habits	Number of Psammophils	Number of taxa reproducing on sandy bottoms
	Percent Phytophils	Ratio of individuals that reproduce on plants to total abundance

Different indices summarize different aspects of biological condition, and respond differently to different stressors; therefore, choosing an appropriate set of indices to characterize the biological community at a site is both challenging and critical.

Selecting Biological Indices

In RCA assessments, a set of indices is generally used (e.g., Plafkin *et al*. 1989), to enable detection of impairment from multiple stressors. Single index approaches are rarely used because different stressors impact the benthic community in different ways (e.g., Karr 1993, Resh and Jackson 1993). For example, a **richness** index may suggest an impact, but gives no information about the type of disturbance. A biotic index may suggest impairment from one type of disturbance, perhaps organic enrichment (e.g., Hilsenhoff 1987), but may not respond to other types of stress (e.g., habitat degradation, water level manipulation). When several indices are calculated, the pattern of "hits and misses" is a fingerprint that implicates particular stressors (Barton and Metcalfe-Smith 1992, Barton 1996, Fore *et al*. 1996). A list of common stressors and expected index responses for a given region can assist in choosing relevant indices (Barbour *et al*. 1996) and diagnosing impairment types. Unfortunately such lists are generally unavailable (see Appendix 8; if supported by data, we intend to add an appendix on **diagnostic** response signatures in future).

Numerous studies, particularly in the U.S., have attempted to identify optimal sets of indices for **Biomonitoring** programs**.** As a result, the US EPA rapid **bioassessment** protocol (Barbour *et al*. 1999) recommends sets of "best candidate benthic metrics" and "potential benthic metrics" for the U.S. (Table 12). A list of 10 indices is proposed in the BioMAP protocol (Griffiths 1993), including qualitative and quantitative biotic indices (the WQI_q and WQI_d indices), the total number of individuals, the ratio of chironomids to insects, the percent oligochaetes, and characterizing taxa (indicator species: taxa that are abundant and have well known habitat and water quality preferences).

Table 12: Summary biological indices recommended for the U.S. (from Barbour *et al*. 1999)

Category	Recommended Metrics
"Best Candidate Benthic Metrics"	**Richness**, EPT **richness**, Ephemeroptera **richness**, Plecoptera **richness**, Trichoptera **richness**, %EPT, % Ephemeroptera, intolerant taxa **richness**, % tolerant organisms, % dominant taxon, % filterers, % grazers and scrapers, number of clinger taxa, % clingers
"Potential Benthic Metrics"	Number of *Pteronarcys* species, number of Diptera taxa, number of Chironomidae taxa, % Plecoptera, % Trichoptera, % Diptera, % Chironomidae, % Tanytarsini, % other Diptera and non-insects, % *Corbicula*, % oligochaetes, number of sensitive snail and mussel species, % sediment tolerant organisms, Hilsenhoff Biotic Index, Florida Index, % Hydropsychidae to Trichoptera, % omnivores and scavengers, % gatherers and filterers, % predators, % shredders, % multivoltine, % univoltine

Comparative studies have shown that the information content of different groups of indices depends on factors like **test site** location and disturbance type. For example, Barbour *et al*. (1992) examined the U.S. EPA's recommended metrics and advocated the use of taxonomic **richness**, the EPT index (proportion of mayflies, stoneflies and caddisflies combined), the Hilsenhoff Index, and percent shredders, but considered the ratio of scrapers to filtering collectors, the ratio of EPTs to chironomid abundances, the percent dominants, and the Community Similarity Index too variable to be useful. Furthermore, Hannaford and Resh (1995) evaluated the US EPA metrics in a northern California stream and reached similar conclusions, although they retained 2 of the 4 metrics that Barbour's group rejected. In another example, Barton and Metcalfe-Smith (1992) evaluated 8 biological indices and recommended taxonomic **richness**, a modified Hilsenhoff Index, and percent oligochaetes, because of their ability to discriminate sites with known degradation. Similarly, an evaluation of 17 indices using MOE rapid **bioassessment** data from 5 south-central Ontario lakes indicated that percent amphipods, percent insects, and a multivariate metric best distinguished the 5 lakes (David *et al*. 1998).

A comparison of the sensitivity of summary benthic community indices to the impacts of mines, pulp and paper mills, and urbanization (Kilgour *et al*. 2004) provides additional justification for the use of multiple indices in **biomonitoring** surveys. Their analyses showed that estimates of effect size varied by index, by stressor, and by sampler type. They found some indices more sensitive to certain types of disturbances, and found that certain collection methods are more likely to demonstrate the effects of specific impacts than other methods. In the case of the Yamaska River urbanization data in Figure 18, the artificial **substrate**s suggested relatively little impact, with only one index, multivariate CA axis 2, being >2 standard deviations from the upstream control site mean. Surber samples demonstrated a much larger biological response to urbanization, with 4 indices (**Richness**, Hilsenhoff index, BioMAP WQI_d index, and Correspondence Analysis Axis 1) showing effect sizes of greater than 2 standard deviations, and two indices showing effect sizes >4 standard deviations.

The above examples show that in most cases we won't know *a priori* what the best set of summary metrics is for a given **test site**. For this reason, we recommend using a large set of metrics in order to contribute as much information as possible to assessments. Well known metrics such as taxonomic **richness**, percent oligochaeta, percent EPT, percent Chironomidae, percent Insecta, and percent dominant taxa should be used as a minimum. Biotic indices (weighted summaries), multivariate summaries, and various proportional indices (e.g., feeding guild indices) should also be used, although their use will require supplementary information on pollution tolerances (e.g., Bode *et al*. 1990, Klemm *et al*. 1990), feeding behavior (e.g., Cummins and Wilzbach 1985), and some familiarity with advanced statistical methods (e.g., Norris and Georges 1993).

Ultimately, the OBBN database will contain a metrics selection tool that suggests a suite of metrics based on test site location and likely stressors, and ensures the ratio of metrics to number of reference sites used is appropriate. The database will also calculate the values for all selected abundance, **richness**, diversity, biotic, proportional, and multivariate metrics.

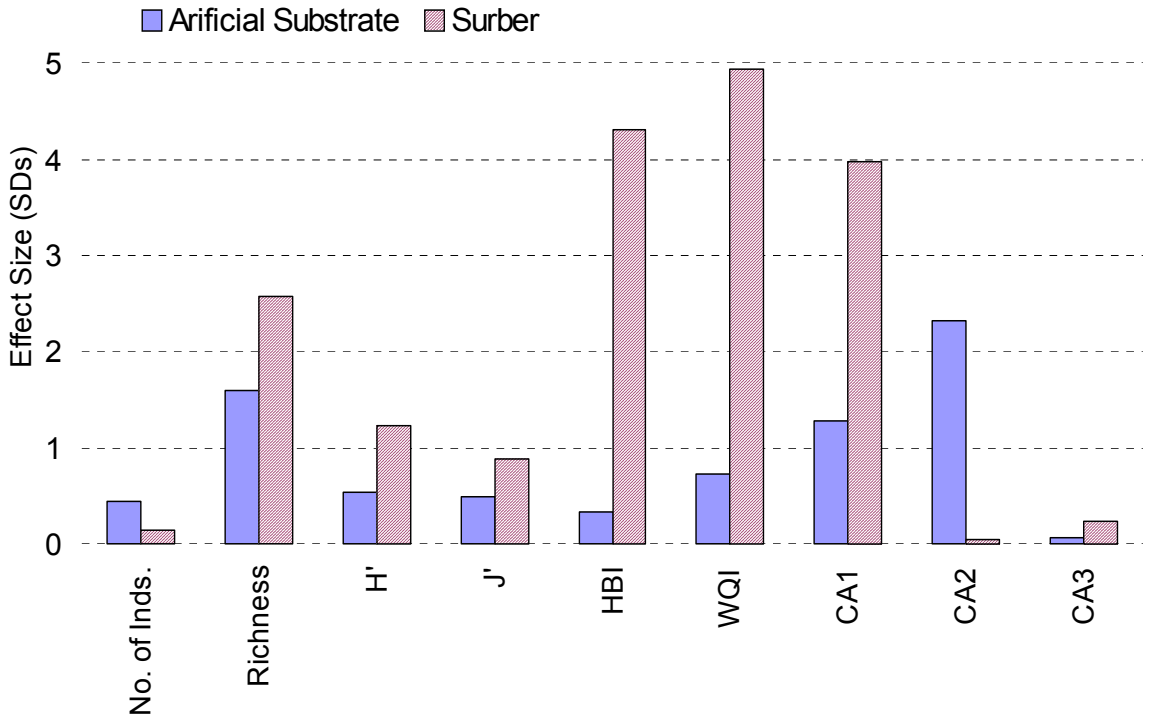


Figure 18: Biological-summary-index responses to urbanization along the Yamaska River, Quebec, using Surber Sampler and artificial substrate collection methods. Effect sizes were rescaled as standard deviations from the upstream control site mean (Kilgour *et al.* 2004).

7.6.2 Biocriteria: Establishing the Normal Range

Just as there are no universal criteria for defining *minimally* ***impacted***, there are no universal guidelines on how many reference sites are required to characterize the **normal range**. We do know that a single control site is unacceptable (Wright *et al*. 1984, Reynoldson *et al*. 1999, Hawkins *et al*. 2000, Bailey *et al.* 2004). Biological community composition at minimally **impacted** sites is a manifestation of both deterministic and stochastic mechanisms that result in considerable natural variability; a single site fails to account for this variability and is unable to establish the expected natural range of biological condition on its own. It is possible for natural communities at two areas to diverge or converge over time in the absence of any environmental impact (Underwood 1991), which would potentially obscure biotic responses to anthropogenic stress. A single reference area also is prone to confounding (Underwood 1993, Resh 1995, Megraw *et al.* 1997), limits our capacity for extrapolation to other sites, and limits our ability to calculate natural variability (Reynoldson *et al*. 1997). Research will be required to determine how best to classify or otherwise select reference sites for **bioassessment**s. A key question is, how many reference sites are required to adequately describe the **normal range**? Another is, which **niche variable**s best discriminate between reference communities (Appendix 8). Several authors have suggested a minimum of 10 reference sites are required to define a reference site group and thereby define the **normal range** (e.g., David *et al*. 1998, Reynoldson *et al*. 2003). Although preliminary studies with simulated data (Bowman and Somers 2005) showed that

many more sites (i.e. 20-50 sites) from a population must be sampled before the mean ($\bar{x}_{ref}$) and standard deviation (SD) can be estimated with acceptable confidence.

Biocriteria, in the form of critical index values, are easily derived from reference site data; however, the decision of where to set an impairment threshold requires consideration of the trade-off between Type I (false positive) and Type II (false negative) error rates associated with the test of the null hypothesis (i.e., H_0: test site is normal [in reference condition]). The consequences of making such errors should also be considered. Paraphrasing Bailey et al. (2004), if environmental protection is a primary concern, we should err on the side of failing sites that may not be damaged; we can justify this approach because the consequences of damage can be serious and costly, and because management activities are likely to include review of the data and more detailed assessment before intervention, and those steps are likely to be inexpensive compared to rehabilitation. If the assessment is principally aimed at guiding mitigation, we may want to err on the side of passing atypical sites because of the high cost of rehabilitation. Because of these considerations, there is no standard definition of the **normal range**, and critical values have been set differently by different authors. For example, as their pass/fail threshold, some authors (e.g., Bailey et al. 1998, Linke et al. 1999, Barbour et al. 1999, Gerritsen et al. 2000) have used "statistically liberal (but environmentally conservative)" (Bailey et al. 2004) criteria, set as the 25th percentile of reference site variability (α=0.25; 25% of reference sites would be mistakenly identified as atypical). In the U.K., the decision threshold for the RIVPACS O:E score is the 5th percentile from reference sites (Clarke 2000). Reynoldson's BEAST method (Reynoldson *et al*. 1995, 2000, Bailey *et al*. 2004) fails sites that fall outside the ordinated reference sites' 90% probability ellipse.

We define the **normal range** (for any given biological index), as the range of values that includes 95% of the data from the regional reference sites (e.g., Thompson 1938, Leffler 1978, Kersting 1984, 1988, and Kilgour *et al*. 1998). For any normally distributed variable that is standardized to have a mean of zero and a variance of one, the area under the normal curve that is bounded by the mean ± 1 standard deviation will enclose approximately 68% of the data points (see Figure 19). The area under the normal curve bounded by the mean ± 2 standard deviations encloses 95% of the data points. Thus, the mean ± 2 standard deviations provides a logical (albeit arbitrary) definition of the **normal range** of variation for any normally distributed biological index from a set of reference sites. The remaining 5% of the values lying outside this range are unusual or atypical relative to the majority of the values.

Given a normally-distributed variable, the mean ± 3 standard deviations encloses 99.9% of the data points. We consider index values lying beyond 3 standard deviations from the mean to be *extreme* because they occur at only 1 in 1000 randomly selected reference sites. Using this construct, we recognize that 5 out of every 100 reference sites will be incorrectly designated as atypical (i.e., will fall outside of the **normal range** by chance) and that 1 in 1000 reference sites will be incorrectly identified as extreme. Although there is a low probability of identifying a healthy site as unusual, defining unusual values on the basis of the **normal range** aids interpretation: it provides a standard screening threshold (biocriterion) that is associated with an expected error rate.

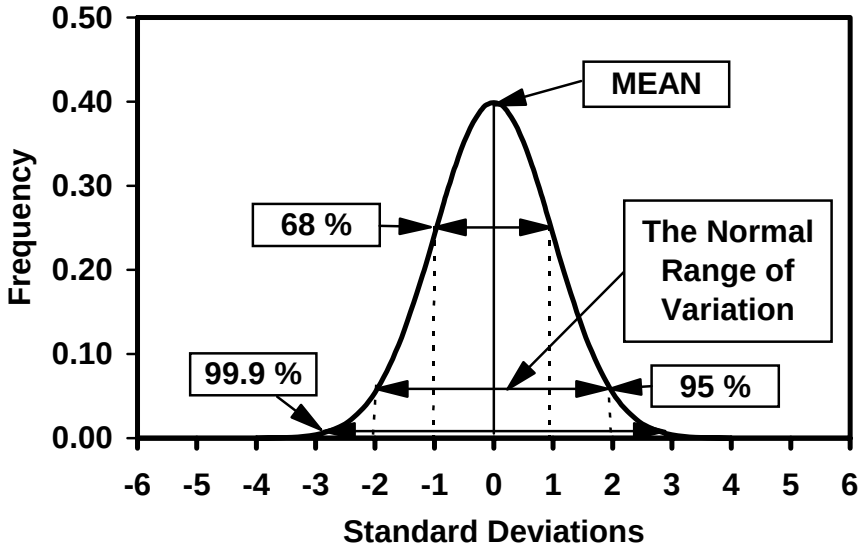


Figure 19: A standard normal curve (mean=0, variance=1) showing the normal range, which encloses 95% of the data points from reference sites and is bounded by the mean +/- 2 standard deviations.

(NB: Our definition of the **normal range** and significant ecological effect is subject to evaluation [see Appendix 8]; it is provided as a guideline for **bioassessment**, however pass/fail criteria for test statistics can be tailored to study designs. OBBN analytical software will allow users to specify α-levels for tests)

7.6.3 Hypothesis Testing: Does the Test Site Lie Within the Normal Range?

Judging impairment is a relatively simple task when only one measure is used and appropriate standards exist, but how do we assess impairment when several indices are used and no standards exist? Below are examples of two different assessment approaches. The first example is non-statistical, and shows the dangers of not recognizing redundancies in information provided by summary indices. The second example, from Bowman *et al.* (2003), describes Test Site Analysis (TSA), our recommended statistical procedure for OBBN assessments.

A Non-statistical Bioassessment Approach (from David *et al*. 1998)

Consider that triplicate samples were collected from three **test site**s, at the same time of the year as 10 minimally **impacted** reference sites were sampled (for this example, we will assume that reference sites were matched to the test site using appropriate habitat variables). All sites were sampled using the standard Transect Kick Method (section 7.5.1). Samples were processed as per section 7.5.5, and a suite of indices were calculated to summarize biological condition. Figure 20 gives a map of the hypothetical test and reference sites referred to in this example.

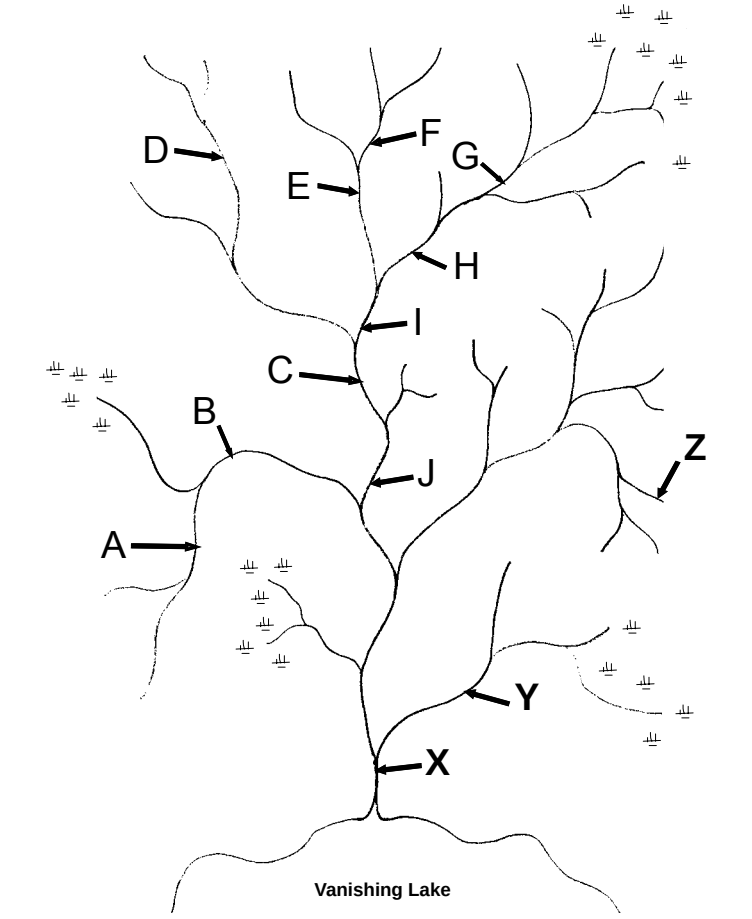


Figure 20: Hypothetical stream system with reference sites (A-G) and test sites (X-Z).

Raw taxa abundance information for the hypothetical test streams is given in Table 13.

Because some within-site variation was expected, the 3 **replicate** samples at each **test site** were averaged to produce a mean abundance for each taxonomic group, as shown in Table 14. A subsequent QA/QC check revealed that participants processing the samples often confused oligochaetes and nematodes, so these 2 groups were combined under the heading of "worms" in the final edited data table.

The mean values from Table 14 were then used to calculate a suite of indices to summarize biological condition at each **test site**. To simplify this example, just 6 biological indices were calculated: number of groups, % EPTs, % worms, % dominants, % diptera, and % insects.

Table 13: Raw taxa abundance data for three hypothetical stream test sites.

Taxonomic Group	Hypothetical "Test" Streams								
	X			Y			Z		
	1	2	3	1	2	3	1	2	3
Coelenterata	0	0	0	0	0	0	0	0	0
Turbellaria	0	0	0	0	0	0	0	0	0
Nematoda	0	0	1	3	0	0	0	0	1
Oligochaeta	21	53	35	24	11	3	0	0	0
Hirudinea	0	0	0	1	0	0	0	0	0
Isopoda	0	0	0	1	0	0	0	0	0
Amphipoda	3	1	11	1	0	1	0	0	0
Decapoda	0	0	0	0	0	1	0	0	0
Hydracarina	0	0	0	0	0	1	0	1	1
Ephemeroptera	0	0	0	1	2	0	34	28	31
Anisoptera	0	1	0	0	0	0	0	0	0
Zygoptera	0	0	0	0	0	0	0	0	0
Plecoptera	0	0	0	2	0	0	12	4	11
Hemiptera	3	1	0	0	0	0	0	0	0
Megaloptera	0	0	0	0	0	0	1	3	1
Trichoptera	1	2	0	13	26	32	20	19	11
Lepidoptera	0	0	0	0	0	0	0	0	0
Coleoptera	0	1	1	10	12	13	35	19	33
Chironomidae	10	14	11	33	45	43	2	10	11
Tabanidae	2	1	1	0	0	0	0	0	0
Culicidae	0	0	3	3	5	0	0	3	1
Ceratopogonidae	0	0	0	0	0	0	3	3	0
Tipulidae	0	0	0	0	0	0	1	0	1
Simuliidae	0	0	0	0	0	0	0	0	0
Gastropoda	40	22	30	3	8	3	0	2	1
Pelecypoda	13	7	5	3	5	5	1	1	1
Total Count	93	103	98	98	114	102	109	93	104

Once raw data were summarized for **test site**s, the reference data were summarized in the same manner (Table 15). A table of critical (expected) values that characterize "normal" was then established as a series of percentiles defining atypical and extreme threshold values for each index (Table 16).

Table 14: Summarized taxa abundance data for a hypothetical stream test site.

Combined Groups	Hypothetical "Test" Streams		
	X	Y	Z
	Mean	Mean	Mean
Worms	36.3	12.7	0.0
Leeches	0.0	0.3	0.0
Sowbugs	0.0	0.3	0.0
Scuds	5.0	0.7	0.0
Crayfish	0.0	0.3	0.0
Mites	0.0	0.3	0.7
Mayflies	0.0	1.0	31.0
Dragonflies	0.3	0.0	0.0
Damselflies	0.0	0.0	0.0
Stoneflies	0.0	0.7	9.0
Bugs	1.3	0.0	0.0
Alderflies	0.0	0.0	1.7
Caddisflies	1.0	23.7	16.7
Beetles	0.7	11.7	29.0
Diptera	14.0	43.0	11.7
Snails	30.7	4.7	1.0
Clams	8.3	4.3	1.0
Total Count	97.7	103.7	101.7

There are known differences in the way the six chosen indices respond to human disturbance and these response patterns had to be taken into account when defining the **normal range**s shown in Table 16. In the case of Taxa R**ichness** and % EPTs, large values imply a healthy biological community and low values imply reduced health. As a result, the critical percentiles for these metrics were set at the low end of the scale (i.e., extreme below the 0.1 percentile and atypical between the 0.1 and 5th percentiles). Because of the limited range of values for the Number of Groups index, the 5th percentile (i.e., 13 groups) and 0.1 percentile were the same value; consequently, an intermediate (Atypical) range could not be distinguished. The critical values for Percent EPTs are 9% (i.e., the 5th percentile) for Atypical and 7% (i.e., the 0.1 percentile) for Extreme, since low values tend to indicate stress. For the small gravelly streams in our example, large values for Percent Worms and Percent Dominants indicate degraded biological condition, and small values reflect a healthy community. Thus, critical values for these 2 metrics lie at the high end of the scale at the 95th and 99.9th percentiles; therefore, healthy sites should support less than 9% worms and the most abundant taxonomic group should comprise less than 45% of the total number of animals.

Table 15: Data processing steps for characterizing biological condition at reference sites.

DATA FROM BENCH SHEETS

Taxonomic Group	Hypothetical 10 Reference Area Streams																														Mean Value
	A			B			C			D			E			F			G			H			I			J			
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Coelenterata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Turbellaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Nematoda	0	2	1	2	3	1	0	0	0	1	0	2	3	1	0	1	0	1	0	1	0	0	3	0	0	3	1	1	2	3	1.1
Oligochaeta	2	2	1	5	6	4	1	1	3	2	0	2	6	1	1	4	2	1	2	0	0	1	4	3	1	0	1	3	8	5	2.4
Hirudinea	1	0	2	0	0	1	0	2	0	0	1	0	2	0	3	3	5	2	2	3	1	2	2	4	2	1	0	0	0	0	1.3
Isopoda	3	4	2	0	0	0	4	8	2	0	0	0	2	2	1	6	8	5	5	3	5	3	7	8	2	0	0	5	11	9	3.5
Amphipoda	0	0	0	15	11	8	0	0	0	6	3	3	0	0	3	0	0	0	0	4	0	0	0	0	0	1	0	0	0	1	1.8
Decapoda	1	0	1	0	0	2	3	1	3	2	0	1	0	2	1	0	1	1	0	0	1	1	0	1	1	2	0	2	1	0	0.9
Hydracarina	0	2	3	2	0	4	6	2	1	5	8	3	2	0	0	3	6	2	0	0	1	3	1	6	3	4	6	6	4	0	2.8
Ephemeroptera	12	15	16	6	8	11	19	13	7	12	21	14	6	16	7	8	4	11	14	18	16	11	3	4	11	7	13	21	17	28	12.3
Anisoptera	0	1	0	0	1	0	2	0	2	0	1	0	0	1	0	1	0	2	0	0	2	0	1	0	0	0	1	1	0	1	0.6
Zygoptera	2	1	1	0	1	2	2	2	1	0	1	0	2	3	1	0	1	0	0	0	0	1	0	1	1	0	0	0	0	1	0.8
Plecoptera	11	7	9	3	3	8	17	8	6	15	17	10	2	16	14	9	6	13	14	17	21	9	4	6	15	12	19	5	7	11	10.5
Hemiptera	2	1	4	0	8	5	0	0	2	0	4	0	0	3	2	1	0	1	0	1	1	4	1	0	4	9	3	3	1	1	2.0
Megaloptera	0	1	3	0	1	2	2	4	0	3	5	2	0	3	2	0	0	3	1	2	3	1	0	0	3	3	5	1	1	0	1.7
Trichoptera	10	7	5	1	0	5	13	12	16	3	17	2	0	3	9	2	2	11	7	11	14	8	0	2	12	8	4	3	6	0	6.4
Lepidoptera	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Coleoptera	4	5	2	3	3	0	2	0	1	3	4	6	5	0	3	3	0	6	2	0	6	2	3	0	2	0	0	5	3	0	2.4
Chironomidae	32	41	29	26	19	23	24	31	28	35	22	36	34	27	27	39	27	44	23	17	18	19	36	22	12	26	19	34	22	27	27.3
Tabanidae	0	1	0	6	1	0	2	0	1	0	0	0	2	0	0	3	0	0	2	1	0	1	3	0	0	0	0	1	0	0	0.8
Culicidae	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0	1	0	1	1	0.3
Ceratopogonidae	2	3	2	4	0	6	0	3	2	4	3	6	1	0	0	1	6	2	2	3	2	2	1	4	2	6	3	5	4	9	2.9
Tipulidae	0	0	0	2	7	3	0	0	0	1	0	0	0	0	0	3	0	0	0	0	0	1	3	0	0	0	0	0	1	0	0.7
Simuliidae	0	0	0	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0.2
Gastropoda	14	8	11	18	24	15	12	7	18	13	5	7	21	10	13	13	18	4	11	12	12	23	22	26	9	6	11	1	6	3	12.4
Pelecypoda	7	0	6	2	4	6	6	7	2	4	11	10	7	6	11	5	9	1	14	8	3	11	4	9	11	15	9	0	1	1	6.3
Total Count	103	101	98	96	100	106	119	101	95	110	125	104	95	94	98	107	95	110	99	101	106	104	100	96	92	103	96	97	96	101	101.6

EDITED DATA

Combined Groups	A			B			C			D			E			F			G			H			I			J			Mean Value
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Worms	2	4	2	7	9	5	1	1	3	3	0	4	9	2	1	5	2	2	2	1	0	1	7	3	1	3	2	4	10	8	3.5
Leeches	1	0	2	0	0	1	0	2	0	0	1	0	2	0	3	3	5	2	2	3	1	2	2	4	2	1	0	0	0	0	1.3
Sowbugs	3	4	2	0	0	0	4	8	2	0	0	0	2	2	1	6	8	5	5	3	5	3	7	8	2	0	0	5	11	9	3.5
Scuds	0	0	0	15	11	8	0	0	0	6	3	3	0	0	3	0	0	0	0	4	0	0	0	0	0	1	0	0	0	1	1.8
Crayfish	1	0	1	0	0	2	3	1	3	2	0	1	0	2	1	0	1	1	0	0	1	1	0	1	1	2	0	2	1	0	0.9
Mites	0	2	3	2	0	4	6	2	1	5	8	3	2	0	0	3	6	2	0	0	1	3	1	6	3	4	6	6	4	0	2.8
Mayflies	12	15	16	6	8	11	19	13	7	12	21	14	6	16	7	8	4	11	14	18	16	11	3	4	11	7	13	21	17	28	12.3
Dragonflies	0	1	0	0	1	0	2	0	2	0	1	0	0	1	0	1	0	2	0	0	2	0	1	0	0	0	1	1	0	1	0.6
Damselflies	2	1	1	0	1	2	2	2	1	0	1	0	2	3	1	0	1	0	0	0	0	1	0	1	1	0	0	0	0	1	0.8
Stoneflies	11	7	9	3	3	8	17	8	6	15	17	10	2	16	14	9	6	13	14	17	21	9	4	6	15	12	19	5	7	11	10.5
Bugs	2	1	4	0	8	5	0	0	2	0	4	0	0	3	2	1	0	1	0	1	1	4	1	0	4	9	3	3	1	1	2.0
Alderflies	0	1	3	0	1	2	2	4	0	3	5	2	0	3	2	0	0	3	1	2	3	1	0	0	3	3	5	1	1	0	1.7
Caddisflies	10	7	5	1	0	5	13	12	16	3	17	2	0	3	9	2	2	11	7	11	14	8	0	2	12	8	4	3	6	0	6.4
Beetles	4	5	2	3	3	0	2	0	1	3	4	6	5	0	3	3	0	6	2	0	6	2	3	0	2	0	0	5	3	0	2.4
Diptera	34	45	31	39	27	32	30	34	31	41	26	42	37	27	27	48	33	46	27	21	20	24	45	26	15	32	23	40	28	37	32.3
Snails	14	8	11	18	24	15	12	7	18	13	5	7	21	10	13	13	18	4	11	12	12	23	22	26	9	6	11	1	6	3	12.4
Clams	7	0	6	2	4	6	6	7	2	4	11	10	7	6	11	5	9	1	14	8	3	11	4	9	11	15	9	0	1	1	6.3
Total Count	103	101	98	96	100	106	119	101	95	110	124	104	95	94	98	107	95	110	99	101	106	104	100	96	92	103	96	97	96	101	101.6

EXAMPLE BIOLOGICAL INDICES

Metric	A			B			C			D			E			F			G			H			I			J			Mean Value
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Number of Groups	14	16	17	15	15	17	17	14	16	16	17	14	14	14	15	18	13	17	13	14	15	19	17	13	17	14	14	16	17	14	15.4
EPTs (%)	32	29	31	10	11	23	41	33	31	27	44	25	8	37	31	18	13	32	35	46	48	27	7	13	41	26	38	30	31	39	28.5
Worms (%)	2	4	2	7	9	5	1	1	3	3	0	4	9	2	1	5	2	2	2	1	0	1	7	3	1	3	2	4	10	8	3.5
Dominants (%)	33	45	32	41	27	30	25	34	33	37	21	40	39	29	28	45	35	42	27	21	20	23	45	27	16	31	24	41	29	37	31.8
Diptera (%)	33	45	32	41	27	30	25	34	33	37	21	40	39	29	28	45	35	42	27	21	19	23	45	27	16	31	24	41	29	37	31.8
Insects (%)	73	82	72	54	52	61	73	72	69	70	77	73	55	77	66	67	48	85	66	69	78	58	57	41	68	69	71	81	66	78	67.7

Sites having 9-10% worms should be considered atypical, and sites with more than 10% worms and more than 45% dominants should be considered extreme. For % Diptera and % Insects, thresholds for atypical and extreme occur at both high and low ends of the reference stream distribution because both high and low values indicate an unhealthy community, and

intermediate values imply a healthy community. To define critical values for these indices, we used two-tailed criteria such that the **normal range** fell between the 2.5 and 97.5 percentiles. Atypical values lie outside these 2 percentiles, whereas extreme values fall outside the 0.1 and 99.9 percentiles (actually 0.05 and 99.95 given rounding errors such that 0.1% of the expected values lie outside of this range).

Table 16: Reference values for six compositional indices, based on data from ten hypothetical reference streams.

Metric	Percentiles using the 30 Samples from the Reference-Area Streams												
	0.1	1	2.5	5	10	25	50	75	90	95	97.5	99	99.9
Number of Groups [1]	13	13	13	13	14	14	15	17	17	18	18	19	19
% EPT	7	7	8	9	11	23	31	37	42	45	46	47	48
% Worms	0	0	0	0	1	1	2	5	8	9	10	10	10
% Dominants	16	17	19	20	21	27	31	39	42	45	45	45	45
% Diptera	16	17	18	20	21	27	31	39	42	45	45	45	45
% Insects	41	43	46	50	54	62	69	73	79	82	83	84	84

Legend:	Colour and Interpretation	Action to be Taken
Green	Normal Range of Variation - Unimpacted	No further action necessary
Yellow	Atypical Value beyond the Normal Range	Repeat assessment and continue to monitor
Red	Extreme Value - Potentially Impacted	Repeat assessment - follow-up suggested

Care must be taken when calculating the number of groups[1] (or **richness**) in this analysis. By taking average counts for each test stream and then calculating **richness**, you actually base **richness** on a 300-count. If each of the reference site 100-count samples is used to calculate "normal", then test site **richness** should also be based on 100-count samples (i.e., **richness** for the test site should be calculated for each 100-count sub-sample and then averaged).

Having specified the regional reference values for the 6 metrics, individual indices were evaluated in relation to the **normal range** as shown in Table 17.

A different assessment pattern emerged for site Y because four indices fell within the **normal range** established by the regional reference sites; only **richness** (Number of Groups) was atypical and % Worms was extreme. Site Z was intermediate to sites X and Y because three indices (% EPTs, % Worms and % Dominants) fell within the **normal range** of variation, and three indices (Number of Groups, % Dipterans, and % Insects) were beyond the extreme threshold. Results from Sites Y and Z demonstrate that each of the different indices may classify a site somewhat differently. This does not mean that some indices are right and others are wrong. Rather, it means that taxa exhibit a range of responses to each type of stressor. Thus the impacts of different stressors will be manifested as different patterns of index passes and failures.

Table 17: Single index comparisons of 3 hypothetical test sites (X, Y, and Z) in relation to the normal range of biological condition defined by reference sites. Unshaded index values fall within the normal range for a given index, lightly shaded values are considered atypical, while darkly shaded values are considered extreme.

SUMMARIZED DATA			
Combined Groups	**Hypothetical "Test" Streams**		
	X	**Y**	**Z**
	Mean	**Mean**	**Mean**
Worms	36.3	12.7	0.0
Leeches	0.0	0.3	0.0
Sowbugs	0.0	0.3	0.0
Scuds	5.0	0.7	0.0
Crayfish	0.0	0.3	0.0
Mites	0.0	0.3	0.7
Mayflies	0.0	1.0	31.0
Dragonflies	0.3	0.0	0.0
Damselflies	0.0	0.0	0.0
Stoneflies	0.0	0.7	9.0
Bugs	1.3	0.0	0.0
Alderflies	0.0	0.0	1.7
Caddisflies	1.0	23.7	16.7
Beetles	0.7	11.7	29.0
Diptera	14.0	43.0	11.7
Snails	30.7	4.7	1.0
Clams	8.3	4.3	1.0
Total Count	97.7	103.7	101.7
SELECTED METRICS AND SITE STATUS			
Metric	**X**	**Y**	**Z**
Number of Groups	**9**	**13**	**9**
EPTs (%)	**1**	**24**	**56**
Worms (%)	**37**	**12**	**0**
Dominants (%)	**37**	**41**	**30**
Diptera (%)	**14**	**41**	**11**
Insects (%)	**18**	**77**	**97**

Simple single-index comparisons allow us to make some inferences about the condition of our **test site**s. At site X, values for 5 of the 6 metrics were extreme (as shown in Table 17) and Percent Dominants was the only index value that fell within the **normal range**. Here 5 of 6 metrics give the same result.

Single-index comparisons to regional reference values suggested that all 3 **test site**s were unusual to some degree: 5 of 6 indices at site X were extreme, half of the indices at Z were extreme, and Y had 1 extreme value and 1 atypical value out of the 6 indices. One might be tempted to rank the 3 **test site**s in order of biological health from site X as poor, site Z also poor but better than X, and site Y as better than X and Z, but not within the **normal range**.

We caution against this simplistic 1-index-at-a-time approach because each biological index responds to different stressors in different ways. Moreover, some metrics may be correlated with certain stressors; a pass or fail on 1 metric is not equal to a pass or fail on a different index, and a fail for one index may be redundant with the fail of another. The number of passes or fails should not be used to compare sites. Returning to the specifics of the hypothetical data, the benthic community at site X was simulated to resemble a community from a site **impacted** by urban development. The benthic community at site Y was simulated to resemble a site modestly **impacted** by agricultural runoff. The macroinvertebrate community at site Z was chosen to represent a cold headwater stream. Thus, the benthic community at site Z was truly atypical of the regional reference sites, not because of human impact, but because of an inappropriate reference group (reference sites should have been cold-water streams). This example highlights two concepts discussed earlier: (1) regional reference sites must be appropriate for the **test site**s, and (2) a cumulative total based on the number of passes or failures is not appropriate because the pattern of passes and fails will be different for different sets of indices.

Test Site Analysis (Bowman et al. 2003, Appendix 9)

A more rigorous approach to assessments is clearly needed to deal with the problem of judging impairment (i.e., concluding an overall pass or fail) when several indices are used. We propose the following two-step approach:

1. Use a non-central multivariate test to determine if the **test site** lies outside of the **normal range;** here we test the null hypothesis, H_0: $\left| D_{test} - D_{reference\ mean} \right| \leq$ critical effect size (in effect, we are testing whether the test site lies outside the 95th percentile of the distances between each reference site and the reference-site-group centroid). The default critical effect size for the OBBN is the 95th percentile of the reference-site distances, but this criterion can be modified to suit study objectives.
2. Use a discriminant analysis to determine which indices best distinguish the **test site** from the reference condition.

Below we work through a hypothetical example that shows how our recommended approach is applied.

Data from 15 reference sites are plotted in Figure 21A. Only two indices are shown: the total number of chironomids, and the total number of mayflies, stoneflies and caddisflies combined (i.e., EPTs).

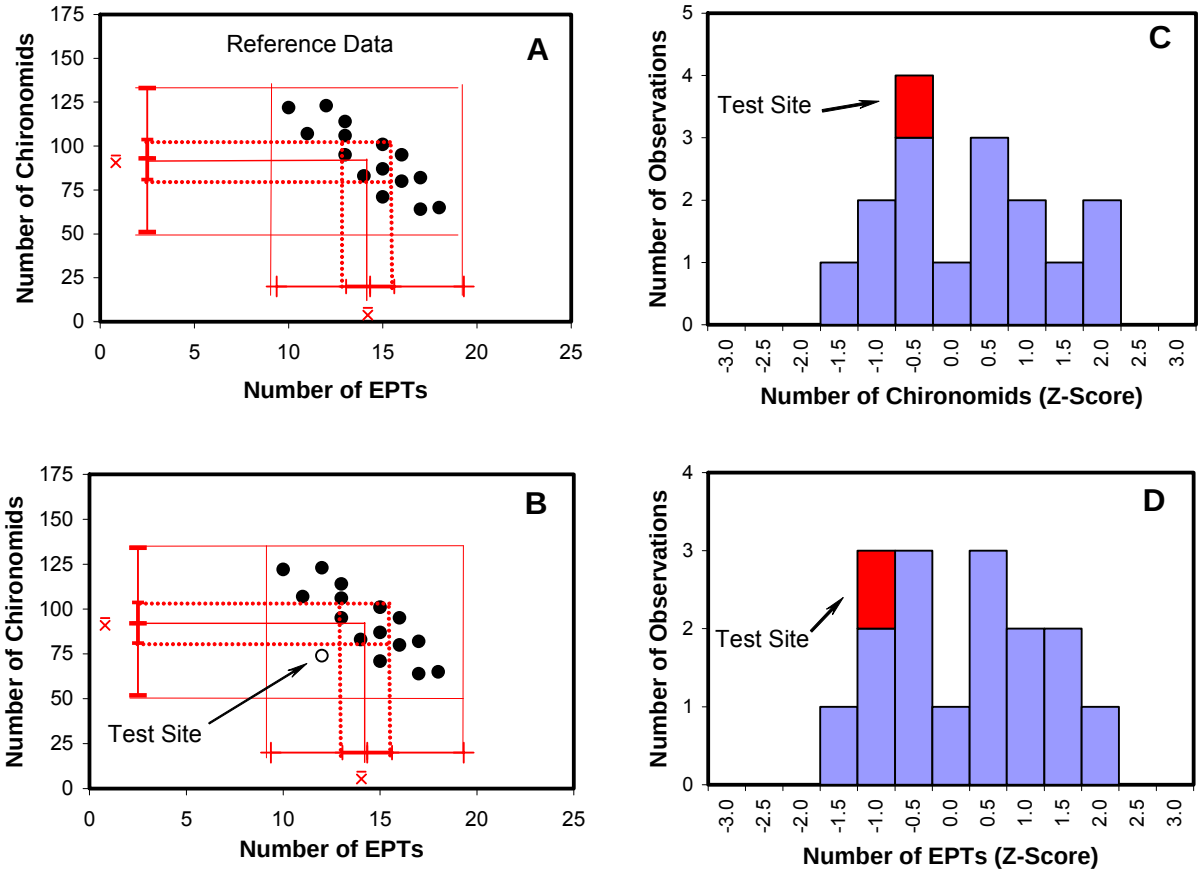


Figure 21: Example reference data set: (A) showing $\bar{x}$±2SE (dotted lines) and 2SDs (solid lines) for each variable, with a test site (B), and associated histograms for the 2 variables after standardization to Z-scores (C & D, $\bar{x}$=0, SD=1)

Mean values for the 15 reference sites are indicated, as are the 95% confidence limits for the mean ($\bar{x}$ ± 2 SEs) and the individual data points ($\bar{x}$ ± 2 SDs). In Figure 21B, a **test site** has been added. The **test site** lies outside of the region defined by ± 2 SEs, but inside the region defined by ± 2 SDs for both variables (as shown in Figure 21C). If we define the **normal range** for reference samples as the reference site mean plus or minus two standard deviations ($\bar{x}_{ref}$± 2 SDs), then the **test site** falls within the **normal range** for both indices, as shown in Figure 21C & D. Based on these graphical results, we would incorrectly conclude that the **test site** is normal. This graphical approach treats each index as if it summarizes independent information, and ignores correlations between variables; it is flawed because the two variables are highly correlated (r = -0.85 for the 15 reference sites in Figure 21A). When we consider covariance we reach a different conclusion.

By definition, multivariate analyses consider multiple indices simultaneously; therefore, they must consider correlations between variables. In the Figure 21 example, a better approach is to use a multivariate t-test to determine whether the **test site** is significantly different from the 15 reference sites using both (all) indices simultaneously (e.g., Kilgour *et al*. 1998). This statistical assessment is illustrated using a discriminant analysis, which maximally separates the **test site**

from the mean of the reference sites (Figure 22). All 16 sites are projected onto the resultant discriminant axis and a histogram of the discriminant scores for all 16 sites is produced (Figure 22B). Using the discriminant scores and the **normal range**, defined as $\bar{x}_{ref} \pm 2$ SDs, the **test site** is clearly unusual because it lies beyond 3.5 SDs from the multivariate reference site mean.

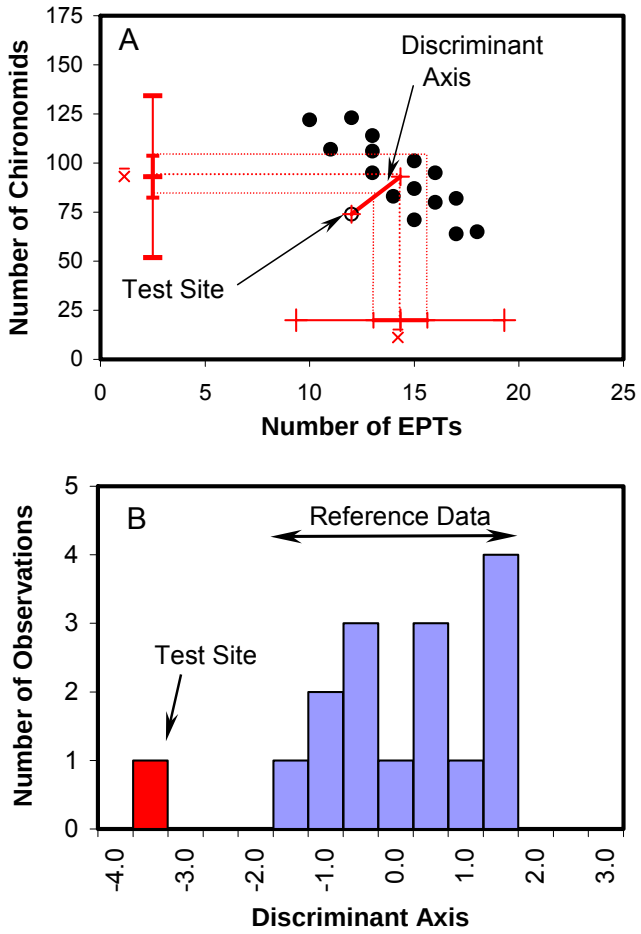


Figure 22: Placement of the discriminant axis (A), and the position of the test site on the discriminant axis (B). Discriminant axis scores re-scaled as standard deviations from the mean.

Discriminant analysis provides an objective way to statistically evaluate a **test site** by combining information from a series of different indices. A variety of statistical tests are associated with the procedure such that traditional probability estimates are produced (Rencher and Scott 1990). In addition, discriminant analyses indicate which indices are most important for distinguishing a **test site** from reference sites. The use of discriminant analysis in the rapid **bioassessment** of a Muskoka stream is described below.

In spring 1995, we collected rapid **bioassessment** data (100 counts, coarse taxonomic level) from CB1, a Muskoka stream with a history of water-quality impairment. We also collected data from 10 minimally **impacted** reference streams surrounding lakes Muskoka, Rosseau, and Joseph. Eleven biological indices (shown in Table 18) were calculated for each stream. These summary indices included the proportions of various taxonomic groups found at each site, as

well as correspondence analysis (CA) ordination scores based on both abundance of the different taxa (Abundance CA axes 1 through 3) and taxa presence-absence data for each site (P/A CA axes 1 through 3).

Results of a comparison between CB1 and the reference streams are presented in Table 18. For each index, the mean for the reference streams ($\bar{x}_{ref}$) is listed followed by the **test site** mean ($\bar{x}_{CB1}$). The standardized difference between the CB1 and reference-stream means ($\bar{x}_{CB1}$-$\bar{x}_{ref}$) is tabulated in units of reference-group standard deviations (σ). Using the concept of the **normal range** ($\bar{x}_{ref}$± 2 SDs), standardized differences (i.e., [$\bar{x}_{CB1}$-$\bar{x}_{ref}$] / SD_{ref}) greater than 2 lie outside of the **normal range** of biological condition. Only Abundance CA axis 1, with ($\bar{x}_{CB1}$-$\bar{x}_{ref}$)/SD_{ref} = 2.76 SDs, suggested that the benthic community at CB1 was unusual.

Table 18: A discriminant analysis summary comparing a test site (CB1) to a series of Muskoka reference streams.

Biological Index	Reference Site Mean ($\bar{x}_{ref}$)	CB1 Test Site Value ($\bar{x}_{CB1}$)	Standardized Difference, ($\bar{x}_{CB1}$-$\bar{x}_{ref}$)/SD_{ref}	*t* (σ=0)	*P* (σ=0)	Partial T^2
% Crustacea	6.626	0.639	-0.690	3.778	0.001	0.242
% Chironomidae	36.578	12.460	-1.289	7.059	<0.001	6.011
% Diptera	44.923	59.744	0.747	4.094	<0.001	4.609
% Gastropoda	1.433	0.000	-0.561	3.070	0.005	1.539
% Pelecypoda	3.243	1.597	-0.599	3.281	0.003	0.908
Abundance CA, Axis 1	-0.316	0.848	2.757	15.101	<0.001	5.408
Abundance CA, Axis 2	-0.179	0.090	0.363	1.989	0.056	3.163
Abundance CA, Axis 3	0.055	-0.111	-0.326	1.785	0.085	0.974
P/A CA, Axis 1	0.0324	0.411	1.637	8.964	<0.001	4.591
P/A CA, Axis 2	0.038	-0.564	-1.399	7.661	<0.001	1.049
P/A CA, Axis 3	-0.052	0.230	0.578	3.166	0.004	3.256

Simple t and associated P values for each summary index are also presented in Table 18. Each t test is the traditional one-sample test that evaluates whether the observed CB1 value is significantly different from the reference-site mean (i.e., whether [$\bar{x}_{CB1}$-$\bar{x}_{ref}$] > 0 SD_{ref}). Nine of the tests were significant at the P<0.05 level. When the critical P value was adjusted for multiple comparisons using a **Bonferroni Correction**, such that significance occurred when P<0.004, eight tests remained significant. Abundance CA axis 1, P/A CA axes 1 and 2, and % chironomids were the 4 most deviant indices based on the t values; however, only abundance CA axis 1 suggested that CB1 was outside of the **normal range** (i.e., [$\times_{CB1}$-$\bar{x}_{ref}$] >2 SDs). To summarize, t tests indicated that $\bar{x}_{CB1} \neq \bar{x}_{ref}$ for 8 of 11 indices, but only one index (CA axis $1_{abundance}$) suggested that CB1 lies outside the **normal range**. Simple t-tests therefore left us with considerable uncertainty regarding whether CB1 was unusual.

The t values in Table 18 represent independent tests that do not account for correlations (or redundancies) among the 11 indices. The multivariate t test associated with the discriminant

analysis incorporates correlations among the variables and was highly significant ($F = 482.0$, $P<0.001$), suggesting that CB1 was quite unusual. The complementary non-central test to determine if CB1 was outside of the **normal range** was also highly significant ($P<0.001$).

To assist in interpreting the discriminant analysis, Partial T^2 values were calculated. Partial T^2 values incorporate inter-variable correlations and provide estimates of the unique contribution of each index to the discriminant axis. In the CBI example, % Chironomids had the greatest Partial T^2, indicating that it was the most important index distinguishing CB1 from the reference group when all 11 indices are considered simultaneously. Abundance CA Axis 1, the % Dipterans, and P/A CA Axis 1 were the next most important indices for discriminating CB1 from the reference-site mean.

To summarize, TSA uses a multivariate t test and associated discriminant analysis to provide quantitative and probabilistic indications of the relative biological condition of a **test site** with respect to reference sites. Recognizing that correlations may exist between indices, the multivariate t test is applied across all indices simultaneously to determine the probability of the site falling within the **normal range**. In the case of unusual sites, subsequent tests within the discriminant analysis are useful because they indicate which indices best demonstrate **test site** community divergence from normal.

When the biological condition at a **test site** falls outside the **normal range** of variation defined by reference sites, further investigation is required to determine if the observed differences were caused by human activities. **Test site**s that fall within the **normal range** require no further investigation (i.e., we have no reason to believe that they are not healthy). Atypical test sites that fall outside the **normal range** should be re-sampled and monitored on a regular basis to watch for changes in their status. Extremely atypical **test site**s deserve more detailed benthic invertebrate assessments and water quality testing.

Ultimately, TSA will be automated in the OBBN database, meaning that the approach can be used by practitioners who have minimal statistical expertise. Until then, calculations must be done manually. Appendix 9 describes a relatively simple method for calculating TSA scores in Microsoft Excel.

8 Glossary

aspect the compass direction toward which the slope of the **longest catchment axis** faces; measured to OFAT (Chang *et al*. 2002) map north

bank-full width stream width measured at the elevation of the high water marks on each bank; the width of the stream at the highest stage that can be confined within the stream banks

base-flow index the modeled ratio of average annual base flow volume to total flow volume (Chang *et al*. 2002)

basin relief the total elevation change for a watershed

biomonitoring the process of sampling, evaluating and reporting on ecosystem condition using biological indicators

bioassessment evaluating degree of impairment using biological indicators

bog a wetland with rarely flooded, but always saturated organic peat substrate; ≥ 40 cm of Sphagnum peat; pH moderate to highly acidic (< 4.2); water obtained primarily from rain, rather than from groundwater, as in a **fen**; tree cover (trees > 2 m tall) ≤ 25% (Lee *et al.* 1998)

Bonferroni Correction a correction that reduces the occurrence of false positives in multiple tests; applied by dividing desired false-positive rate (probability of type I error, α) by the number of tests (k), and then using that modified α for all tests in the series; for example, in a study using α=0.05 with ten tests, the Bonferroni Correction for α (α_β) is $\alpha_\beta=\alpha/k$, so $\alpha_\beta=0.05/10=0.005$; Applying a significance level of 0.005 to each of the ten tests gives a true 5% chance that the null hypothesis will be falsely rejected on any 1 test

catchment an area of land draining to a common outlet (synonym of **drainage basin**)

catchment land cover areal proportions of 28 land-cover types as interpreted from 1997 LandSat imagery (Chang *et al.* 2002); land-cover classes include: water, coastal mudflats, intertidal **marsh**, supertidal **marsh**, freshwater coastal **marsh**/inland **marsh**, deciduous swamp, conifer swamp, open **fen**, treed **fen**, open **bog**, treed **bog**, tundra heath, dense deciduous forest, dense coniferous forest, coniferous plantation, mixed forest (mainly deciduous), mixed forest (mainly coniferous), sparse coniferous forest, sparse deciduous forest, recent cutovers, recent burns, old cuts and burns, mine tailings, quarries, and bedrock outcrop, settlement and developed land, pasture and abandoned fields, cropland, alvar, and unclassified

catchment perimeter the length of the watershed boundary line (Chang *et al*. 2002)

cross-over a location in a stream channel where the **thalweg** is in the center of the channel during the bank-full discharge; where the **thalweg** crosses from one side to the other side of the longitudinal mid-line of the channel; in streams defined by alluvial processes, typically occur at intervals of ½ the meander wavelength; often associated with **riffle**s where stream banks on either side of the channel are about the same height (Stanfield 2005)

cultivated intensively managed vegetation; row-cropped field or lawn (Stanfield 2005)

DEM (digital elevation model) a topographic surface specified as a dataset of regularly spaced x, y, and z coordinates (where z represents elevation)

detritus organic fragments of decomposing plant or animal matter

diagnostic useful in determining cause (often of biological impairment)

drainage basin an area of land draining to a common outlet (synonym of **catchment**)

fen a rarely flooded but always saturated wetland with organic substrate (≥ 40 cm of brown moss or sedge peat); pH slightly alkaline to mildly acidic; water obtained primarily through mineral soils (i.e., groundwater) rather than from direct precipitation, as in a **bog**; tree cover (trees > 2 m tall) ≤ 25%; sedges, grasses and low (< 2 m tall) shrubs dominate (Lee *et al.* 1998)

fetch length of the longest linear uninterrupted wind flow path across a lake

hydraulic head a surrogate for current speed; measured as the height of water "piled up" (above water's surface) against the wide side of a meter stick that is held vertically in the stream; always measured in the **thalweg** (Stanfield 2005)

impacted exposed to a stressor

impaired showing a biological response to imposed stressors; exhibiting a changed biological community brought about by degradation in water or habitat quality (Parsons and Norris 1996)

length of main channel the length of the longest continuous defined channel within a **catchment**

longest catchment axis a line connecting the centroid of the sampling unit with the intersection of the watershed boundary and longest flow path to the sample point)

marsh a wetland with a variable flooding regime, water depth < 2 m, tree and shrub cover ≤ 25%, and with a plant community dominated by emergent hydrophytic macrophytes (Lee *et al.* 1998)

maximum depth the depth of the deepest standing water encountered during sampling; recorded for each **sub-sample** (Transect Kick) or **replicate** (lakes and wetlands)

maximum flow distance the longest water flow path from the **drainage basin** outlet to the watershed divide (Chang *et al*. 2002)

maximum watershed elevation the maximum **DEM** elevation value in a defined **catchment** (Chang *et al*. 2002)

meadow an open terrestrial community characterized by grasses and broad-leafed herbaceous plants; usually originating from or maintained by cultural disturbances such as mowing, burning or grazing (Lee *et al*. 1988); includes pasture

mean annual lake evaporation the average amount of annual evaporation from lakes within a defined **drainage basin**

mean annual precipitation the average amount of annual precipitation within a defined **catchment**

mean annual run-off the average annual amount of run-off within a defined **catchment** (Chang *et al*. 2002)

mean annual snowfall the average annual amount of snowfall within a defined **catchment** (Chang *et al*. 2002)

mean elevation the average elevation value of the **DEM** for a defined **catchment** (Chang *et al*. 2002)

mean slope of watershed the average slope of the **catchment** based on the OFAT slope grid associated with the **DEM** (Chang *et al*. 2002)

meander belt in plan view, the area enclosed by lines drawn tangential to the points of maximum amplitude of stream meanders

minimum watershed elevation the minimum **DEM** elevation value for a defined **catchment** (Chang *et al*. 2002)

niche variable a natural, often physiographic (e.g., elevation, stream channel slope, lake **order)** habitat variable that accounts for a significant portion of the variance between different biological assemblages (e.g., between reference-site groups)

normal range the central n % of a variable's distribution (n typically equal to 95)

order a stream segment or lake classification based on the number and size of contributing tributaries; a stream with no tributaries (headwater stream) is a first-**order** stream; a segment downstream of the confluence of two first-**order** streams is a second-**order** stream; an n^{th}-

order stream is always located downstream of the confluence of two (n-1)th**-order** streams (Strahler 1952); lakes and wetlands take the **order** of their outlet stream (e.g., Riera *et al*. 2000, Quinlan *et al*. 2003); if no outlet, order is zero.

pool a stream segment characterized by slow flow and a constant surface elevation; in alluvial systems, typically occur along the outside bend of a meander, where the **thalweg** is adjacent to the stream bank at bank-full discharge

richness the number of taxa found

riffle a stream segment having fast, sometimes turbulent flow and typically shallow depth; typically exibits an obvious local surface elevation change; in alluvial systems, typically occurs at a **cross-over** (Stanfield 2005)

replicate a sample from a specific lake or wetland sampling unit

riparian vegetation vegetation growing adjacent to a stream, lake or wetland; includes trees, shrubs, and grasses (other types may be specified)

Sampling Reach sampling unit for streams; a segment of stream containing a minimum of 2 **riffle**s and one **pool**; in alluvial streams, often defined as 1 meander wavelength, beginning and ending at a **cross-over;** where there is no discernable **pool-riffle** sequence, may be defined as 14-20 times the **bank-full width**

scrub land a terrestrial community of small trees and shrubs, interspersed with grasses and sedges; transitional between **meadow** and forest, with trees generally less than 10 cm in diameter at breast height (Stanfield 2005)

shape factor the square of the length of the main channel divided by the drainage area (Chang *et al*. 2002)

slope of main channel the slope of the longest continuous channel within a defined **catchment** (Chang *et al*. 2002)

stream reach a geomorphic unit delimited by changes in slope, stream bank vegetation or width of the valley floor; part of a stream segment (Frissell *et al*. 1986, Stanfield 2005)

substrate bottom material at a lake, stream, or wetland sampling location; includes several particle size classes: clay (hard pan), silt (gritty, < 0.06 mm particle diameter), sand (grainy, 0.06 - 2 mm), gravel (2 - 65 mm), cobble (65 - 250 mm), boulder (> 250 mm), bed rock, and organic

sub-sample a benthos sample collected from either a **pool** or **riffle transect** in a stream **Sampling Reach**; a portion of a sample to be picked (e.g., the contents of 1 Marchant Box cell)

swamp a wetland with a variable flooding regime, and with standing water or vernal pools providing > 20% of surface cover; water depth < 2 m; tree or shrub cover > 25%, and dominated by hydrophytic tree and shrub species (Lee *et al.* 1998)

test site a site where biological condition or health is questioned

thalweg the longitudinal riverine flow path that represents the main concentration of flow; normally located along the deepest part of the channel.

transect a line upon which benthos samples are collected. In streams, **transect**s are located in **pool**s and **riffle**s and run perpendicular to the **thalweg**. In lakes and wetlands, **transect**s typically run perpendicular to the shoreline and extend out to the maximum wadeable depth (usually approximately 1 m)

tributary density the ratio of the sum of the length of all stream channels in a **catchment** to the area of that **catchment**; typically expressed in km/km^2

wetted width bank to bank stream width; measured perpendicular to current flow at the water's surface

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Appendix 1: Guide to Coarse-level Benthos Identification

In this section, we describe the **diagnostic** features that distinguish the 27 taxa groups (a mixture of Classes, Orders, sub-Orders, and Families) that comprise our minimum requirement for benthos identification in the Ontario Benthos Biomonitoring Network (Figure 23).

MOE Rapid Bioassessment Protocol taxonomic Level		
Coelenterata, Hydras	Ephemeroptera, Mayflies	Tabanidae, Horseflies
Turbellaria, Flatworms	Anisoptera, Dragonflies	Culicidae, Mosquitos
Nematoda, Roundworms	Zygoptera, Damselflies	Ceratopogonidae, Biting Midges
Oligochaeta, Aquatic Earthworms	Plecoptera, Stoneflies	Tipulidae, Craneflies
Hirudinea, Leeches	Hemiptera, True Bugs	Simuliidae, Blackflies
Isopoda, Sow Bugs	Megaloptera, Fishflies	Other Dipterans, Other True Flies
Amphipoda, Scuds	Trichoptera, Caddisflies	Gastropoda, Snails and Limpets
Decapoda, Crayfish	Lepidoptera, Moths	Pelecypoda, Clams
Hydracarina (Trombidiformes), Aquatic Mites	Coleoptera, Beetles	
	Chironomidae, Midges	

Figure 23: The 27 taxa group minimum taxonomic resolution (a mix of Classes, Orders, sub-Orders, and Families).

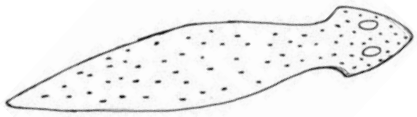
This appendix is intended only as an aid to understanding diagnostic characters of the groups listed in Figure 23; when identifying benthos, we recommend consulting appropriate keys (e.g., see Merrit and Cummins 1996, Pennak 1987, Logan 2003, Voshell 2002).

Taxa are listed in order of appearance on OBBN Tally Sheet. Quoted lengths are sizes at maturity.

Coelenterata

- Tube with tentacles
- Asexual reproduction by budding
- Inconspicuous, 2-25 mm long
- Variable colouration: often clear to whitish
- Sessile

Turbellaria (Flatworms)

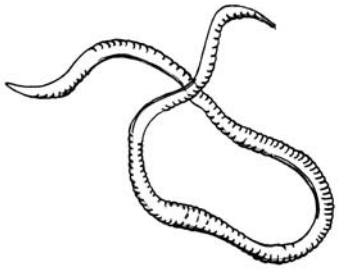


- Very flat 'worms', heads with eyespots
- Ventral mouth; may have pharynx
- 5-20 mm long, usually dark in colour: mottled grayish-brown to black dorsally
- Non-swimmers; creep slowly on bottom of sorting tray

Nematoda (Roundworms)

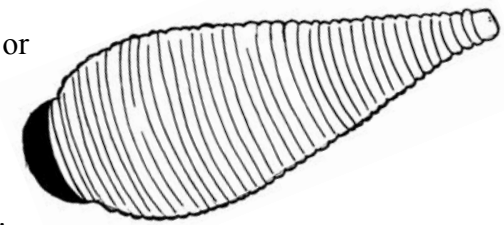
- Unsegmented, frequently clear
- Head usually tapered, tail pointed
- Often <1 cm long
- Rapid, whip-like movements

Oligochaeta (Aquatic Earthworms)



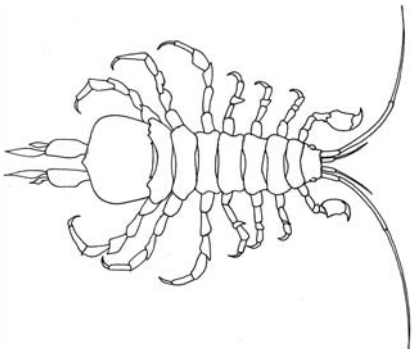
- Bundles of hairs on each segment behind the first
- Segmented bodies are round, soft, muscular and elongate Look 1 to 30 mm long, often pinkish
- Look like like earthworms
- May crawl along bottom of tray but often coiled up
- No suckers or eyes

Hirudinea (Leeches)



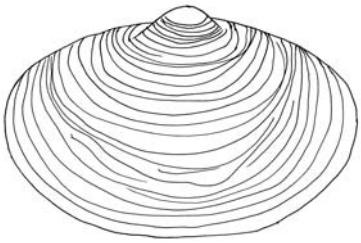
- Suckers at both ends, move by inching along or swimming
- 34 annulated segments, no chaetae
- ~5mm-30 cm long
- Head often with several pairs of eyes
- Colour varies, brown, olive and black common; typically patterned dorsally

Isopoda (Sow Bugs)



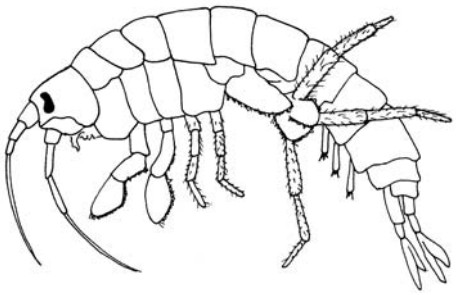
- 5-20 mm long; mini armadillos
- Dorso-ventrally compressed; 7 pairs of legs, adapted for crawling (first pair sub-chelate, others with simple claws)
- 1st antennae longer than 2nd
- Usually gray in colour
- Often associated with organic matter
- Uropods bifid

Bivalvia (Clams and Mussels)



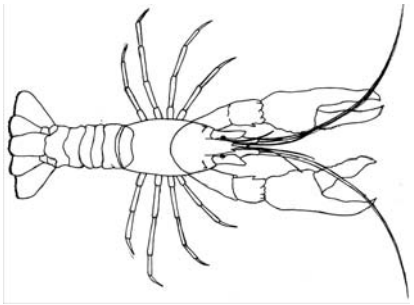
- Hard oval shell hinged in two halves
- 2 - 250 mm; colour variable
- Found in bottom of tray in sand or gravel
- Watch for (and don't count) empty shells

Amphipoda (Scuds)



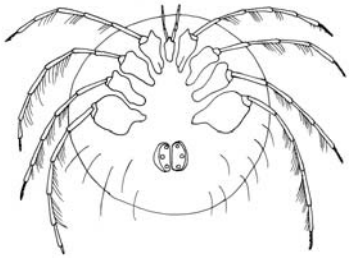
- Laterally compressed
- 2 Long antennae of approx. equal length
- 7 pairs of walking legs
- 6-segmented abdomen
- 5-20 mm long, colour variable
- Usually a translucent grey or light brown
- Catch with small piece of screen

Decapoda (Crayfish)



- Look like small lobsters; front half of body cylindrical, rear half dorso-ventrally flattened
- 5 pairs of walking legs: first 3 pairs chelate (claws of forelegs enlarged)
- Hard-shelled, eyes on stalks
- Broad telson used in backward-swimming escape
- 1 to 15 cm long, often green, brown, blue

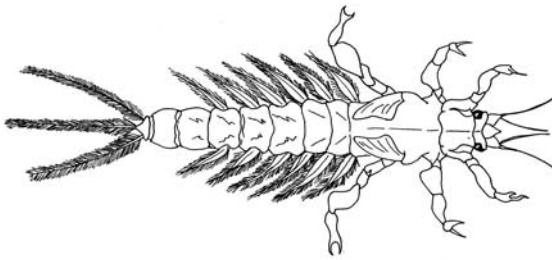
Hydrachnida (Mites)



- Adults with 4 pairs of segmented legs (larvae with 3)
- Body a sphere without visible segments
- Anterior finger-like pedipalps; simple eyespots; no antennae
- Often brightly coloured (red, green, blue, brown)

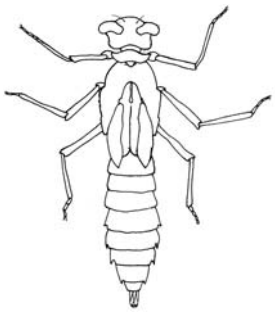
- Look like small (1-7 mm) spiders
- Uncoordinated, scrambling swimming motion

Ephemeroptera (Mayflies)



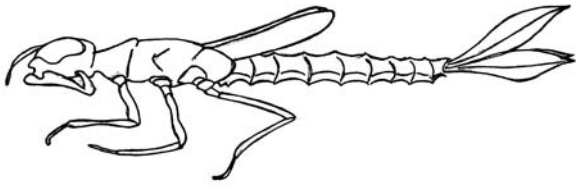
- Usually 3-tailed (sometimes 2-tailed)
- Single tarsal claw
- Gills held dorso-laterally on abdomen
- 3-28 mm long (not including tails)
- Swim using dorso-ventral undulations

Anisoptera (Dragonflies)



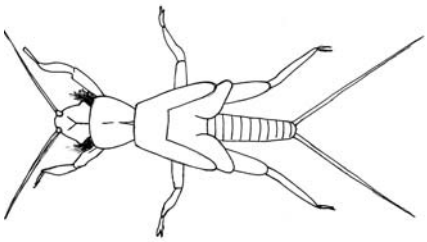
- Modified labium for catching prey
- Larger and heavier-bodied than mayflies; No visible external gills;
- Big head and eyes
- 15-45 mm; drab colours, often green to greenish brown
- Often flattened; Jet propulsion

Zygoptera (Damselflies)



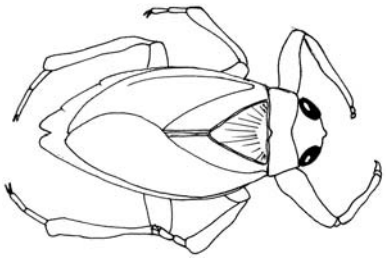
- Bodies more tubular, thinner than dragonflies
- 3 gills at terminus of abdomen
- Same modified raptorial labium as dragonflies
- Ten to 22 mm long, drab cryptic colours

Plecoptera (Stoneflies)



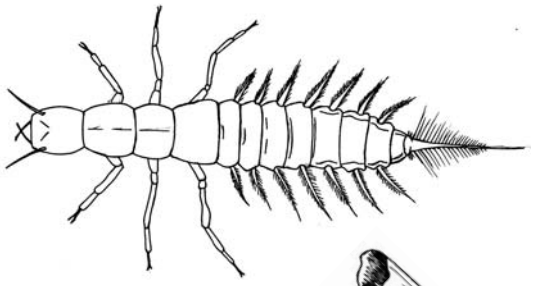
- 2 tails
- Gills may be abdominal, thoracic, and on the ventral head or neck region (gills never insert dorso-laterally on abdomen)
- Tarsi with 2 claws
- 6-50 mm, yellowish, brown or blackish

Hemiptera (True Bugs)



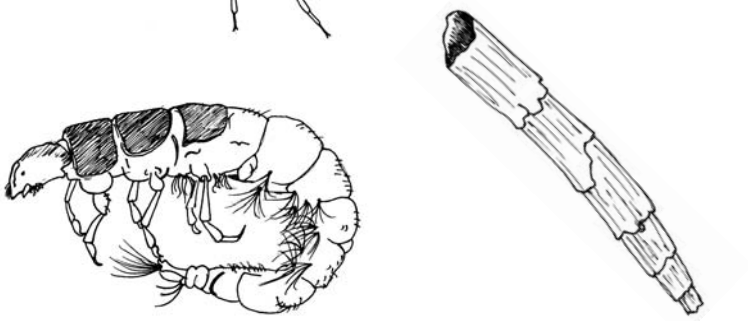
- 15-40 mm
- Sucking mouth parts (beak)
- No gills
- 2 claws on at least some legs
- Base of forewings leathery, otherwise membranous wings
- Often two pair of membranous wings
- Often with well developed breathing appendages

Megaloptera (Fishflies, Alderflies)



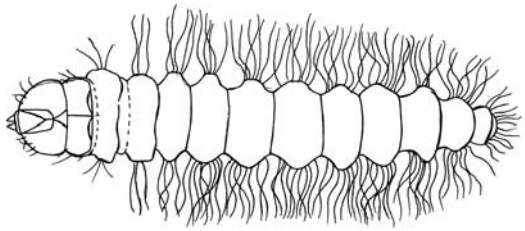
- Large: 25-90 mm long
- Lateral abdominal gill filaments
- Well developed mandibles
- Either with anal prolegs or a long terminal filament

Trichoptera (Caddisflies)



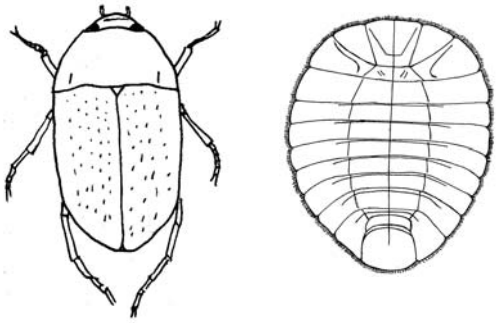
- Anal prolegs with hooks
- Often with portable case or fixed- retreat
- Dorsal thoracic plates variously sclerotized
- 2-50 mm long, head and thorax compressed into anterior portion of body

Lepidoptera (Aquatic Moths)



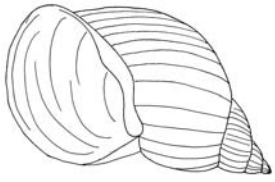
- Head with ring of ocelli
- 3 pairs of short, segmented, thoracic legs
- Ventral, abdominal prolegs
- 10 - 25 mm, crawl like a caterpillar

Coleoptera (Beetles)



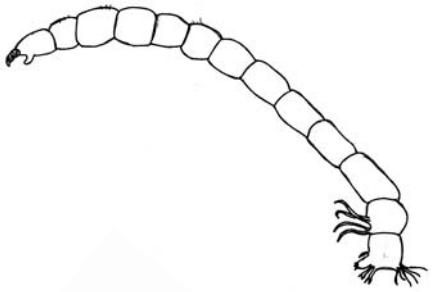
- 2 - 20 mm
- 3 pairs of thoracic legs
- Adults: Fore-wing modified as elytra, and extends posteriorly to cover all or most of the body
- Antennae with 11 or fewer segments
- Larvae: Sclerotized head with mandibles, maxillae, labium and 2- or 3-segmented antennae; May have unsegmented terminal abdominal appendages

Gastropoda (Snails and Limpets)



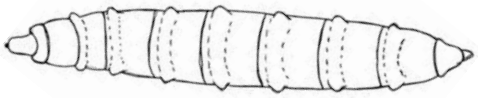
- 2 - 70 mm
- hard spiral or cap-shaped shell
- Bodies with prominent head and tentacles
- May have operculum

Chironomidae (Midges)



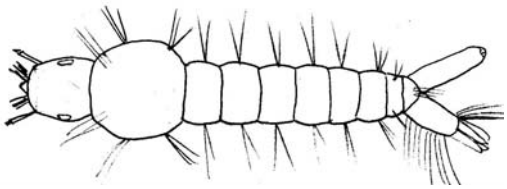
- 2 - 30 mm long, red, white, olive or yellowish
- Well developed, sclerotized head with eyes
- Anterior and posterior parapods with hooks
- Characteristic shape like letter "J"
- May be in a tube made of fine dirt particles
- Often caught in surface film

Tabanidae (Horse Flies, Deer Flies)



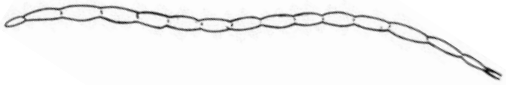
- 3 or 4 pairs of creeping welts with hooks on each of the first 7 abdominal segments
- Pointed at both ends, leathery texture
- Head retracted into thorax
- 15 - 40 mm

Culicidae (Mosquitoes)



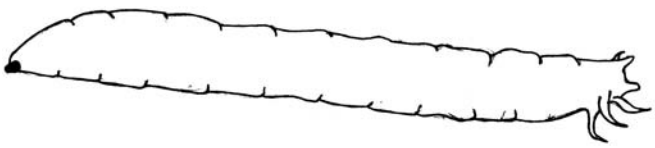
- 3-15 mm
- Fused thoracic segments are wider than abdomen
- Brushes of hairs at front of head and sides of mouth
- Posterior respiratory siphon

Ceratopogonidae (No-see-ums, Biting Midges)



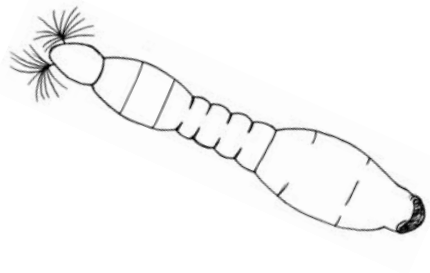
- very slender, pointed at both ends, segmented; small pointed sclerotized head
- No abdominal appendages but may be a tuft of terminal abdominal hairs
- 3-13mm; skin smooth shiny and creamy white
- remain stiff when picked up with forceps
- move by "whipping"

Tipulidae (Crane Flies)



- 10-50 mm, white, yellowish or brown
- Reduced head is retracted into thorax
- Membranous body; may have creeping welts
- Posterior respiratory disc with lobes

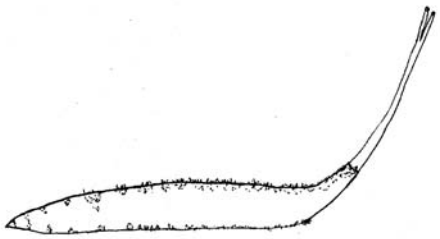
Simuliidae (Black Flies)



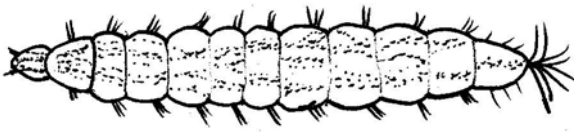
- Often with labral fans
- like flattened maggot with one end 1/3 fatter
- Sessile with posterior attachment organ
- move with looping (inch-worm) movements
- 3 - 15 mm, brown or greyish clour

Diptera, Miscellaneous (Other True Flies)

- Adults with single pair of wings
- May have parapods, pseudopodia, creeping welts or other appendages, but no jointed thoracic legs



- Often maggot-like; head may be retracted into thorax



Appendix 2: Checklist of Ontario Benthos Families

A List of Ontario benthos Families is provided in Table 19.

Table 19: Checklist of Ontario benthos families (Logan 2003)

Alderflies & Fishflies
Corydalidae
Sialidae

Beetles
Carabidae
Curculionidae
Dryopidae
Dytiscidae
Elmidae
Gyrinidae
Haliplidae
Hydrophilidae
Psephenidae

Bugs
Belostomatidae
Corixidae
Hebridae

Caddisflies
Apataniidae
Brachycentridae
Dipseudopsidae
Helicopsychidae
Hydropsychidae
Hydroptilidae
Lepidostomatidae
Leptoceridae
Limnephilidae
Molannidae
Philopotamidae
Odontoceridae
Phryganeidae
Polycentropodidae
Psychomyidae
Rhyacophilidae

Dragonflies & Damselflies
Aeshnidae
Calopterygidae
Coenagrionidae
Cordulegastridae
Corduliidae
Gomphidae
Lestidae

Mayflies
Baetidae
Baetiscidae
Caenidae
Ephemerellidae
Ephemeridae
Heptageniidae
Isonychiidae
Leptophlebiidae
Leptohyphidae

Moths
Pyralidae

True Flies
Athericidae
Ceratopogonidae
Chaoboridae
Chironomidae
Empididae
Enchytraeidae
Ephydridae
Muscidae
Psychodidae
Simuliidae
Stratiomyidae
Tabanidae
Tanyderidae
Tipulidae

Stoneflies
Capniidae
Chloroperlidae
Leuctridae
Perlidae
Perlodidae
Nemouridae
Pteronarcyidae
Taeniopterygidae

Crustaceans
Asellidae
Cambaridae
Gammaridae
Hyalellidae
Haustoriidae
Bopyridae

Molluscs
Ancylidae
Bithyniidae
Dreissenidae
Hydrobiidae
Physidae
Planorbidae
Sphaeriidae
Unionidae
Valvatidae
Viviparidae
Lymnaeidae
Pleuroceridae

Segmented Worms
Erpobdellidae
Glossiphoniidae
Lumbriculidae
Naididae
Piscicolidae
Tubificidae
Spionidae
Sparganophilidae
Sabellidae

Horsehair Worms
Gordiidae

Flatworms
Planariidae

Mites
Hydrachnidae
Lebertiidae
Anisitsiellidae
Arrenuridae
Aturidae
Hydrodromidae
Hydryphantidae
Hygrobatidae
Limnesiidae
Oxidae
Sperchontidae
Torrenticolidae
Pionidae
Unionicolidae
Hydrozetidae
Trhypachthoniidae

Appendix 3: Taxa Tally Sheet

A tally sheet for use when picking samples and identifying invertebrates to the coarsest permitted level is provided below. OBBN partners that are making more detailed identifications are advised to develop custom tally sheets or lists for their area.

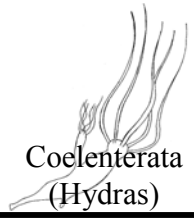
A digital version of the field sheet is available, contact: Chris Jones at (705) 766-1724 or chris.jones@ene.gov.on.ca.

Water Body Name: _____ Site #: _____ Replicate #: _____ Date (mm/dd/yyyy) and Time: _____

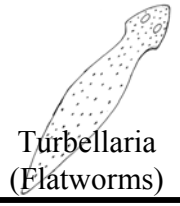
Organization: _____ Department_____ Address:_____

Contact: _____ Phone: _____ E-mail: _____ % picked for 100-count _____ # of vials: _____

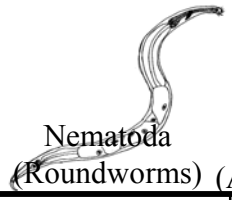
Circle Method: (Sub-sampling) Marchant Box / Teaspoon (Location) Field / Lab (Preservation) Live / Preserved (Magnification) Microscope / Unaided



Coelenterata (Hydras)

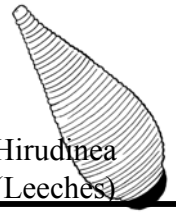


Turbellaria (Flatworms)

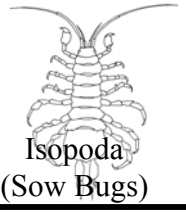


Nematoda (Roundworms)

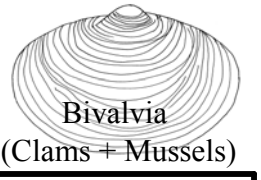
Oligochaeta (Aquatic Earthworms)



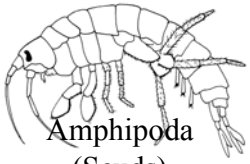
Hirudinea (Leeches)



Isopoda (Sow Bugs)



Bivalvia (Clams + Mussels)

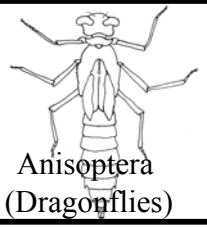


Amphipoda (Scuds)

Decapoda (Crayfish)

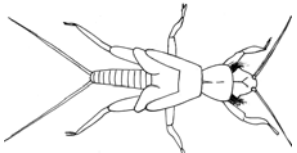
Hydrachnida (Mites)

Ephemeroptera (Mayflies)



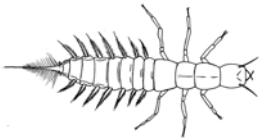
Anisoptera (Dragonflies)

Zygoptera (Damselflies)



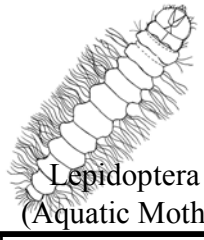
Plecoptera (Stoneflies)

Hemiptera (True Bugs)

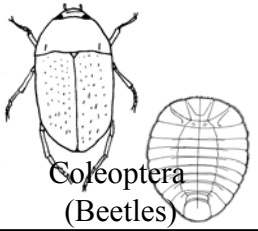


Megaloptera (Fishflies, Alderflies)

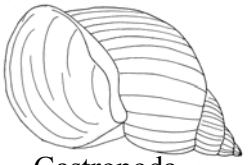
Trichoptera (Caddisflies)



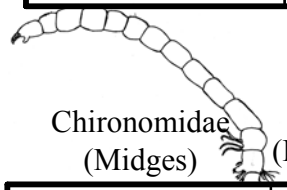
Lepidoptera (Aquatic Moths)



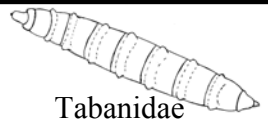
Coleoptera (Beetles)



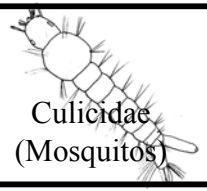
Gastropoda (Snails, limpets)



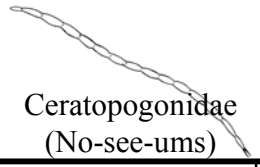
Chironomidae (Midges)



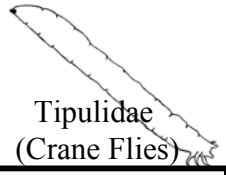
Tabanidae (Horse and Deer Flies)



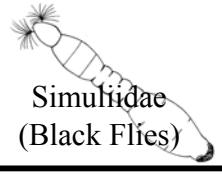
Culicidae (Mosquitos)



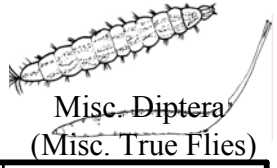
Ceratopogonidae (No-see-ums)



Tipulidae (Crane Flies)



Simuliidae (Black Flies)



Misc. Diptera (Misc. True Flies)

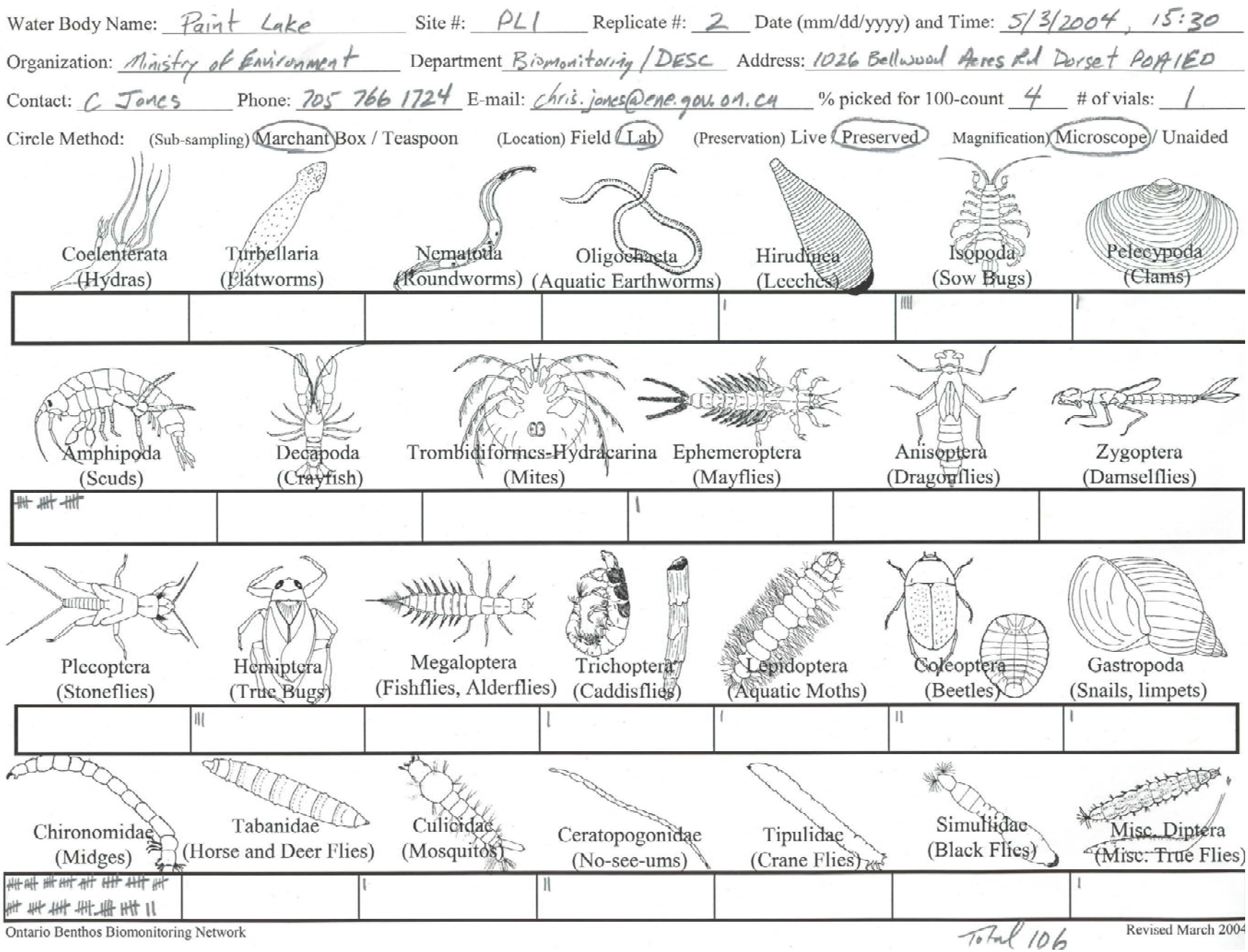
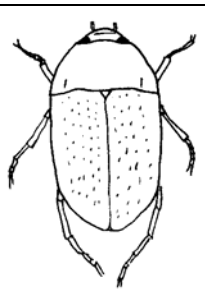


Figure 24: Example of completed tally sheet (hypothetical data).

Appendix 4: Lake Field Sheet

A field sheet for use when sampling lakes is provided below. A digital version is available, contact: Chris Jones at (705) 766-1724 or chris.jones@ene.gov.on.ca.

Ontario Benthos Biomonitoring Network Field Sheet-LAKES



Date:	Lake Name:
Time	Site #:
Agency:	Location (centroid of 3 replicates, use deg./min./sec. or specify other)
Investigators:	Latitude: Elevation (m asl):
Water Quality	Longitude:
Water Temperature (^{o}C):	Conductivity (uS/cm): pH:
DO (mg/l):	Alkalinity (mg/l as $CaCO_3$):

Site Description and Map

Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)
Show north arrow.

Benthos Collection Method (circle one):

- Traveling Kick & Sweep
- Other (specify):

Gear Type (circle one)

- D-net
- Other (*specify*):

Mesh Size: 500 micron (or specify)

Replicates	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	Replicate locations (Degrees/Minutes/Decimal seconds or specify): Latitude	Longitude
Sample 1					
Sample 2					
Sample 3					

Substrate

Enter dominant substrate class and second dominant class for each sub-sample

	Sample 1	Sample 2	Sample 3
Dominant			
2nd Dominant			

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock

Substrate Notes:

Organic Matter-Areal Coverage

Use 1: Abundant, 2: Present, 3: Absent

	Sample 1	Sample 2	Sample 3
Woody Debris			
Detritus			

Riparian Vegetative Community

Use: 1 (None), 2 (cultivated), 3 (meadow), 4 (scrubland), 5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (dist. From water's edge)	Sample 1	Sample 2	Sample 3
1.5-10 m			
10-30 m			
30-100 m			

Aquatic Macrophytes and Algae (Use: 1 (Abundant), 2 (Present), 3 (Absent). Circle dominant type.

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent			
Rooted Floating			
Submergent			
Free Floating			

Algae	Sample 1	Sample 2	Sample 3
Floating Algae			
Filaments			
Attached Algae			
Slimes or Crusts			

Lake Morphometry (optional, will be calculated by OBBN Coordinator using OFAT)

Perimeter (m): Volume (m^3): Fetch (m): Surface area (m^2): Order:

Notes (esp. related to land-use, habitat, obvious stressors)

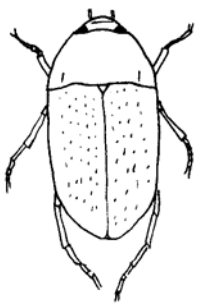
Candidate reference Site - Minimally Impacted? (circle one) Yes No

General Comments

Appendix 5: Stream Field Sheet

A field sheet for use when sampling lakes is provided below. A digital version is available, contact: Chris Jones at (705) 766-1724 or chris.jones@ene.gov.on.ca.

Ontario Benthos Biomonitoring Network Field Sheet: STREAMS



Date:	Stream name:
Time	Site #:
Agency:	Location: centroid of 3 replicates; Lat/Long or UTM
Investigators:	Elevation (m asl):
Water Quality	Datum/zone:
Water Temperature (^{o}C):	Conductivity (uS/cm): pH:
DO (mg/l):	Alkalinity (mg/l as $CaCO_3$):

Site Description and Map

Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)
Show north arrow.

Benthos Collection Method (circle one)**:**

- Traveling Kick & Sweep
- Grab Sample
- Other (specify):

Gear Type (circle one)

- D-net
- Ponar
- Other (*specify*):
- Ekman
- Rock Baskets

Mesh Size: 500 micron (or specify)

Sub-samples	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	Wetted Width (m)	Max. Hydraulic Head (mm)	# Grabs pooled per sample
Sample 1: Riffle (cross-over)		KICK AND SWEEP ONLY				GRAB SAMPLING ONLY
Sample 2: Pool						
Sample 3: Riffle (cross-over)						

Substrate

Enter dominant substrate class and second dominant class for each sub-sample

	Sample 1	Sample 2	Sample 3
Dominant			
2nd Dominant			

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock

Substrate Notes

Organic Matter-Areal Coverage

Use 1: Abundant, 2: Present, 3: Absent

	Sample 1	Sample 2	Sample 3
Woody Debris			
Detritus			

Riparian Vegetative Community

Use: 1 (None), 2 (cultivated), 3 (meadow), 4 (scrubland), 5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (dist. From water's edge)	Left Bank	Right Bank (facing downstream)
1.5-10 m		
10-30 m		
30-100 m		

% Canopy Cover (circle one)

0-24 25-49 50-74 75-100

If instrument used, record type:

Aquatic Macrophytes and Algae (Use: 1 (Abundant), 2 (Present), 3 (Absent). Circle dominant type.

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent			
Rooted Floating			
Submergent			
Free Floating			

Algae	Sample 1	Sample 2	Sample 3
Floating Algae			
Filaments			
Attached Algae			
Slimes or Crusts			

Stream Size/Flow

Bank Full Width (m): Discharge (m^3/s, optional, indicate method):

River Characterisation (*circle one*) Perennial Intermittent Unknown

Notes (esp. related to land-use, habitat, obvious stressors)

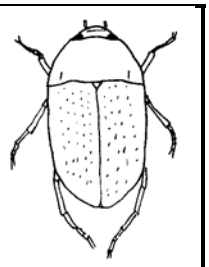
Candidate reference Site - Minimally Impacted? (circle one) Yes No

General Comments

Appendix 6: Wetland Field Sheet

A field sheet for use when sampling lakes is provided below. A digital version is available, contact: Chris Jones at (705) 766-1724 or f.chris.jones@ontario.ca.

Ontario Benthos Biomonitoring Network Field Sheet-WETLANDS



Date:	Wetland Name:
Time	Site #:
Agency:	Location: centroid of 3 replicates; Lat/Long or UTM
Investigators:	Elevation (m asl):
Water Quality	Datum & zone:
Water Temperature (^{o}C):	Conductivity (uS/cm): pH:
DO (mg/l):	Alkalinity (mg/l as $CaCO_3$):

Site Description and Map

Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)
Show north arrow.

Benthos Collection Method (circle one):

- Traveling Kick & Sweep
- Jab & Sweep
- Coring
- Artificial Substrate
- Other (specify):

Gear Type (circle one)

- D-net
- Corer
- Rock Baskets
- Other

Mesh Size: 500 micron (or specify)

Corer/Artificial Substrate specifications:

Replicates	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	# Pooled per replicate	Location (UTM or Lat./Long; note datum, zone)
Sample 1	TRAVELING KICK AND JABS ONLY			CORES OR JABS ONLY	
Sample 2					
Sample 3					

Substrate

Enter dominant substrate class and second dominant class for each sub-sample

	Sample 1	Sample 2	Sample 3
Dominant			
2nd Dominant			

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock
8	Organic

Substrate Notes

Organic Matter-Areal Coverage		Sample 1	Sample 2	Sample 3
Use 1: Abundant, 2: Present, 3: Absent	Woody Debris			
and circle dominant type	Detritus			

Riparian Vegetative Community

Use: 1 (None), 2 (cultivated), 3 (meadow), 4 (scrubland), 5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (dist. From water's edge)	Sample 1	Sample 2	Sample 3
1.5-10 m			
10-30 m			
30-100 m			

Aquatic Macrophytes and Algae (Use 1: abundant, 2: Present, 3: Absent. Circle dominant type)

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent			
Rooted Floating			
Submergent			
Free Floating			

Algae	Sample 1	Sample 2	Sample 3
Floating Algae			
Filaments			
Attached Algae			
Slimes or Crusts			

Wetland Description (Circle)

- Marsh
- Fen
- Other
- Swamp
- bog

Physiographic location

- Riverine, floodplain
- Coastal (lakeshore)
- Riverine, headwater
- Inland

Presence of Standing Water:

- Seasonal
- Unknown
- Permanent

Wetland Morphometry (optional, will be calculated by OBBN Coordinator using OFAT)

Surface area (m^2): Perimeter (m):

Notes (esp. related to land-use, habitat, obvious stressors)

Candidate reference Site - Minimally Impacted? (circle one) Yes No

General Comments

Appendix 7: Recommended Catchment-scale Habitat Measures

Table 20: Catchment-scale habitat variables calculated by OBBN coordinator using OFAT (Chang *et al.* 2002). NV=candidate niche variable; D=may be diagnostic

Feature	Application	Value
Drainage area	All samples	Measure of stream size; related to size, nutrient status and various physiographic variables for lakes and wetlands; NV
Base-flow index	**Catchment** scale variable; all samples	Related to a variety of physiographic variables, flow regime, water temperature, stream size; NV
Basin relief		
US Soil Conservation Service (SCS) Run-off Curve Number (CN) under antecedent moisture condition (AMC) I		
US SCS Runoff Curve Number under AMC II		
US SCS Runoff Curve Number under AMC III		
Mean annual lake evaporation		
Length of main channel		
Mean annual precipitation		
Mean Annual Run-off		
Mean Annual Snowfall		
Maximum Watershed Elevation		
Mean Elevation		
Maximum Flow Distance		
Minimum Watershed Elevation		
Mean Slope of Watershed		
Catchment Perimeter		
Shape factor		
Slope of main channel		
Tributary density		
Catchment land cover (areal proportions of 28 land cover types)		D
Order	All samples	Related to lake, stream, and wetland size; correlated with a variety of water quality variables; NV
Aspect		Related to a variety of micro-climate effects including sun exposure (warming), wind speed, precipitation and run-off; NV
Area	Lakes and Wetlands	Measure of size; NV
Perimeter		Measure of size; NV
Fetch	Lakes	Measure of size; related to wave action, shoreline erosion; NV

Appendix 8: OBBN Research Questions

Below is a partial list of OBBN research questions that is intended to illustrate uncertainties, stimulate discussion and highlight opportunities for collaborative research.

Experimental Model

1. What is an ecologically significant effect?
2. What is the minimum effect size that can be detected with each of the sampling methods?
3. Do order-level, family-level and genus/species-level assessments give equal ability to detect an ecologically significant effect?
4. Are biological community "response signatures" at **impaired** sites **diagnostic** for impairment factors?
5. What is the best way to classify reference sites for matching to a test site?
6. What physiographic variables account for differences in biological condition among minimally **impacted** sites? What are appropriate surrogates (that can be repeatably measured) for these variables? Can an appropriate reference group for a **test site** be predicted based on physiographic attributes alone?
7. Is 100 animals enough?
8. Is $\bar{\times}_{ref} \pm 2$ SDs or the 95^{th} percentile of among-reference-site-group distances reasonable definitions of the **normal range**?
9. What is an acceptable quantitative definition of minimal impact? Does this definition change regionally in Ontario?
10. How many samples is enough to screen for biological health (spatially and temporally)? How many samples are enough for whole lake, whole river, or whole wetland assessments?

Sampling and Sample Processing

1. How much of the variance in estimates of benthos community composition is due to different collection methods (e.g., CABIN Travelling-Kick vs. OBBN Transect Kick vs. fixed-area methods [e.g., 1 m^2 stationary-kick-and-sweep, Surber], different processing methods (e.g., Marchant vs. Bucket sub-sampling, use of microscope for picking vs. visually unaided) and sampling-crew-specific biases? How do these sources of variation compare to variation between waterbodies, and between samples collected in the same waterbody at different times (using the same methods each time)?
2. How reproducible are OBBN-recommended methods (i.e. how important is between-crew variance in relation to other sources of variation)?

Analysis

1. What is the ideal ratio of reference sites to number of metrics used in the analysis? Can a metric screening procedure be developed?
2. Is the suite of recommended indices sufficient to summarize biological condition and characterize response signatures?

Appendix 9: Test Site Analysis Using Microsoft Excel

(Reprinted from: Bowman, M.F., K.M. Somers, and R.A. Reid. 2003. A Simple Method To Evaluate Whether A Biological Community Has Been Influenced By Anthropogenic Activity. In Hedley, K., S. Roe, and A.J. Niimi (Eds). 2003. Proceedings for the 30th Annual Aquatic Toxicity Workshop: September 28 to October 1, 2003, Ottawa, Ontario. Pp. 62-72. Figure and table numbers have been updated to fit this document's series)

Introduction

To determine whether the biological community at a test site has been influenced by human activity, the community at the test site can be compared to communities found at minimally **impacted** reference sites in what is generally called the reference condition approach (e.g., Hughes et al. 1986, Hughes 1995). Currently, there is no consensus on the most effective type of data analysis to use in order to conclude whether a test site has been **impacted** by anthropogenic activities (Reynoldson and Wright 2000). Numerous indices, each summarizing different aspects of biological condition, are commonly used in bioassessments. However, the techniques used to evaluate test sites with multiple indices or multivariate methods often: (i) involve subjective interpretation, (ii) do not use all biological information available or use redundant information, (iii) are difficult to calculate and explain, and (iv) do not provide probabilities of incorrectly classifying test sites. Herein we demonstrate a test-site analysis (TSA) method that is objective, uses all biological information available, accounts for and identifies redundant information, and therefore, decreases the probability of misclassifying a test site (e.g., see Somers et al. 2003). The TSA method provides a single probability that the test site differs from the reference sites. In addition, a second statistical test can be used to assess whether the test site is **impaired** to a degree considered ecologically important (Kilgour et al. 1998). Furthermore, our TSA method is applied using Microsoft Excel® and add-ins that are freely available on the internet.

Methods

Sample Dataset: In an assessment of the impacts of acid precipitation on Dorset-area streams in south-central Ontario, MacKay and Kersey (1985) found that macroinvertebrate communities in streams with low pH were less diverse than communities in streams with higher pH. To illustrate the TSA method, we re-sampled one of MacKay and Kersey's acidified streams (Dickie 6) and ten reference streams in May 1999 after high spring flows had receded. All 11 streams are within 35 km of Dorset, are of comparable size (1st and 2nd **order**) and have similar land-use characteristics (> 90% of the **catchment** is forested). The pH of all 10 reference streams (Blue Chalk, Bona Vista, Britannia, Fletcher, Harp, Longline, Portage, Robertsons, St. Mary and Tramway) was greater than 6.0. By contrast, the pH of the historically acidified test stream (Dickie) was 4.4.

Benthic macroinvertebrates were collected using a standardized, **bioassessment** protocol (David et al. 1998). In each stream, three **riffle**s were sampled using a one-minute, kick-and-sweep method (1 m^2 quadrat, 250 μm-mesh D-net). Each sample was sieved in the field and then taken to a laboratory where the debris and associated organisms were randomly subsampled and live

sorted until a minimum of 100 animals was obtained. Most organisms were identified to order or coarser taxonomic level, although dipterans were identified to family. Results for the 3 quadrats were combined to produce total counts of approximately 300 organisms for each stream.

Indices: To determine if a test site was **impacted**, we would normally calculate a number of summary biological indices (10-15) that are appropriate indicators for the suspected stressor(s). Here we selected only 4 metrics to simplify our demonstration of the TSA method. In their original study, MacKay and Kersey (1985) found that in acidic streams, the percentage of plecopterans (i.e., stonefly larvae) was lower, and the percentage of chironomids (i.e., midge larvae) was higher, relative to benthic communities in circum-neutral pH streams. However, Yan et al. (1996) found that multivariate ordination scores were generally better than simple summary metrics as indicators of community change. Therefore, we selected the following indices: 1) the total number of Plecoptera and the total number of Chironomidae found in the 3 quadrats divided by the total number of organisms counted for each stream, and 2) the first and second axis scores of a correspondence analysis (CA) ordination of the streams-by-taxa, presence-absence data.

Multivariate ordinations are often used to summarize large matrices of sites-by-taxa data into a smaller set of axis scores that represent the dominant trends of variation among sites. Of the various types of ordinations, correspondence analysis (CA) is appropriate for abundance and presence-absence (P/A) data (Legendre and Legendre 1998). When CA is used to summarize P/A data, the resultant scores generally reflect patterns in community **richness** associated with species occurrence and co-occurrence. Because CAs can be strongly influenced by rare taxa, the rare taxa are generally removed or down-weighted prior to analysis (ter Braak and Prentice 1988). Here, we used the full P/A data matrix with rare taxa down-weighted by adding 0.2 to all values in the matrix (e.g., see Keller et al. 2002). The CA ordination was calculated in a simple spreadsheet (Figs. 25 & 26) using the Biplot add-in for Excel® (Lipkovich and Smith 2001). The resultant CA bi-plot shows the relative positions of the reference and test streams as well as the taxa that are important in defining the first and second axes (Figs. 27 & 28).

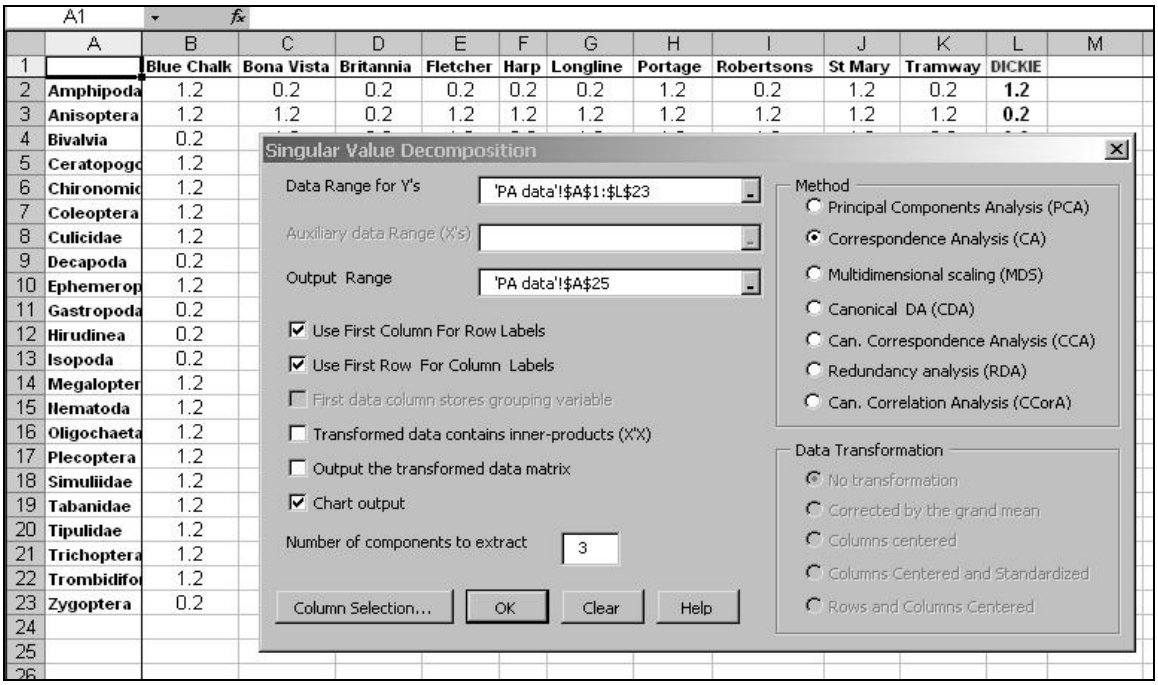


Figure 25: Input information required to perform a correspondence analysis in Biplot.

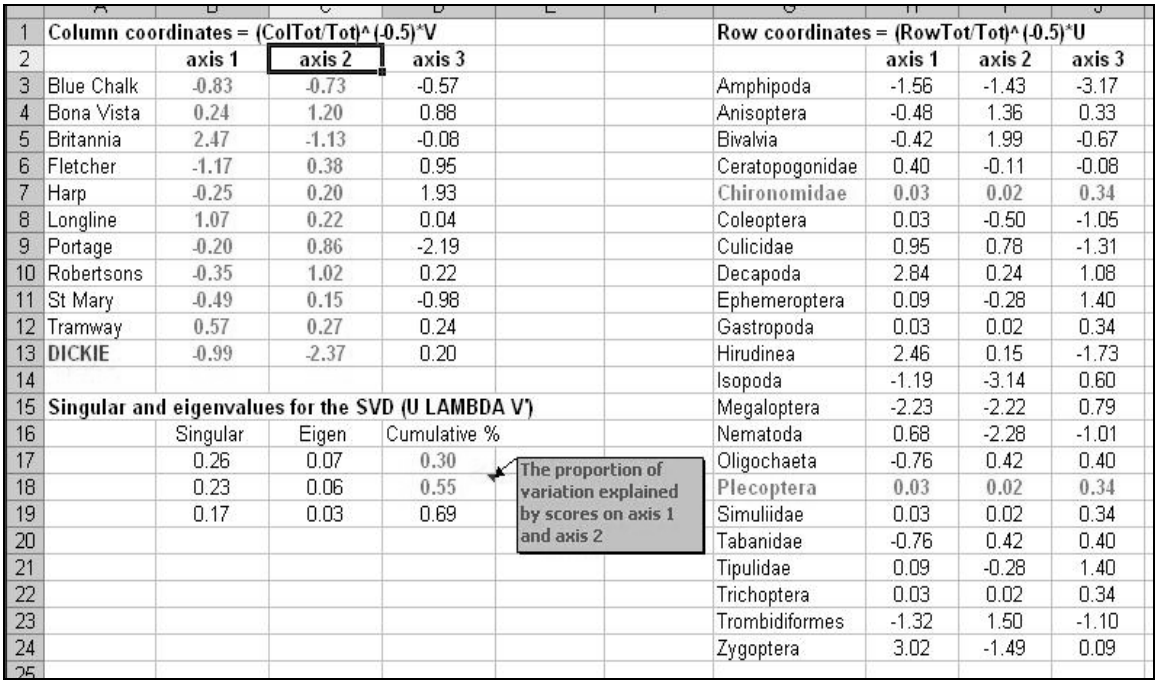


Figure 26 Output from a correspondence analysis performed in Biplot.

Table 21: Sequence of calculations used in each step of TEST SITE ANALYSIS (TSA)

STEP 1. Calculate Indices that will Characterize Biological Community Structure

[a] Appropriate metrics	e.g., %Plecoptera, %Chironomidae (Barbour et al. 1999)
[b] CA axes	e.g., CA1, CA2 (Fig. 25 & 26)

STEP 2. Calculate the Generalized Distance (D) Between Reference Site Mean and Test Site

[a] standardize data	(value $_{observed}$ – **AVERAGE** $_{reference}$) / **STDEV** $_{reference}$ (Fig. 29)
[a] covariance matrix	Tools/Data analyses/covariance on standardized data or **COVAR** ($index_1$, $index_2$) (Fig.30)
[a] full covariance matrix (corrected for appropriate n)	multiply each cell in the covariance matrix by number of reference sites (n_{ref}) and divide by n_{ref} -1 (Fig. 30)
[a] matrix inverse	**MINVERSE** (full covariance matrix) (Fig. 30)
[a] matrix products	**MMULT** (standardized test data , inverse matrix) (Fig. 31) **MMULT** (resultant product, transposed test data)

STEP 3. Assess the Statistical Significance of D (using central and / or non-central tests)

Numerator df	number of indices (p)
Denominator df	number of reference sites (n_{ref}) – number of indices
[a] F	$((n_{ref} - p) * n_{ref} * D^2) / (p * (n_{ref} - 1))$
[a] Central P	**FDIST** (F, p, n_{ref} - p)
[a] χ^2	**CHIINV** (0.05, p)
[a] λ	$\chi^2 * n_{ref}$
[c] Non-central P	1 - (**NCF** (F, p, n_{ref} - p, λ, $1e^{-8}$, 400))

STEP 4. Determine the Contribution of Each Index to the TSA (in uni- and multivariate analyses)

Univariate	
[a] Standardized difference (δ)	($value_{test}$ – $mean_{reference}$) / $standard\ deviation_{reference}$
[a] t	δ * **SQRT** (n_{ref})
[a] P	**TDIST** (**ABS** (t), n_{ref} - 1, 2)
Multivariate	
[a] T^2	$n_{ref} * D^2$
[a] Partial T^2 (pT^2)	redo calculations for T^2, omitting one index at a time (T^2_{p-1}) **SQRT** $((n_{ref} - p) * (T^2 - T^2_{p-1}) / (n_{ref} + T^2_{p-1}))$
[a] Partial F	(F_{p-1}) $pT^2 * pT^2$
[a] P	FDIST(F_{p-1}, 1, n_{ref} - p)

[a] standard excel worksheet functions (in bold) required (Microsoft Corporation 2003)
[b] biplot add-in required (Lipkovich and Smith 2001)
[c] pie-face add-in required (Lenth 2003)

Generalized Distance (**D**): In order to estimate the biological similarity among test and reference streams using all summary indices simultaneously, we calculated the generalized or Mahalanobis distance (e.g., Legendre and Legendre 1998). The generalized distance (**D**) is a standardized Euclidean distance that accounts for correlations or redundancies among indices. By using **D**, the estimated biological distance among streams is not biased by our choice of metrics if these indices measure redundant aspects of the benthic community. In this demonstration, **D** is calculated using information associated with 4 indices (i.e., the number of variables, p = 4).

To calculate generalized distance, all data associated with each summary biological index were centred by subtracting the average value for the 10 reference streams and then standardized by dividing by the standard deviation associated with the 10 reference streams (Table 21, Fig. 29). The standardization step was included because many biological indices are measured in different units (or on different scales) and this step weights the indices equally in the analysis. The standardized data for the reference streams were used to calculate a variance-covariance matrix among the 4 metrics (Table 21, Fig. 30 - matrix 1). The full, corrected 4 x 4 variance-covariance matrix ($\mathbf{S}_{ref}$ - Fig. 30 - matrix 2) was used to calculate an inverse matrix ($\mathbf{S}^{-1}_{ref}$ - Fig. 30 - matrix 3) and the vector of standardized values for the test stream ([test – $\mathbf{X}_{ref}$]/$\mathbf{SD}_{ref}$ - Fig. 30 – matrix 4) was multiplied by the inverse matrix. Subsequently, $\mathbf{D}^2$ was calculated by multiplying the resultant product (Fig. 30 – matrix 5) by the transposed vector associated with the test stream (([test – $\mathbf{X}_{ref}$]/$\mathbf{SD}_{ref}$)′ - Fig. 30 – transposed matrix 4). Thus, **D** was calculated using the following equation:

$$D = \sqrt{\left(\frac{test - \overline{X}_{ref}}{SD_{ref}}\right) * S_{ref}^{-1} * \left(\frac{test - \overline{X}_{ref}}{SD_{ref}}\right)'}$$

To determine whether the biological condition of the test stream was significantly different from the reference-stream mean, we could evaluate whether the distance ***D*** was significantly different from zero. If this test is based on $\boldsymbol{D^2}$, then $\boldsymbol{D^2}$ multiplied by the number of reference streams (n_{ref}) approximates the standard multivariate $\boldsymbol{T^2}$ test (Legendre and Legendre 1998). The significance of $\boldsymbol{D^2}$ is assessed using an ***F*** value calculated as:

$$F = \frac{\left(\left(n_{ref} - p\right) * n_{ref} * D^2\right)}{\left(p * \left(n_{ref} - 1\right)\right)} \quad \text{with } p \text{ and } (n_{ref} - p) \text{ df.}$$

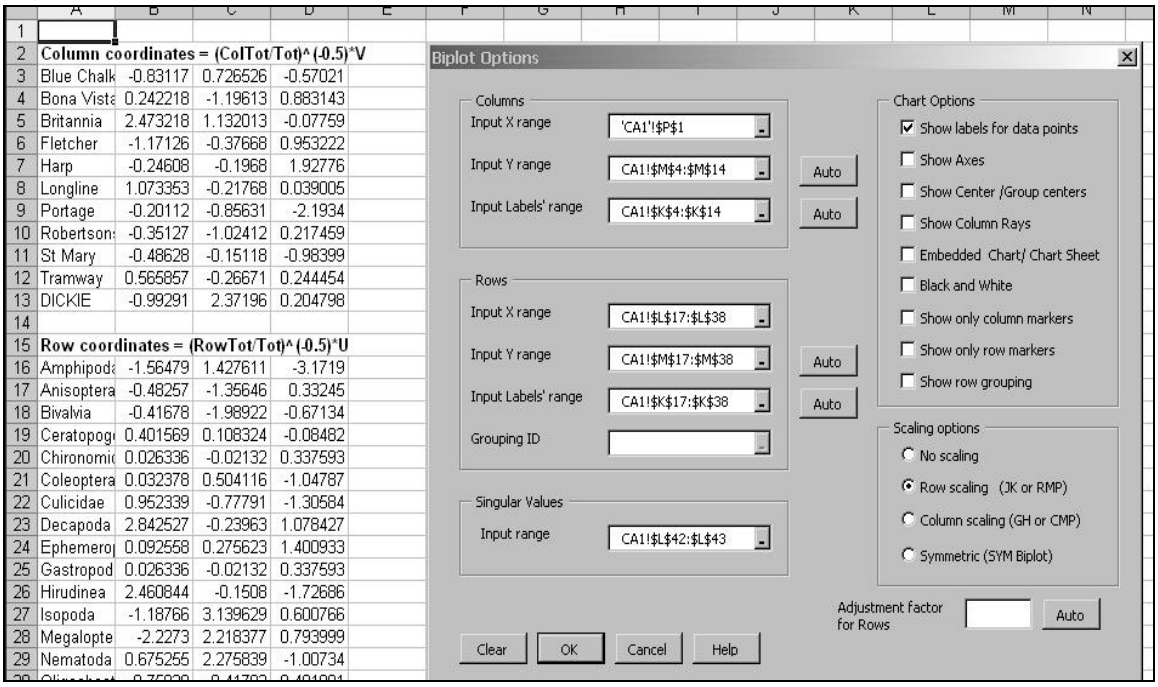


Figure 27: Graphing window and input information that automatically appears in Biplot following a correspondence analysis.

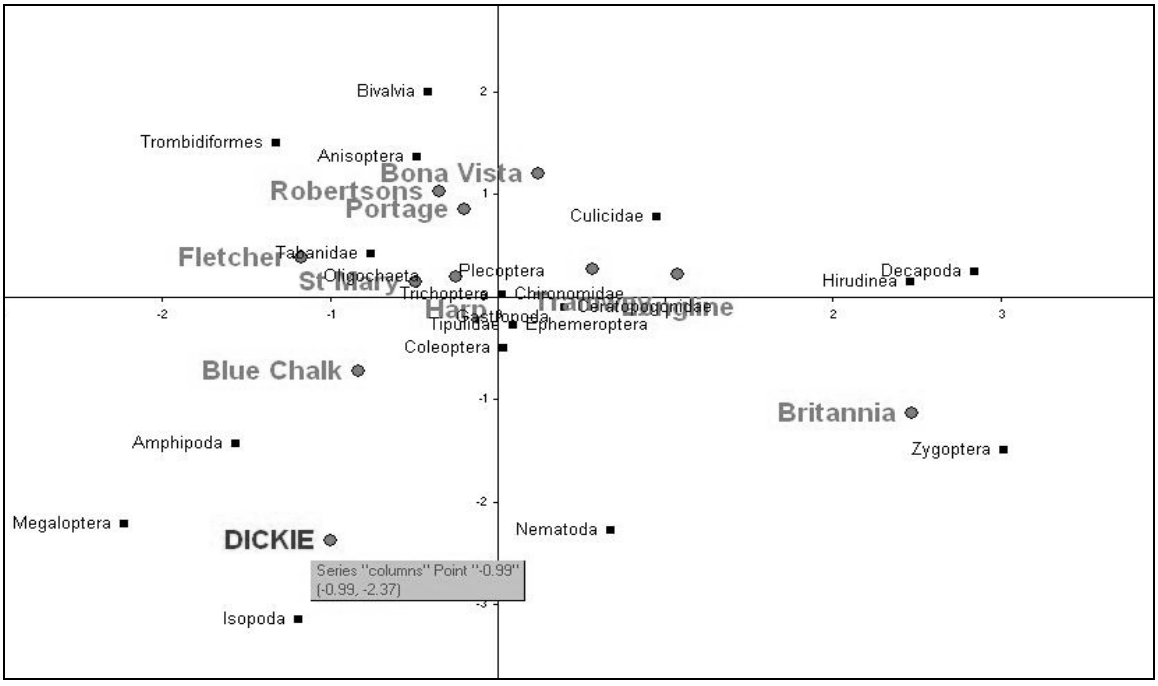


Figure 28: Graph of correspondence analysis (CA) axes one versus axes two, showing the position of the Dickie test site relative to reference sites, and the taxa important in defining the CA axes.

G13 =(B13-B$16)/B$17

	A	B	C	D	E	F	G	H	I	J
1		Data					Standardized Data			
2		%P	%Chir	CA 1	CA 2		%P	%Chir	CA 1	CA 2
3	Blue Chalk	39.6	14.1	-0.83	-0.73		1.35	-0.94	-0.89	-1.33
4	Bona Vista	26.2	23.2	0.24	1.20		0.50	-0.37	0.13	1.31
5	Britannia	12.0	33.3	2.47	-1.13		-0.40	0.27	2.24	-1.89
6	Fletcher	47.4	5.2	-1.17	0.38		1.85	-1.49	-1.21	0.18
7	Harp	26.0	24.8	-0.25	0.20		0.49	-0.27	-0.33	-0.06
8	Longline	8.9	28.8	1.07	0.22		-0.59	-0.02	0.91	-0.03
9	Portage	0.3	61.8	-0.20	0.86		-1.14	2.05	-0.29	0.84
10	Robertsons	6.3	19.5	-0.35	1.02		-0.76	-0.60	-0.43	1.08
11	St Mary	9.0	35.5	-0.49	0.15		-0.59	0.40	-0.56	-0.13
12	Tramway	7.2	44.4	0.57	0.27		-0.71	0.96	0.43	0.03
13	DICKIE	**0.6**	**17.8**	**-0.99**	**-2.37**		**-1.13**	**-0.70**	**-1.04**	**-3.60**
14										
15	**Reference**									
16	**site mean**	**18.3**	**29.1**	**0.1**	**0.2**					
17	**&standard**	**15.7**	**16.0**	**1.1**	**0.7**					
18	**deviation**									
19										

Figure 29: Raw index values, and index values standardized by subtracting the reference site mean and dividing by the reference site standard deviation (P = Plecoptera, Chir = Chironomidae, CA 1 = scores on correspondence axes 1).

N10 {=MINVERSE(T3:W6)}

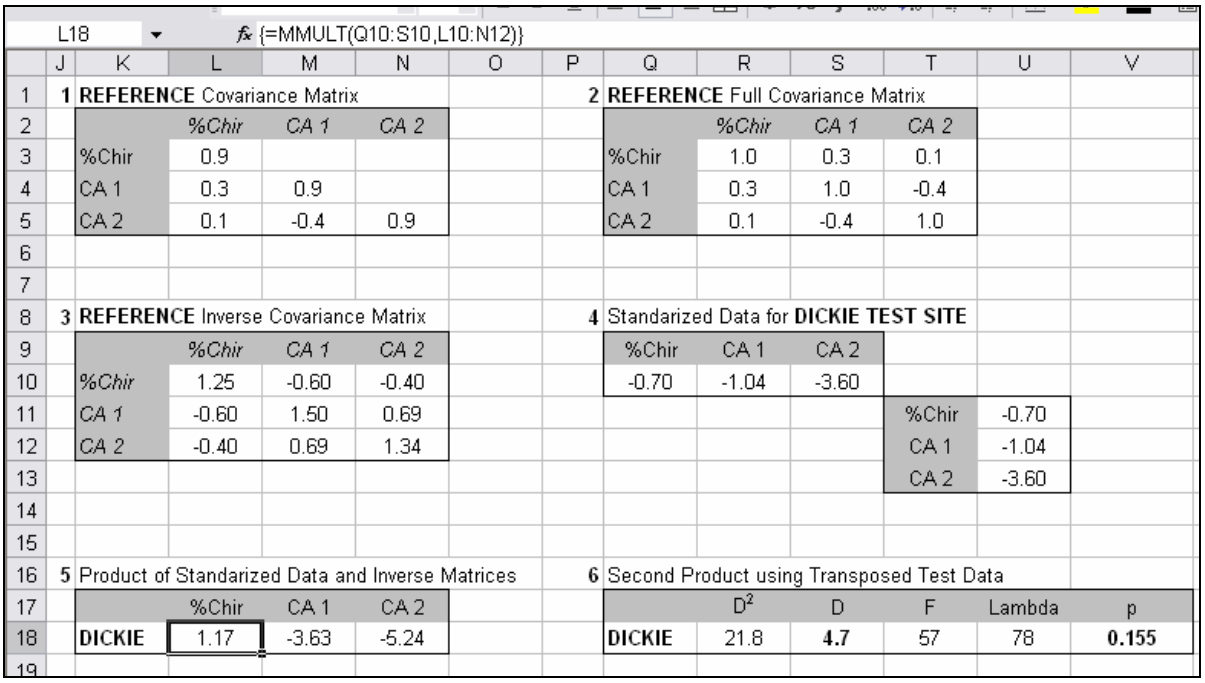
	L	M	N	O	P	Q	R	S	T	U	V	W	X
1	1	**REFERENCE** Covariance Matrix					2	**REFERENCE** Full Covariance Matrix					
2			*%P*	*%Chir*	*CA 1*	*CA 2*			*%P*	*%Chir*	*CA 1*	*CA 2*	
3		%P	0.9					*%P*	1.0	-0.8	-0.5	-0.2	
4		%Chir	-0.7	0.9				*%Chir*	-0.8	1.0	0.3	0.1	
5		CA 1	-0.4	0.3	0.9			*CA 1*	-0.5	0.3	1.0	-0.4	
6		CA 2	-0.2	0.1	-0.4	0.9		*CA 2*	-0.2	0.1	-0.4	1.0	
7													
8	3	**REFERENCE** Inverse Covariance Matrix					4	Standarized Data for **DICKIE TEST SITE**					
9			*%P*	*%Chir*	*CA 1*	*CA 2*		%P	%Chir	CA 1	CA 2		
10		*%P*	3.97	2.55	1.47	1.10		-1.13	-0.70	-1.04	-3.60		
11		*%Chir*	2.55	2.89	0.34	0.31						%P	-1.13
12		*CA 1*	1.47	0.34	2.04	1.10						%Chir	-0.70
13		*CA 2*	1.10	0.31	1.10	1.64						CA 1	-1.04
14												CA 2	-3.60
15													
16	5	Product of Standarized Data and Inverse Matrices					6	Second Product using Transposed Test Data					
17			*%P*	*%Chir*	*CA 1*	*CA 2*			D^2	D	F	Lambda	p
18		**DICKIE**	-11.75	-6.37	-7.98	-8.50		**DICKIE**	56.6	**7.5**	94	95	**0.048**
19													

Figure 30: Six matrices used to calculate the generalized distance (D) between a test site and the reference site mean (P = Plecoptera, Chir = Chironomidae, CA 1 = scores on correspondence axes 1).

In this ***F*** test, we are evaluating whether the difference (or biological distance) between the test stream and the reference-stream mean is significantly different from zero. Because statistical significance is affected by power (i.e., significance is a function of the number of reference streams and number of metrics, as well as the effect size), Kilgour et al. (1998) recommended that we focus on whether there is an ecologically meaningful rather than a statistically significant difference between the test and reference streams. As a result, Kilgour et al. proposed that the observed difference between the test stream and the reference-stream mean should be significantly greater than the **normal range** of variation among the reference streams. Kilgour et al. defined the **normal range** as the confidence region enclosing 95% of the reference streams, and hence, the appropriate test assesses whether the test stream is significantly outside of the **normal range**. That is, we test whether the difference between the test stream and the reference-stream mean is greater than the **normal range** instead of the traditional test that the observed difference is greater than zero. This type of statistical test is a non-central test, whereas the traditional test evaluating a difference of zero is a central test.

To statistically evaluate a non-central test, the critical difference (or critical effect size) must be defined *a priori*. Following Kilgour et al. (1998), we based our critical effect size on the **normal range** of variation among reference streams. In non-central tests, this effect size is typically expressed as a function of the non-centrality parameter (λ). Because we are using the generalized distance, the non-centrality parameter associated with the distance enclosing 95% of the reference-stream observations is defined by the 95th percentage point of the chi-square distribution with p df, where p is the number of metrics used to calculate the generalized distance (i.e., $\lambda = \chi^2_{(0.05,p)} * n_{ref}$; Kilgour et al. 1998). Having determined the non-centrality parameter, the probability that a test stream lies significantly outside of the **normal range** is readily calculated using the *πface* add-in for Excel® (Lenth 2003) and the observed ***F*** value defined above. As a result, the probability associated with the traditional ***F*** test indicates whether the test stream is significantly different from the reference-stream mean (i.e., $\boldsymbol{D} \neq 0$), and the non-central test probability indicates whether the test stream lies significantly outside of the **normal range** of variation among the reference streams (i.e., ***D*** > **normal range**).

To determine the relative importance of each biological index in separating the test stream from the reference streams, calculations for ***D*** and $\boldsymbol{T^2}$ can be repeated p times leaving out a different metric each time. The differences between the original analyses and the analyses using one fewer index are used to calculate *partial* $\boldsymbol{T^2}$ values for each index that is omitted (e.g., Rencher and Scott 1990, Table 1). The *partial* $\boldsymbol{T^2}$ values indicate the amount of unique information that a given index adds to the analysis given the variation already explained by the other metrics. The index with the highest *partial* $\boldsymbol{T^2}$ contributed the most unique information to the multivariate assessment (Table 1, Figs. 31 & 32), whereas metrics with small *partial* $\boldsymbol{T^2}$ values add very little to the analysis.



L18 =MMULT(Q10:S10,L10:N12)

1 REFERENCE Covariance Matrix

	%Chir	CA 1	CA 2
%Chir	0.9		
CA 1	0.3	0.9	
CA 2	0.1	-0.4	0.9

2 REFERENCE Full Covariance Matrix

	%Chir	CA 1	CA 2
%Chir	1.0	0.3	0.1
CA 1	0.3	1.0	-0.4
CA 2	0.1	-0.4	1.0

3 REFERENCE Inverse Covariance Matrix

	%Chir	CA 1	CA 2
%Chir	1.25	-0.60	-0.40
CA 1	-0.60	1.50	0.69
CA 2	-0.40	0.69	1.34

4 Standarized Data for **DICKIE TEST SITE**

%Chir	CA 1	CA 2
-0.70	-1.04	-3.60

%Chir	-0.70
CA 1	-1.04
CA 2	-3.60

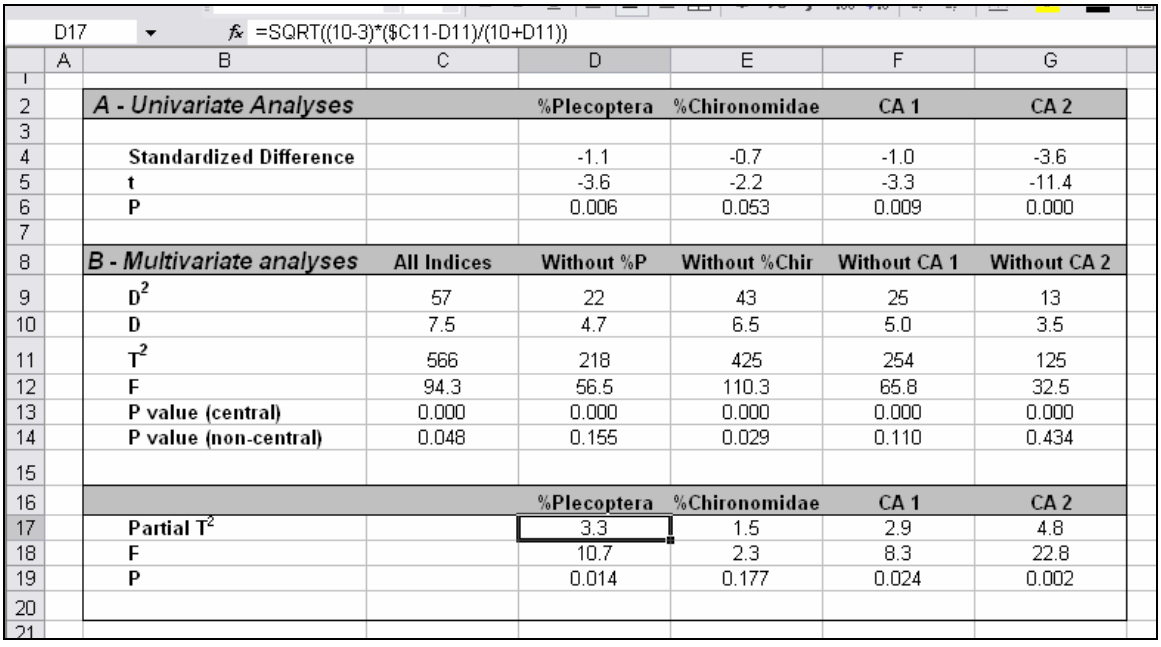
5 Product of Standarized Data and Inverse Matrices

	%Chir	CA 1	CA 2
DICKIE	1.17	-3.63	-5.24

6 Second Product using Transposed Test Data

	D^2	D	F	Lambda	p
DICKIE	21.8	**4.7**	57	78	**0.155**

Figure 31: Initial six steps used to calculate partial T^2 for % Plecoptera metric (Chir = Chironomidae, CA 1 = scores on correspondence axes 1).



D17 =SQRT((10-3)*($C11-D11)/(10+D11))

A - Univariate Analyses		%Plecoptera	%Chironomidae	CA 1	CA 2
Standardized Difference		-1.1	-0.7	-1.0	-3.6
t		-3.6	-2.2	-3.3	-11.4
P		0.006	0.053	0.009	0.000
B - Multivariate analyses	**All Indices**	**Without %P**	**Without %Chir**	**Without CA 1**	**Without CA 2**
D^2	57	22	43	25	13
D	7.5	4.7	6.5	5.0	3.5
T^2	566	218	425	254	125
F	94.3	56.5	110.3	65.8	32.5
P value (central)	0.000	0.000	0.000	0.000	0.000
P value (non-central)	0.048	0.155	0.029	0.110	0.434
		%Plecoptera	**%Chironomidae**	**CA 1**	**CA 2**
Partial T^2		3.3	1.5	2.9	4.8
F		10.7	2.3	8.3	22.8
P		0.014	0.177	0.024	0.002

Figure 32: Summary of TSA results (P = Plecoptera, Chir = Chironomidae, CA 1 = scores on correspondence axes 1).

Results and Discussion

The first 2 axes of the CA ordination accounted for 55% of the variation in the P/A data matrix (Fig. 26). Most of the reference sites clustered together in the ordination (Fig. 28), although the

Britannia stream was separated from the others along the first CA axis because of the presence of zygopteran nymphs and the absence of anisopterans, tabanids and oligochaetes that were found in all other reference streams. The relative proportions of plecopterans and chironomids in the reference streams averaged 18 and 29%, respectively. By contrast, both insect groups were less frequent in the test stream, with <1% plecopterans and 18% chironomids. When expressed as differences from the reference-stream mean in units of reference-stream standard deviations, the % plecopterans was -1.1, the % chironomids was -0.70, CA 1 was -1.0, and CA 2 was 3.6 (Fig. 32A). This summary indicated that the test stream was most different from the reference streams on CA 2 (t = -11.4, $P < 0.001$). This difference is likely due to the occurrence of isopods, megalopterans and amphipods in the test stream. No reference streams supported isopods, and relatively few reference streams had megalopterans (2) or amphipods (3).

Using the 4 metrics and the TSA method, we found that the benthic community in the test stream was significantly different from reference-stream communities (D = 7.5, T^2 = 566, F = 94.3, $P < 0.001$; Fig. 32B). Moreover, the test stream was significantly outside the **normal range** of reference streams (non-central test, P = 0.048). Based on the *partial* T^2 values, CA 2 added the most unique information (*partial* T^2 = 4.8, F = 22.8, P = 0.002), whereas the % Chironomidae metric failed to add a significant amount of unique information to the multivariate analysis (*partial* T^2 = 1.5, F = 2.3, P = 0.177). The test stream was most different from reference streams on CA 2 (highest univariate t, $P < 0.001$), and the CA 2 metric contained the most unique information relative to the other 3 indices (highest ***partial*** T^2, P = 0.002). However, P values for the univariate t tests (e.g., $P < 0.001$ for CA 2) were smaller than P values for the *partial* T^2 tests (e.g., P = 0.002 for CA 2), suggesting there was some correlation or redundant information among the indices we used. Omitting any one of the 4 indices from the multivariate T^2 test did not change our conclusion that the test stream was significantly different from reference streams (i.e., $D \neq 0$); however, 3 of the 4 non-central tests (without %P, CA 1 and CA 2) failed to indicate that the test stream was outside of the **normal range** for reference streams when any one index was removed (i.e., $D \not\geq$ **normal range**, $P > 0.05$). This result underscores the importance of our choice of summary indices in benthic community assessments and highlights the fact that our statistical power will depend on that choice.

This simple demonstration illustrates that the TSA approach is an objective way to assess whether a test site differs from a set of reference sites. The resultant P value based on the multivariate T^2 provides a single probability to evaluate a test site using a suite of summary biological indices simultaneously. Redundancies or correlations among the indices are factored out of the assessment by using the generalized distance (D). The non-central test evaluates the degree of impairment relative to a benchmark derived from the **normal range** of variation in the reference sites. Because the magnitude of D depends, in part, on the number of indices used in the assessment, we suggest using the P values associated with the tests as a means of comparing different test site analyses. We believe that the TSA method provides a relatively easy way to assess and interpret the degree of impairment at a test site. We predict this approach will also allow us to: 1) set critical effect sizes to suit the objectives of particular study design or management practice; 2) test existing knowledge about the response of benthic invertebrates to anthropogenic stressors; and 3), improve monitoring and rehabilitation endeavours by clearly identifying significant differences between test and reference sites.

Summary

To illustrate the TSA approach, we compared the benthic macroinvertebrate community from a test stream that was historically **impacted** by acid precipitation with benthic communities from a set of minimally **impacted** reference streams. Using calculations in a simple spreadsheet, we evaluated the biological condition of the test stream based on a number of summary biological indices, both individually and simultaneously. We also illustrated how to evaluate the contribution of each summary index to the assessment. Our use of a variety of summary indices to obtain a single statistical test of significance within the context of the reference condition approach provides a simple and unambiguous framework for evaluating the biological condition of a test site.

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Appendix 10: Equipment Checklist

Below is a recommended equipment list for participants in the Ontario Benthos Biomonitoring Network.

Sample Collection

- ☐ Map
- ☐ Field Sheets (lake, stream, and/or wetland), ideally printed on waterproof paper
- ☐ Multi-probe or similar device(s) for measuring pH, water temperature, conductivity and dissolved oxygen (optional)
- ☐ Metre stick
- ☐ Pencils
- ☐ Sampling device (e.g., 500 µm mesh D-net, corer, grab sampler, or artificial substrate)
- ☐ Buckets
- ☐ Squeeze bottle (for rinsing samples into jars for transportation)
- ☐ Waders
- ☐ Boat
- ☐ Safety Equipment (pylons, traffic vest, PFD, insect repellent, throw rope, etc.)
- ☐ Camera
- ☐ Stopwatch
- ☐ Labeling tape

Habitat Characterization

- ☐ Measuring tape
- ☐ Densiometer (optional)
- ☐ Current speed meter (optional)

Sample Transportation

- ☐ Permanent marker (for labeling plastic jars)
- ☐ Plastic jars (recommend 1 L to 4 L wide mouth HDPE jars)
- ☐ Alcohol or formalin preservative

Sample Processing

- ☐ White sorting trays
- ☐ Fine tipped forceps
- ☐ Small pieces of screen for scooping fast moving animals from sorting trays
- ☐ Taxonomic keys
- ☐ Petri dish
- ☐ Marchant Box and cell extraction equipment
- ☐ Ladle (or similar) for "Bucket method"
- ☐ Random number generator
- ☐ Dissection microscope (optional)

Sample Archiving

- ☐ Alcohol (isopropanol or ethanol) for preserving samples and for cleaning labels from plastic sample jars used to transport samples
- ☐ Vials for preserving samples (recommend ~ 1 oz. glass vials with plastic "polyseal caps", or similar)
- ☐ Acid resistant paper for labels inside sample container

Notes:

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