Water Quality Division

Field Methods Manual

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VERMONT

DEPARTMENT OF ENVIRONMENTAL CONSERVATION

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1. Introduction

This document replaces the 1989 Vermont Department of Environmental Conservation (VTDEC) Field Methods Manual. That document covered all VTDEC field methods for collecting solids, liquids and air samples. Since then, other Divisions within the Department have written their own field methods; narrowing the scope of this document to include only the field procedures typically used by the Water Quality Division. Many of the methods remain in their original form (Secchi, temperature, pH) while others have evolved (biological monitoring) and some are brand new (river morphology methods and multiprobes). Because many of the programs have developed detailed protocols, this document will provide an overview and refer to those detailed Quality Assurance Plans, protocols, methodologies when appropriate.

This manual does not include methods used by the River Management Program within Water Quality. Their field methods are described in two separate documents:

> (a) the Vermont Stream Geomorphic Assessment Phase 2 Handbook. Rapid Stream Assessment Handbook (2003) and;
> (b) the Vermont Stream Geomorphic Assessment Phase 3 Handbook. Survey Assessment. Field and Data Analysis Protocols (2003).

Other program-specific field methods are referenced individually through out this document.

2. Water Quality Division Fieldwork Code of Practice

These guidelines are intended for anyone conducting fieldwork in aquatic habitats. Aquatic non-native species, such as non-native fishes, zebra mussels, and Eurasian milfoil have been frequently reported from across the state. Fish diseases such as Whirling Disease are less extensive but when present in a waterbody can be of great concern. These guidelines are intended to prevent the spread of non-native species or transmission of disease from one locale to another.

Non-native species or disease can be carried in a variety of ways between waterbodies via hands, footwear or equipment of fieldworkers. This can result in the detrimental introduction of exotic species into localities containing native species which have had no prior contact with the pathogens or non-native species.

Therefore it is important for those involved in fieldwork in aquatic habitats where non-native species or diseases are present, to take steps to minimize the spread of disease or nuisance species between study sites.

1. Arrange field visits so that uncontaminated sites are visited first and sites with known non-native species are visited last in the day.
2. Inspect all equipment prior to and after use. Remove mud, snails, algae, aquatic plants and other debris from equipment if present. This includes, but is not limited to: nets, traps, boots, float tubes, vehicle tires, boats, trailers, anchors, anchor chains and all sampling equipment.
3. Avoid cleaning equipment in the immediate vicinity of the waterbody. All equipment should be washed and dried before using it again. Allow equipment to dry in the sun, but if limited by time, or if between sampling sites, a towel may be used.
4. These procedures are used for sites known to have zebra mussels or whirling disease. When working at sites with known or suspected nuisance species and/or disease problems, or when sampling populations of rare or isolated species, wear disposable gloves and change them between handling each animal. (Used disposable gloves should be retained for safe disposal in sealed bags.) Dedicate sets of nets, boots, traps and other equipment to each site being visited. Clean and store them separately at the end of each field day.
5. Drain water from motorboats and leave drain plug out. Run engine with salt water which is stored on the VTDEC Laboratory loading dock. Use every day to kill zebra mussel veligers and other non-native larva within the boat engine.
6. See Section 4.4.2. Biological contamination for specific recommendations on preventing the spread of whirling disease.

3. Physical Parameter Methods

3.1. TEMPERATURE

3.1.1. Field Thermometer (non-mercury)

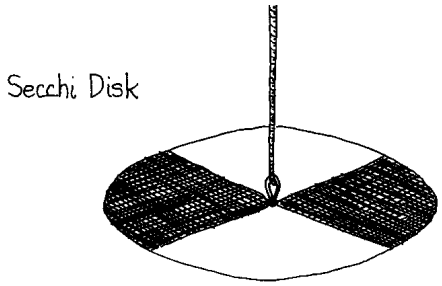
1) Thermometers must first be calibrated against a National Bureau Standards (NBS) thermometer and the correction factor should be recorded on the upper end of the thermometer.
2) Keep field thermometers in a protective case at all times.
3) To determine the temperature, immerse thermometer in the water sample for at least one minute. Keep the thermometer immersed in the water while reading the temperature. Record to the nearest degree, after adding the correction factor to the thermometer reading.

3.1.2. Thermistor

1) Thermistors must be calibrated electronically every year by certified technicians. A two-point calibration bracketing the expected temperature range should be conducted. The probe line should be measured every year and re-marked if necessary.
2) To operate meter:
   a) Plug the probe into the meter.
   b) Unwrap cable from the spool and lower the weighted probe into the water.
   c) Turn the meter on with the toggle switch.
   d) The probe line is marked in meters. Lower the probe to the desired depth, beginning at the lake surface if a profile is to be taken. Record the temperature in degrees Centigrade, to the nearest tenth degree. Be sure the meter has stabilized before taking each reading.
   e) When finished, turn the meter off, unplug the probe, and rewind the cable on the spool.

3.2. WATER TRANSPARENCY

3.2.1. Lentic Waters: Secchi Disc Transparency.

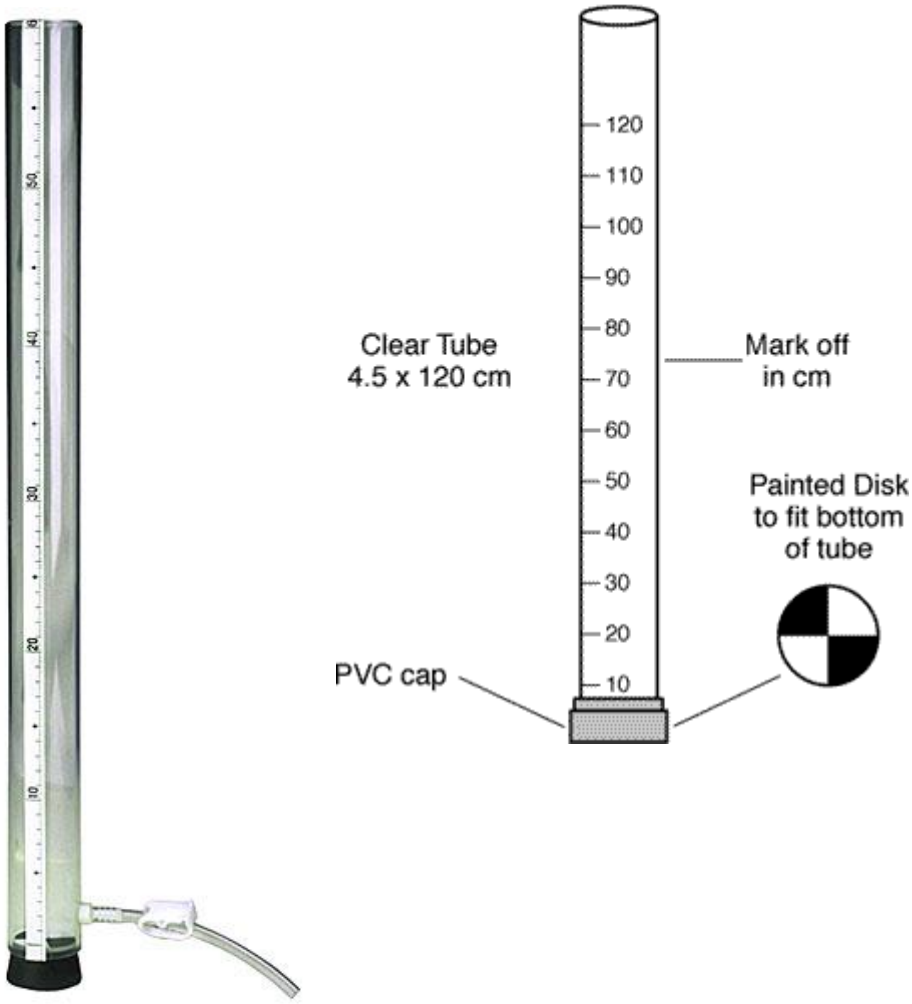


1) Background: On lakes and ponds, the most common measurement for water transparency is the Secchi disk. It consists of a 20 cm metal disk painted with alternating black and white quarters. This disk is attached to a line marked in meter increments. The optimum conditions for determining Secchi disc transparency are at mid-day on calm water.
2) Method:
   a) Lower the Secchi disc over the shaded side of the boat and remove sunglasses. Sun glare off the surface of the water interferes with accurate measurement.
   b) Lean over the side of the boat with your face as close to the water's surface as possible.
   c) Lower the disc slowly until it just disappears from view, and then raise it back up until it is barely visible. The Secchi disc transparency is the deepest depth at which the disc is still visible.
   d) The line attached to the Secchi disc is marked in meters. Estimate the Secchi disc depth to the nearest tenth meter by reading the marked line at the water's surface.
   e) If the Secchi disk is visible resting on the bottom, the actual Secchi disk transparency cannot be measured. It is important to note that the transparency was at least the depth to the bottom of the lake. Record the Secchi transparency as “>” the total depth or by placing a “B” to indicate that the Secchi hit bottom.

For Secchi instructions specific to the Vermont Laying Monitoring Program, please refer to the Vermont Lay Monitoring Program Manual, 2000 edition.

3.2.2. Lotic Waters: Transparency Tube.

1) Background
   Transparency tubes are used on running waters to measure clarity and suspended solids. The tube allows the user to collect shallow water and determine its transparency and relative turbidity. It consists of a clear plastic tube, marked off in centimeters with a Tygon tube closure valve on a Tygon release tube at the bottom.
2) Method:
   a) Fill the transparency tube with river or stream water, making sure to stand downstream of the sampling point.
   b) Peer into the top of the tube. If the black and white painted disk is visible, record the transparency as “>” the total depth in cm.
   c) If the painted disk is not visible, slowly release water through the valve at the bottom of the tube, until the disk is visible. Record the transparency in cm.



3.3. STREAM FLOW

3.3.1. Background

Stream flow, or discharge, is defined as the volume rate of flow, normally expressed in cubic feet per second (cfs). Conversion to metric units of cubic meters per second may be made by dividing by 35.31 cfs/cms.

3.3.2. Principals of flow measurement:

1) Stream flow estimation is made by summing the products of the partial areas of the stream cross-section and their respective average velocities.
2) Hydraulically, at each subsection the point velocity at 60% of the depth (0.6d) below the water surface is approximately equivalent to the average velocity in the vertical velocity profile. Place the measuring rod and meter at this location in the water column. This measurement represents the average velocity for a subsection starting midway between the last location of the meter and the present location and ending midway to the next location. Remember the subsection limits when deciding where to position the rod when irregularities in the streambed (i.e. boulders) are encountered.
3) Measure as many subsections as is practical in order to get reasonably accurate results. The general rule is to have no more than 5% of the flow in any given subsection. Therefore, readings should be closer together where there are greater depths and/or velocities. The 5% rule is primarily for larger streams. On small streams, measurements 1 to 2 feet apart will suffice. Take a minimum of ten readings.
4) It is good practice to make two discharge measurements using two different cross sections of the stream as a check on accuracy.
5) Current meters can be used effectively in most stream situations and in canals and flumes; however, if the velocities and/or depths are too low or the streambed too irregular, alternate techniques should be used in order to achieve a reasonable level of accuracy. Weirs, flumes, or volumetric techniques (i.e., a bucket or graduated cylinder) should be used. Generally, flow meters should not be used where the stream depths and velocities are less than 0.3 feet and 0.2 feet per second (fps) across most of the section.
6) Occasionally shallow or slow velocity conditions may be dealt with by physically constricting the flow in order to increase depths and/or velocities.
7) If possible, large rocks and debris should be removed from the area of the section. After the section is modified, measurements should not be started until flow conditions have stabilized. Try to select a section that does not have complex flow patterns, eddies, or cross currents.

3.3.3. SonTek Acoustic Doppler Current Meter - Flowtracker.
The flowtracker unit will be used in the field as described by Section 5 of the Operation Manual [FlowTracker Handheld ADV® Operation Manual Firmware Version 2.4 Software Version 1.20 (December 2003)]. Data processing and download will be performed per Section 6 of the Operation Manual.

3.4. TIME OF TRAVEL MEASUREMENTS

3.4.1. Background

Note: The Water Quality Division no longer routinely conducts time of travel measurements. These historical methods are included in this version of the Field Methods Manual for reference purposes only.

Time of travel measurements provide information regarding the time required for a discrete mass of water to pass through a particular river reach and is dependent upon a number of physical variables including stream gradient, velocity, flow and river bed characteristics. Time of travel measurements involve the slug injection of a fluorescent tracer dye (Rhodamine WT) at the head of the river reach during stable flow conditions and the subsequent fluorometric detection of the dye at the tail end of the reach.

3.4.2. Dye Injection

1) Equipment
    a. Rhodamine WT dye
    b. flow measuring apparatus
    c. notebook
2) Obtain the following information on the study reach:
    a. The discharge at the time of dye injection.
    b. The length of the reach in miles.
    c. The estimated velocity within the reach. This should be based on actual velocity measurements combined with knowledge of the physical characteristics of the reach.
3) Choose a dye injection site where good mixing can be achieved quickly. Dumping the dye immediately above a riffle will facilitate distribution of the dye.
4) Determine the amount of dye to dump using the following equation and the information obtained in (1) above.

$$\text{Volume Dye} = 3.4 \times 10^{-4} \frac{(Q-L)}{V}$$

Where:

Q = river discharge (cfs)

L = length of reach (miles)

V = mean velocity (fps)

5) Apply appropriate amount of dye to the river. Avoid getting the dye on skin or clothing.

6) Record the time and site of dye injection in a field notebook.

3.4.3. Direct Dye Measurement

1) Equipment

a) Fluorometer (Turner Model III) equipped with the following accessories:

- Flow-through sample door
- Primary filter #110-823 (Corning 546)
- #590 combination secondary filter
- #110-851 lamp (carry one spare)
- Strip chart recorder

b) Submersible pump with waterproofed extension cord.

c) Intake hose requires female connectors on both ends.

d) Outlet hose - female connector on one end.

e) Gasoline powered 120 volt AC generator.

f) Plug-in volt meter.

g) Floatation buoy and anchor.

h) Fluorometer operation manual.

i) Chart paper.

j) Gasoline and oil for generator.

k) Extension cord for fluorometer.

l) Stop watch.

m) Tools and spare parts necessary for equipment repair.

2) Procedure

a) Set up the fluorometer and generator near the stream bank. Attach the intake hose to the lower part of the fluorometer door and the outlet hose to the upper part. Attach the other end of the inlet hose to the submersible pump.

b) Using the float and anchor, suspend the pump in the water column in the main flow of the stream. Be sure the pump is not in contact with the bottom to avoid the intake of sediment.

c) Check the generator gas and oil. Start the generator and check the voltage. It should be between 110 and 130 volts. If not, adjust the idle until the output is within this range.

d) Plug in the fluorometer and pump. The pump will immediately begin to draw water. At all times, plug in the pump before turning on the fluorometer and turn the fluorometer off before unplugging the pump so as to avoid damage to the fluorometer due to voltage fluctuations.

e) Turn on the fluorometer and start the lamp by holding the lamp toggle switch up for five seconds and releasing. Open the door and check to make sure the lamp is lighted.

f) Set the sensitivity setting to the most sensitive position (30x). Adjust the detector dial to a reading of about five using the adjustment knob next to the dial. This is to adjust for background fluorescence.

g) Check the strip chart recorder to insure an adequate supply of paper. Record the date, river reach, river flow, time of dye injection and staff names on the strip chart.

h) Turn on the strip chart recorder and run until a steady baseline is established for five to ten minutes. Record the sensitivity setting on the strip chart. The recorder may be turned off at this point until the leading edge of the dye plume is first detected by the fluorometer.

i) When the detection dial of the fluorometer begins to move upward, indicating the arrival of the leading edge of the dye plume, turn the recorder back on and record the time on the strip chart. Do not shut off the equipment until the entire dye plume has passed.

j) The dye concentration should increase rapidly. As the dial approaches 90, quickly change the sensitivity level to the next setting (10x) and record the new sensitivity setting on the strip chart. Record the time on the strip chart periodically. Repeat to the 3x and 1x levels as needed. If the reading goes off the high end of the scale at the 1x sensitivity level, record the time on the strip chart, turn off the strip recorder and wait for reading to come back on scale. Then turn on the strip chart again and record the time.

k) At some point, the concentration will peak and then decrease. In most cases the decrease will be much slower than the increase as the dye has a tendency to tail off behind the initial concentration. As the concentrations decrease, adjust the sensitivity settings to maintain a dial reading above 10. Be sure to record all sensitivity changes on the strip chart.

l) At the detection point, the dye plume will not be visible. The dispersion of the dye plume will vary depending on the physical characteristics of the reach. A series of pools and riffles will tend to stretch the plume out, whereas more uniform velocity characteristics

will maintain a more compact plume, in most cases, the shape of the plume.

m) When the concentration approaches the background level turn off recorder and record the time on the strip chart, turn off the fluorometer BEFORE unplugging the pump. Remove the strip chart from the recorder.

3.4.4. Indirect Dye

1) Equipment

a) ISCO automatic sampler with individual bottles. (See section 7.2. for operating instructions.)

b) Fluorometer (Turner Model III) equipped with the following accessories:

- cuvette sample door
- primary filter #110-823 (Corning 546)
- #590 combination secondary filter
- #110-851 lamp
- cuvettes

2) Procedure

a. Estimate when the leading edge of the dye will reach the sampler location and program an appropriate delay into the sampler.

b. Estimate the time necessary for all of the dye to pass the sampler. The sampler holds 28 bottles. Use the first bottle to determine if the sampler is functioning properly and for use as a background fluorescence blank. Divide the time by 27 to determine the sampling interval and program the interval into the sampler. An example: The dye will take about 9 hours to pass through - 9 hours x 60 min./hour - 540 min./27 = 20 min. sampling interval.

c. Collect the samples when the sampler has finished. Use the fluorometer to determine if the dye concentration is at background level. If it is not, set up the sampler again with new bottles.

d. Use the fluorometer to determine the dye concentration in each bottle.

3.5. FIELD PH

3.5.1. Definition

pH is defined as the negative logarithm of the hydrogen ion concentration. The greater the concentration of hydrogen ions, the lower the pH value. The pH scale ranges from 0 to 14 with 7 being neutral. A pH below 7 is acidic, a pH above 7 is basic or alkaline. The pH scale is logarithmic, therefore a pH of 5 is 10 times more acidic than a pH of 6 and 100 times more acidic than a pH of 7. pH changes immediately with temperature and as it interacts with air and any suspended solids. Readings should be measured as soon as possible, and sample temperature should be recorded.

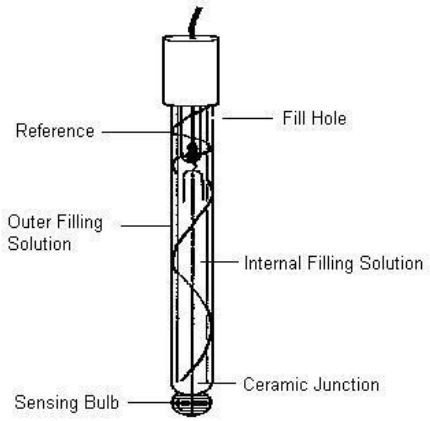
3.5.2. Background

'Measurement of pH is one of the most important and frequently used tests in water chemistry. Practically every phase of water supply and wastewater treatment, e.g., acid-base neutralization, water softening, precipitation, coagulation, disinfection, and corrosion control, is pH-dependent. pH is used in alkalinity and carbon dioxide measurements and many other acid-base equilibria. At a given temperature the intensity of the acidic or basic character of a solution is indicated by pH or hydrogen ion activity.

The pH value of a highly dilute solution is approximately the same as the negative common logarithm of the hydrogen ion concentration. Natural waters usually have pH-values in the range of 4 to 9, and most are slightly basic because of the presence of bicarbonates and carbonates of the alkali and alkaline earth metals.' (Standard Methods for the Examination of Water and Wastewater - APHA (1998) pp. 4-86 to 4-87.)

3.5.3. pH electrodes

1) Selection: For low pH solutions (<pH 6), use a calomel electrode. For high pH solutions (>pH 10), use a "low sodium error" electrode. For solutions closer to neutral, a standard pH probe is adequate.
2) Storage: Note: Never store a pH probe in distilled water.
   For short period of times (<1 week), pH electrodes should be stored with their fill hole covered and the ceramic junction submerged in electrode- storage solution. For non-refillable probes, cover the ceramic junction with a protective cap filled with electrode storage solution.



For longer period of times, the sensing bulb of the pH probe should be covered with a protective cap filled with storage solution and the fill hole covered.

3) Maintenance: The solution level in refillable electrodes should be checked frequently and replenished as needed with the appropriate electrolyte. Gel filled reference electrodes leak the slowest but are not refillable and will generally run out of reference solution in about a year. These should be replaced every 6 months. If crystals form inside a refillable electrode, or if the pH probe starts to drift or respond slowly, drain the filling solution and replace.
4) Care: Avoid wiping the bulb of a pH electrode. Electric static may occur and cause polarization.

3.5.4. Great Lakes pH Meter (Model 819)

1) General
These portable pH meters are available for use by Agency of Natural Resources staff and may be borrowed from the Biomonitoring and Aquatic Studies Section (241-3777). This field meter is highly susceptible to moisture. Do not attempt to perform field pH measurement on wet or rainy days. Simply collect the sample and perform the analysis in a vehicle or nearby building.
2) Equipment needed:
   a) pH meter
   b) pH electrode
   c) Kimwipes
   d) Distilled water
   e) Sample cup (glass or plastic beaker)
   f) pH buffers (4, 7 and/or 10)
3) Calibration and Measurement Procedure
   a) When calibrating a pH meter, use fresh certified standard pH buffers. The two buffers selected should be no more than 3 pH units apart and should bracket the expected pH value. Use pH 4, pH 7 and/or pH 10 buffers dailly. Top off the pH electrode with 4m KCl electrode filling solution if the level drops below the fill hole. Rinse out and refill the electrode if the solution has crystallized at the tip. .
   b) Attach the pH electrode to the BNC connector and turn meter on to "pH".
   c) Using a thermometer, take the temperature of the buffers and set temperature control accordingly.
   d) Rinse electrode with distilled water and gently pat dry with a Kimwipe.
   e) Put electrode in buffer 7 solution and allow reading to stabilize. Then calibrate by adjusting pH knob to 7.00.
   f) Take out the electrode and rinse thoroughly with distilled water. Blot dry and place electrode in buffer 4 or 10 solution (you may need to

reset the temperature control if the buffers are not the same temperature). When the meter has fully stabilized, adjust the mV/pH knob to 4.00 or 10.00.

g) Rinse the thermometer with distilled water and blot dry with a Kimwipe. Determine the temperature of the sample. Collect sample in a well rinsed sample bottle or beaker. Do not attempt to take a pH measurement in flowing water because of the likelihood of drift causing an inaccurate result.

h) Adjust temperature control to proper temperature of the sample. Place rinsed electrode into sample and allow meter to stabilize. This meter may take up to 2 minutes.

3.5.5. Hanna waterproof portable pH meter (Model 9025)

1) General
This meter is primarily used by the Long-Term Monitoring Program for acid sensitive lakes (VLTM). It is equipped with a calomel reference electrode which more accurately measures low ionic strength (low pH) solutions.

2) Equipment needed:
   a) pH meter
   b) pH electrode
   c) Kimwipes
   d) Distilled water
   e) Sample cup (glass or plastic beaker)
   f) pH buffers (4, 7 and/or 10)

3) Calibration
   a) When calibrating a pH meter, use fresh certified standard pH buffers. The two buffers selected should be no more than 3 pH units apart and should bracket the expected pH value. Use pH 4, pH 7 and/or pH 10 buffers daily. Ensure that the electrode filling solution is above the ceramic junction and at least one inch above the sample level when immersed (see pH electrode figure in section 3.5.3). If not, fill electrode with 4m KCl electrode filling solution. Rinse out and refill the electrode if the solution has crystallized.
   b) Attach the pH electrode to the BNC connector and turn meter on.
   c) Remove protective cap from pH electrode tip. Rinse pH electrode and ATC probe with distilled water and pat dry with a Kimwipe.
   d) Put electrode in buffer 7 buffer solution, stir gently and press “CAL” button. Display will flash “CAL” and “NOT READY”. The bottom

of the screen displays the buffer the meter is looking for. The default value for the first buffer is 4. Use the up and down arrow keys (🡅°C or 🡇°C) to bring the value to 7.01. When reading has stabilized, the meter's screen will read "CAL, READY", and "CON" will be flashing. Press "CFM" to confirm the first calibrant.

e) Rinse pH electrode and ATC probe with distilled water and pat dry with a Kimwipe.
f) Put electrode in pH 4 buffer solution and stir gently. Make sure the lower right corner display reads "BUF 2 4.01 pH".
g) Display will flash "CAL" and "NOT READY" until the reading for the second buffer has stabilized. When reading has stabilized, the meter's screen will read "CAL, READY", and "CON" will be flashing. Press "CFM" to confirm the second calibrant. If the second buffer is pH 10, use the up and down arrow to adjust the value for the second buffer. The meter is now calibrated and ready to read samples.

4) Measurement Procedure
   a) Rinse pH electrode and ATC probe with distilled water and pat dry with a Kimwipe. Rinse sample cup with distilled water, then rinse sample cup with sample water.
   b) Dip probe into sample water and gently stir. Wait for reading to stabilize. Record reading.
   c) Turn off meter. Replace cap on probe filled with buffer solution to prevent wick from drying out. Cover filling hole on pH probe with protective sleeve.

3.5.6. Hanna portable waterproof pH meter (Model 9023)

1) Calibration
   a) When calibrating a pH meter, use fresh certified standard pH buffers. The two buffers selected should be no more than 3 pH units apart and should bracket the expected pH value. Use pH 4, pH 7 and/or pH 10 buffers daily. Fill up the pH electrode with 4m KCl electrode filling solution if the level drops below the hole. Rinse out and refill the electrode if the solution has crystallized at the tip.
   b) Attach the pH electrode to the BNC connector and turn meter on with "ON/OFF" toggle button.
   c) Remove protective cap from pH electrode tip. Rinse pH electrode and ATC probe with distilled water and pat dry with a Kimwipe.
   d) Put electrode in buffer 7 buffer solution, stir gently and press "CAL" button.
   e) The temperature compensated pH value will flash on the screen. Wait for the "pH" symbol to stop flashing, then press the "CFM" key.
   f) Rinse pH electrode and ATC probe with distilled water and pat dry with a Kimwipe.
   g) Place the pH probe into the second buffer (4 or 10) and stir gently. When the reading stabilizes, the "pH" symbol will stop flashing. Press

the “CFM” key to complete the two point calibration. The meter is now calibrated and ready to read samples.

2) Measuring the pH of solutions.
   a) Rinse pH electrode and ATC probe with distilled water and pat dry with a Kimwipe. Rinse sample cup with distilled water, then rinse sample cup with sample water.
   b) Place probe in sample water and gently stir. Wait for reading to stabilize. Record reading.
   c) Turn off meter. Replace cap on probe filled with buffer solution to prevent wick from drying out.

3.6. FIELD CONDUCTIVITY

3.6.1. Definition

Conductivity is the ability of an aqueous solution to conduct an electrical charge. Conductivity, or specific conductance can be correlated to the water's ionic strength. By using a conductivity meter the electrical charge is defined by a numerical expression.

3.6.2. Overview

Most freshwater lakes and rivers in Vermont have specific conductance values ranging from 10 to 1000 µS/cm. Remote acid sensitive water bodies which are low in nutrients and buffering capacity typically have values less than 25 µS/cm. The opposite is found in highly buffered, enriched or otherwise polluted water bodies where values will be considerably higher. Runoff of road salt into neighboring waterbodies also yields high conductivities.

The analysis is performed using a conductance meter and a cell (probe). The meter and cell should be calibrated frequently using potassium chloride standards of known conductance. Use standards with values above and below the expected range of the samples.

Suspended solids or humic acids will in time build up on the conductivity cell and periodically need to be removed according to the manufacturer's recommendations.

Conductivity samples have a 28 day holding time, but like most analyses, the sooner analysis is performed the better. Contact with a plastic bottle alone will slowly change the result, and thus immediate conductivity measurements will eliminate this source of error.

In river sample analysis, the principle objective of field conductivity measurements is to locate and trace a pollutant source or "plume". For instance, conductivities should be expectedly higher below a treatment plant outfall or in the vicinity of landfill leachate.

3.6.3. Hanna HI 9033 portable waterproof conductivity meter

1) Calibration in laboratory
   a) Unlike pH meters, a conductivity meter need not be calibrated daily. Turning off the instrument will not affect the meter's accuracy. A standard should be used at least weekly to verify the accuracy of results. Use a calibrant that is close to the conductivity of the waters being measured. The calibration solution should be at 25°C when measured.
   b) Fill a clean beaker with calibrant. Make sure probe is connected to the meter by pushing in the plug and tightening the threaded pin. Rinse conductivity probe with distilled water and pat dry with a Kimwipe. Rinse conductivity probe with sample water. Dip probe into calibrant. Make sure that the sample water is above the air release holes on the probe.
   c) Turn meter on by pressing the "ON/OFF" button and select the appropriate range (e.g.199.9 µS/cm). Tap the conductivity probe on the bottom of the beaker in order to release any air bubbles caught inside the probe.
   d) Allow reading to stabilize.
   e) Use a flathead screwdriver to adjust the calibration trimmer until the meter reads the correct conductivity value. All measurement will be temperature compensated to 25°C.

2) Measuring conductivity
   a) Turn on the meter. Rinse the cell with distilled water and pat dry with a Kimwipe.
   b) Place cell into the sample making sure the water comes above the cell holes. Drift may occur if the cell is placed directly in flowing water. It is best to collect sample in a 1000 ml bottle and place the cell directly into the bottle. After testing and recording result along with temperature toss out the bottle. Do not carry out further parameter analysis after contaminating sample with cell probe.
   c) With cell in sample bottle, select one of the four buttons with the expected range of, i.e. 199.9 µS, 1999 µS, 19.99 mS or 199.9 mS. Wait for the reading to stabilize and recorded the value (typically within one minute).
   d) Remove cell from bottle and rinse with distilled water. Pat dry cell and return meter and cell to carrying case.

3.7. MULTIPLE PARAMETER PROBE

3.7.1. Background

Multiple parameter probes are used in many water quality applications. Currently, within the Water Quality Division, the Lake Assessment and Biomonitoring Programs use them regularly to provide a snapshot of a basic array of water quality parameters. These mutliprobes have two units: (1) a sonde containing multiple probes which is submerged in a waterbody and (2) a surveyor unit which stores the data.

Hydrolab minisonde unit

Model MS4A (c) 2002 Campbell Scientific (Canada) Corp.

Hydrolab surveyor unit

Model S4A (c) 2001 Campbell Scientific (Canada) Corp.

3.7.2. Hydrolab Minisonde 4 and Surveyor 4.

The following protocol is entitled the VTDEC Water Quality Division Hydrolab SOP. It has been reproduced from Appendix C of the Vermont Nutrient Development Project Quality Assurance Project (2004).

1) Equipment
   - Hydrolab Minisonde
   - Hydrolab Surveyor
   - Hydrolab depth cable
   - Altimeter
   - Barometer
2) Parameters measured
   - Temperature
   - Dissolved Oxygen
   - pH
   - Conductivity
   - Depth
3) Calibration
   a) Laboratory calibration. The multiprobe should be calibrated each day in the laboratory for best performance, and a minimum of once per week. Follow the Hydrolab laboratory calibration, maintenance and power charging schedule as outlined in the Hydrolab Standard Operating Procedure in Appendix A of this document.

b) Field Calibration. Calibrate the dissolved oxygen by determining the barometric pressure at each sampling site.
c) Annotating Files. Follow the following key strokes: Files, Svr4a, Annotate, Select Program the data should be filed under (e.g.'BASS REMAP'), Enter name of site, Done, Hit any key, Hit Go Back until Go Back is no longer a selection.
d) Record Data
   i) Hang or lay Hydrolab in stream or lake. Wait until temperature equilibrates. Hit 'Store'. Select 'BASS REMAP' or 'Lake Assessment'. Wait until logging active note disappears.
   ii) Record on backup data sheet. If in lake, drop at 1 m intervals with last reading 1 m above bottom. At each depth, wait for the readings to stabilize (equilibrate) and hit 'Store'.
   iii) If in lake, pull up to 1 m, equilibrate and hit 'Store' to check pH against first reading at this depth.
e) Review Files
   i) In order to review the data that has been logged in, follow these key strokes. Files, Svr4a, Review, Select Program you are working on (e.g. 'BASS REMAP' or Lake Assessment').
   ii) Choose to review from beginning or by date and time, Done, Hit 'Go Back' until Go Back is no longer a selection.

3.7.3. Hydrolab Minisonde 4a and Surveyor 4a.

1) Parameters measured
   - Temperature
   - Dissolved Oxygen (DO)
   - pH
   - Conductivity
   - Depth
   - Chlorophyll a
   - Redox potential (Oxydation Reduction Potential or ORP)
2) Calibration
   a) Laboratory calibration
      The multi-probe should be calibrated each day in the laboratory for best performance. Follow the Hydrolab laboratory calibration and maintenance schedule as outlined in the Hydrolab Standard Operating Procedure in Appendix A of this document.
   b) Field Calibration: LOTIC MEASUREMENTS
      a. At the each sampling site, calibrate the dissolved oxygen.
      b. Connect Sonde to Surveyor (careful to pull plug off – DO NOT TWIST)
      c. Remove black cap off calibration cup. Cover sensors with ambient water to "O" ring on membrane. Set black cap over calibration cup (to keep temperature from fluctuating).

d. TURN STIRRER ON If it is not, use the following steps to turn it on: Setup/Cal on Surveyor, Setup, Sonde, Select 'Circltr: Off/On, Select 1 for On, Done, Hit any key, Hit Go Back until Go Back is no longer a selection.
e. Note BP reading, Setup/Cal, Calibrate, Sonde, Select, 'DO%', Enter BP, Done, Hit any key, Hit Go Back until Go Back is no longer a selection.
f. DO calibration complete.

c) Field Calibration: LENTIC MEASURMENTS. If the Hydrolab is being used in a lake or reservoir, the depth measurement must be calibrated at each lake. Follow these steps:
   i) Setup/Cal, Calibrate, Sonde, Select 'Dep100: meters', Hang Hydrolab sensors to 1 m depth, Select 1.0, Done, Hit any key, Hit Go Back until Go Back is no longer a selection
   ii) ANNOTATE FILES. Complete at each site: Files, Svr4a, Annotate, Select a Program (e.g. W.S. Reservoirs), Enter name of site, Done, Hit any key, Hit Go Back until Go Back is no longer a selection.
   iii) REVIEW FILES. Review the files to check what has been logged in. Complete the following steps: Files, Svr4a, Review, Select Program (e.g. W.S. Reservoirs), Choose to review from beginning or by date and time, Done, Hit Go Back until Go Back is no longer a selection.

3.8. RAIN FALL

3.8.1. HOBO MODEL 400 RAINGAUGE

1) General
   This rain gauge is used primarily by the Lake and Ponds Management and Protection Section in order to measure rainfall volumes during non-freezing conditions for watershed modeling.
2) Equipment

- HOBO Shuttle
- HOBO Data logging rain gauge
- 12" logger interconnect cable
- rigid post or stake
- 4 hose clamps
- BoxCar program

3) Launch
   a) Before heading into the field, the gauge and shuttle must be "launched" using the HOBO launch command.
   b) Connect the gauge to the host computer with the cable. Run BoxCar and select "launch" from the Logger menu. The software automatically synchronizes the Shuttle's clock with the host computer's clock, and displays the Shuttle's battery level. If the battery level is low (32% or less), change them immediately. If batteries are changed in the field, the Shuttle loses its time synchronization and will not offload any loggers until it is launched or read out.
   c) Repeat step 2 with the Shuttle.
   d) Disconnect the Shuttle from the host computer when you are finished. Leaving the Shuttle connected to the computer (or a logger) drains the batteries.
   e) Disconnect the 12" cable from the Shuttle, coil it, and store in the Shuttle case (Disconnecting the cable from the Shuttle prevents the cable from being strained when you close the lid). Be careful to position the cable in the case so that it does not engage the button when the lid is closed.

4) Calibration. Before using rain gauge, calibrate it by allowing 373 mls of water to pass at a slow rate (~1l/hr) through the unit. This should equal 98-102 "tips" of the gauge, or 19.6-20.4 mm of rainfall.

5) Operation
   a) Locate a canopy-free area and install with rain gauge level and above surrounding vegetation. Use a rigid post and secure unit to post with clamps.
   b) Blinking red LED light ensures battery is operating. Close and secure lid.

6) Download Data from rain gauge

   a) Remove any moisture from the Shuttle and/or data logger cases before opening to prevent damage to internal electronics.
   b) Remove rain gauge top with the single thumbscrew.
   c) Plug the 12" cord into logger and into the Shuttle. Offloading begins automatically. An orange "Offloading" light on the Shuttle will flash during this process.
   d) Next the Shuttle automatically checks the logger's battery voltage. If the logger's batteries are below 30%, the Shuttle blinks "Change Logger Battery" but this has not interfered with the data offloading process.
   e) If the logger's batteries are at more than 30%, the Shuttle flashes "Testing". This gives the user an opportunity to test the operation of the logger by manually tipping the rain gauge bucket.
   f) Manually trip the tipping unit. If the logger is working, the "Testing" light will quickly flash twice for each event. These tests are not recorded as rainfall.
   g) When testing is completed, press the button on the Shuttle. The Shuttle then automatically relaunches the logger and synchronizes the logger's clock to the Shuttle's internal clock. The Shuttle's orange "Relaunching" light flashes during this step.
   h) When the relaunch is completed, the "Successful" light flashes until the cable is disconnected from the logger and/or Shuttle.
   i) Store the cable in the Shuttle case without allowing it to press down on the button. To check the Shuttle's batteries, depress the button and hold for three seconds while the shuttle is disconnected and read the battery level indicator chart to the left of the lights.
   j) Rain gauge data now stored in the shuttle must then be loaded into a PC using the "BoxCar" software prior to the next usage.

These sections are excerpted from the Hobo Shuttle Operating Instructions (Part # H09-003-08). 2004. Onset Computer Corporation.

4. General Water Sample Collection Methods

4.1. SAMPLE CONTAINERS AND SAMPLE PRESERVATION

4.1.1. Ambient Water

Through the Water Quality Field Methods Manual, the VTDEC Laboratory provides written protocols for the collection and preservation of samples it accepts. The Laboratory provides specially cleaned non-interchangeable sample containers, preservation materials, and instruction to sampling personnel. It requires sampling personnel to have followed these protocols when samples are collected. Contact the Laboratory to request bottles and approve sampling schedules (802-244-4522). The Laboratory policy is to reject any sample that:

- has been improperly preserved
- has been taken in a non-approved container
- has exceeded the hold time for analysis

1) Preservation: All samples requiring refrigeration (4ºC or freezing) should be stored on ice from the time of sampling until placed in laboratory refrigeration.

2) Labeling: Samples need to be temporarily labeled in the field. Field labels should contain the following information:
   - Location
   - Stream mile or lake sample depth
   - Collection Date
   - Collection Time
   - Tests requested
   - Quality Assurance indicator (e.g. duplicate, spike)

3) Logging in Samples to the VTDEC Laboratory: When samples are logged into the Laboratory Information Management System (LIMS) permanent labels containing the following information are generated:

   - Customer: (Program Number)
   - Collection Date
   - Collection Time
   - Location
   - Test Requested
   - Sample Number
   - Bar Code

See Figure 4.1.1. for an example of a sample label.

Figure 4.1.1. LIMS Sample Label

4.1.2. Metals

1) See Summary Table 4.1.7 for sample volumes required.
2) Preservation: Sampling personnel are responsible for acidifying metal samples to 0.2% HNO_3 (2 ml concentrated HNO_3/ liter sample). Acids for preservation may be found in the Sample Prep room of the VTDEC Laboratory (room 142).
3) Hexavalent chromium samples should NOT be acidified. The laboratory needs prior notice for this parameter.
4) See Section 5.6. for additional instructions.

4.1.3. Bacteriology

1) The laboratory needs prior notice before submitting bacteria samples. Samples should arrive at the Laboratory within 6 hours of sample collection. Separate containers are required for each test requested. An inch of headspace is required to insure proper mixing. Samples must be kept on ice from the time of sampling until refrigerated at the laboratory.
2) Samplers are instructed to use sterilized 120 ml sterile sealed containers unless dechlorination is necessary.
3) Chlorinated samples must be dechlorinated at the time of collection. On request, the laboratory will provide sterile 250 ml containers containing sodium thiosulfate. The sodium thiosulfate in the sample bottle will neutralize 250 ml of water containing up to 15 ppm chlorine. Chlorinated samples typically originate from a wastewater treatment plant or water supply source.
4) Total coliform and *Escherichia coli* samples require a 24 hour incubation period; currently samples are accepted Monday through Wednesday. Samples must arrive at the laboratory prior to 3 p.m. unless special arrangements have been made.
5) Specific sampling instructions can be found in Section 5.2.

4.1.4. Anions/Nutrients.

1) See Summary Table 4.1.7. for sample volumes required.
2) Rinse sampling container three times with sample water before filling with sample water.
3) Acidify Nitrate-Nitrite, Total Nitrogen and Total Kjeldahl Nitrogen to <2 pH units.

4.1.5. Organics-Volatile

1) Samples are collected in 40 ml glass vials with silicone/Teflon septa, reserved specifically for sampling volatile organic chemicals. They are collected without headspace and capped with the Teflon side of the septum down.
2) Two sample vials are required. Acid is added to both vials before capping (5 drops 1:1 HCl/vial).
3) Fill each bottle until the sample crowns over the top of the bottle. When tightly closed, the Teflon cap should bulge slightly. Bottles are then inverted and tapped to reveal hidden air bubbles. If present, bottles should be reopened and filled further with sample.
4) Samples are to be kept on ice from the time of sampling until refrigerated at the laboratory.
5) Each sampling effort will require the sampler to carry two trip blanks which contain laboratory reagent water and label them as trip blanks.
6) More specific sampling instructions can be found in Section 3.3 of this manual.

4.1.6. Organics - Semi-Volatiles (Base Neutrals, Acids); PCBs, Pesticides

1) Water samples are collected in 2.6 liter amber glass containers with Teflon lined caps. If the samples are taken from chlorinated sources, then the laboratory must add sodium thiosulfate (0.008% $Na_2S_2O_3$) to the containers prior to sampling.
2) Sample containers should NOT be pre-rinsed and compositing should be done in the sampling container.
3) Avoid plastic sampling materials, as they will introduce artifacts into the analysis. Sample removal equipment and/or funnels must be made of glass or Teflon. Do not use Tygon tubing.
4) Project plans calling for the use of automatic sampling devices should be discussed with the laboratory before sampling.
5) If the pH of the sample is not between 5.0 and 9.0, the hold time for PCBs and Pesticides is shortened and the laboratory needs to be notified as soon as possible.
6) Procedures 8270 (Semi-volatiles) and 8080 (PCBs, Pesticides) can be performed on this sample. No preservative is added. Refrigeration is required. Samples should be kept on ice from the time of sampling until refrigerated at the laboratory.
7) To perform the complete semi-volatile, PCB and Pesticide analyses one sample is required.
8) See Sections 5.7. (Semi-volatiles) and 5.8. (PCBs, Pesticides) of this manual for more specific instructions.

Table 4.1.7. Parameter Summary Chart: Required Containers, Preservation and Holding Times

Parameter	Sample Type	Container	Preservation	Maximum Hold Time	Note
METALS					
Cations, (calcium, magnesium, potassium, sodium)	Water	P, 125ml	HNO_3 to pH < 2, filter 0.45µm for dissolved	6 months	a, b
Mercury, Low Level	Water	G, 125ml, Teflon lined cap, acid rinsed or 500ml Teflon bottle	12N HCl (5ml/l) and BrCl solution (5ml/l); preserved at the laboratory in original bottle	28 days to preservation – 90 days to analysis	o
Metals, (all metals and cations)	Water	P, 250 ml	HNO_3 to pH < 2, filter 0.45µm for dissolved	28 days for Mercury, 6 months for remainder	a, b
Metals (TCLP - see footnote m)	Liquid Sludge	P, 1000 ml or P, 2000ml (2 containers/sample) Sample size depends on % solids. Consult Lab Supervisor.	Cool 4°C	6 months, Mercury as soon as possible	l
MICROBIOLOGY					
Coliform-Total	Water	P, 250 or 120 ml sterile	Cool, 4°C	8 hours (30 hours drinking water)	c, d, n
E. Coli	Water	P, 250 or 120 ml sterile	Cool, 4°C	8 hours (30 hours drinking water)	c, d, n
INORGANIC CHEMISTRY					
Alkalinity	Water	P, 250 ml	Cool, 4°C	14 days	
BOD Uninhibited 5-Day	Water	P, 2 L	Cool, 4°C	24 hours	c, f
Chloride	Water	P, 50 ml centrifuge tube	none required	28 days	
Chlorine	Water	G, 125ml amber	Cool, 4°C	analyze immediately	
Chloride-(Ion Chromatography)	Water	P, 50 ml centrifuge tube	Cool, 4°C, filter 0.45µm	28 days	j
Chlorophyll-a	Water	glass fiber filter, Whatman GF/A, 4.7mm, 7µm pore size stored in black jar	Freeze filter in black jar -20 to -70°C	21 days	
COD	Water	P, 50 ml centrifuge tube	Cool, 4°C, H_2SO_4 to pH < 2	28 days	e
Conductance	Water	P, 250 ml	Cool, 4°C	28 days	
Cyanide, Total	Water	P, 2 L brown **and** P, 250 ml, with preservative added	Cool, 4°C, NaOH to pH >12, ascorbic acid	n	g, d, n
Dissolved Oxygen, Probe	Water	G, 300 ml D.O. bottle	none required	analyze immediately	
Dissolved Oxygen, Winkler	Water	G, 300 ml D.O. bottle	MnSO4, I-/Azide, dark	8 hours	h
Ignitability	Solid or Liquid	G, 250 ml	Cool, 4°C		

Parameter	Sample Type	Container	Preservation	Maximum Hold Time	Note
Nitrogen, Ammonia	Water	P, 250 ml	Cool, 4°C, H_2SO_4 to pH < 2	28 days	e
Nitrogen, Nitrate (Ion Chromatography)	Water	P, 50 ml centrifuge tube	Cool, 4°C filter (0.45µm) **DO NOT ACIDIFY**	48 hours	j
Nitrogen, Nitrate + Nitrite	Water	P, 250 ml	Cool, 4°C, H_2SO_4 to pH < 2	28 days	e
Nitrogen, Nitrite	Water	P, 50 ml centrifuge tube	Cool 4°C, filter **DO NOT ACIDIFY**	48 hours	c, j
Nitrogen, Total Kjeldahl	Solid	P, 250 ml	Cool 4°C	28 days	
Nitrogen, Total Kjeldahl	Water	P, 250 ml	Cool 4°C, H_2SO_4 to pH < 2	28 days	e
Nitrogen, Total Persulfate	Water	P, 50 ml centrifuge tube	Cool 4°C, H_2SO_4 to pH < 2	28 days	e
pH	Water	**analyze immediately**	none required	analyze immediately.	
Phosphorus-Ortho	Water	G, 75 ml tubes	Filter immediately (0.45µm), Cool, 4°C	48 hours	c, j
Phosphorus, Total	Water	G, 75 ml tubes	none	28 days	k
Phosphorus, Total Dissolved	Water	G, 75 ml tubes	Filter immediately (0.45µm)	28 days	j, k
Silica	Water	P, 50 ml centrifuge tube	Filter immediately for dissolved (0.45µm) Cool, 4°C	28 days	
Solids - Total Dissolved	Water	P, 250 ml	Cool, 4°C	7 days	
Solids - Total Suspended	Water	P, 1 L	Cool, 4°C	7 days	
Solids-Total Volatile	Water	P, 250 ml	Cool, 4°C	7 days	
Sulfate (Ion Chromatography)	Water	P, 50 ml centrifuge tubes	Cool, 4°C, filter 0.45µm	28 days	j
Turbidity	Water	P, 250 ml	Cool, 4°C	48 hours	

ORGANICS					
Pesticides & PCBs	Water	G, 2.6 L amber, Teflon lined cap, methylene chloride rinsed	Cool, 4°C, Pesticides & PCBs need to be pH 5-9	7 days to extraction, 40 days after	d
Semi-volatiles or TPH (nonvolatile)	Water	G, 2.6 L amber, Teflon lined cap, methylene chloride rinsed	Cool, 4°C	7 days to extraction, 40 days after	d
TPH Immunoassay Screen	Solid	P, 50 ml centrifuge tube	Cool, 4°C	14 days	c
TPH Immunoassay Screen	Water	P, 50 ml centrifuge tube	Cool, 4°C	14 days	c
Volatile Organics or TPH (volatile)	Water	G, two 40 ml vials per sample, Teflon lined septa. Trip blanks.	Cool, 4°C, HCl to pH <2	14 days	i, d
Volatile Organics or TPH (volatile)	Solid	G, 40ml vial pre-weighed, with methanol. % solids container without methanol.	Cool, 4°C	14 days	n
Volatile Organics	Air	Air Cannister 6L	Room Temperature	30 days	m

G = Glass
P = Plastic (Nalgene)

NOTES:

a "Dissolved" samples or turbid samples must first be filtered through a 0.45 µm membrane filter followed by acid preservation. A filter blank must be submitted with each batch of filtered samples.

b 2 mls concentrated HNO_3/1000 mls and 0.5 mls/250 mls of sample; 0.2 mls/125 mls.

c Lab needs prior notice for this parameter.

d Chlorinated samples need to be collected in sample bottle containing 0.008% $Na_2S_2O_3$ (0.0032g for 40ml; 0.16g for 2000ml; 0.208g for 2600ml). Sample bottles (250ml) for microbiology tests contain 0.25mls of 10% $Na_2S_2O_3$.

e 0.5 mls concentrated H_2SO_4/250 mls and 0.1 mls/50ml centrifuge tube.

f Hold time is from the end of sample collection period for composite samples. Maximum composite time is 24 hours.

g Test for sulfides in the field prior to preservation. Test kit and instruction provided with sample bottles. If positive for S, bring sample to lab in brown bottle for filtration. If negative, collect in bottle containing ascorbic acid and NaOH.

h Samples must be "fixed" in field with 2 mls of manganese sulfate (DO #1) and then 2 mls of iodide-azide (DO #2), stored in the dark and analyzed within 8 hours.

i No head space, vials pre-acidified with 0.4 mls 1:1 HCl. Two trip blanks must be brought into the field with each sample set.

j Samplers must filter sample through 0.45 um membrane filter. For ion chromatography, a single sample container of 50 mls is sufficient for all three anions (chloride, sulfate and nitrate).

k Samplers must fill tubes to 50 ml line.

l TCLP work requires two separate samples of the listed volume. Sample must undergo TCLP extraction within the following time periods (Table 4.1.8.)

m Sample handling procedures are described in the Air Toxic Quality Assurance Plan.

n Sampling instructions provided by lab must be followed.

o Sampling instructions outlined in the following method should be followed: Method 1669, "Method for Sampling Ambient Water for Determination of Metals at EPA Ambient Criteria Levels", U.S. Environmental Protection Agency, April 1995 with January 1996 revisions.

Table 4.1.8 . SAMPLE MAXIMUM HOLDING TIMES (Days)

	From: Field Collection **To: TCLP Extraction**	**From: TCLP Extraction** **To: Preparative Extraction**	**From: Preparative Extraction** **To: Analysis**	**Total Elapsed Time (Days)**
Volatiles	14	NA	14	28
Semi-volatiles	14	7	40	61
Mercury	28	NA	28	56
Metals except mercury	180	NA	180	360

NA = Not Applicable

4.2. MANUAL WATER SAMPLE COLLECTION PROCEDURES

A summary of acceptable sampling devices for various chemical parameters may be found in section 4.3.

4.2.1. Grab Samples

1) Collect surface grab samples by hand or using a dip sampler. The dip sampler should be used to obtain a more representative sample from a river or lake by collecting the sample further from shore. Rinse the dip sampler with the sample water three times before collecting the sample.
2) To collect the grab sample, place the mouth of the sample container below the water surface keeping hands and clothing away from the mouth of the bottle. In a stream or river, face upstream to avoid contamination of the sample.
3) Rinse bottle three times with sample water and tighten lid securely. DO NOT RINSE the sample container if sampling for the following parameters: Total Phosphorus, Dissolved Phosphorus or *E. coli*.
4) When collecting duplicates, two open sample containers should be submerged side-by-side and collected simultaneously to achieve a true duplicate sample. Field blanks are treated identically, except that distilled water from a lab-cleaned carboy is used in place of ambient sample water.

4.2.2. Hose Sampling

1) Equipment
   a) Weighted Hose with attached rope
   b) 5 gallon plastic bucket (acid rinsed)
2) Procedure
   a) Hose samples are collected to obtain vertical composite samples in lakes or very slow moving rivers. Care must be taken to keep the open ends of the hose free from contamination by covering each end when not in use. The two ends of a garden hose can be screwed together as a method of sealing to protect the ends from contamination.
   b) The hose must be weighted on one end, marked in meters, and have a rope attached to the weighted end. Determine the depth at the sample location prior to lowering the hose. Contamination of the sample by contact with the lake bottom must be avoided. The hose should be lowered to no more than one meter above the bottom.
   c) Rinse the hose and the sample bucket twice prior to sample collection. Lower the rope and hose at a slow, even rate to the desired depth while keeping the hose taut and the rope loose.
   d) Place two crimps in the hose by folding it over against itself just above the water surface when at the correct depth. Slowly pull up the rope until the weighted end reaches the surface, then lift the hose to the sample container.
   e) Raise the pinched end as high as possible, then release the pinched grip and run the hose through both hands until all the water has emptied into the sample container. Do not let the water sample contact the weights on the end of the hose.

f) Swirl the water in the container to mix it thoroughly before filling other sample containers.

4.2.3. Kemmerer Water Sampler

1) Procedure

a) Unwrap sufficient line to reach the desired sampling depth.
b) Cock the Kemmerer and check to be sure it is secure. Check the release valve to ensure it is closed. Rinse the Kemmerer three times with surface water.
c) Lower the Kemmerer smoothly to the desired sampling depth, holding the messenger in hand. The attached line is marked in meters. Some tension should be maintained on the line to ensure that the Kemmerer remains upright and cocked. A jerk on the line could trip the mechanism. If a lake profile is being taken, sample collection should begin at the surface and progress downward so that the lower depths are not disturbed prior to sample collection.
d) Drop the messenger and wait for the sound and the tug on the line that indicates the sampler has closed. If no tug is felt, the sampler may have been tripped earlier. If sampling from a distance above the water surface (such as from a bridge), a lighter messenger should be used to avoid damage to the Kemmerer.
e) Pull the Kemmerer up, maintaining tension on the line. When at the surface, hold the sampler by the rope, by the brass or plastic rod sticking out the top, or with a hand held over the bottom.
f) Push up on the spring, release pinch clamp or turn nozzle (depending on model used) to start the water flowing out the plastic tube on the bottom. Flush out at least the length of the tube before starting to fill a sample bottle to be sure the water in the tube is sample water.
g) Put the tube in the sample bottle and fill the bottle to the proper depth. With a biological oxygen demand (B.O.D.) or dissolved oxygen (D.O.) bottle, the tube should touch the bottom of the bottle while filling. Allow the water to overflow the sample bottle approximately twice its volume to rid container of air bubbles. With all other sample bottles, be sure to keep the tube above the sample water to avoid contamination. Release the spring, replace pinch clamp or turn nozzle to stop the water flow when the bottle is full.
h) When finished, open the top of the Kemmerer and drain remaining water.

2) Storage. Store Kemmerer on its side, in the open position. This allows air to dry the interior and prevents mold or mildew from forming.

4.2.4. Peristaltic Sampling Pump

1) General.
   a) Use of a peristaltic sampling pump in lakes allows for discrete water samples from thin (about 5 cm) depth strata. Water can be filtered in-line without air contacting the sample. A pump is capable of sampling deep wells of small diameter as long as the head difference is less than 10 meters. A temperature/conductance probe can be attached to the flow-through weight for in situ sample measurements.
   b) The peristaltic pump functions by drawing water through two 7 mm orifices in the stainless steel flow-through weight that connects to 50 ft sections of Tygon tubing. The inlet tube connects to malleable silicone tubing that fits around the pump head. This silicone tubing may be used for the outlet for unfiltered samples. Otherwise, connect a more rigid Teflon tube to the silicone section to enable split samples for filtered and unfiltered sampling.
2) Equipment
   a) Peristaltic sampling pump (700 ml/min. when fully charged)
   b) Tygon tubing (water transit delay 1.5 sec/meter)
   c) Teflon tubing
   d) Batteries
      i) Internal
      ii) 12 volt car battery
3) Procedure
   a) To collect unfiltered samples, close the line with the filter assembly to allow maximum flow. Lower the Tygon tubing to the desired depth and allow the pump to run for 30 seconds (15 m hose) to clear the line.
   b) To collect filtered samples, unscrew the collar of the Nucleopore 57 mm filter assembly and place a filter so that it covers the entire grid plate. Make sure that the notches on the upper and lower plates fit tightly when screwing the collar back on. Lower the Tygon tubing to the desired depth and allow the pump to run 30 seconds (15 m hose) to clear the line, with the water released through the unfiltered line to avoid clogging the filter. Shut the unfiltered line to obtain enough pressure to obtain the sample.
   c) Recharge the battery when the continual use period exceeds one hour, or when the amount of existing charge is not known. Occasionally, continual use of at least two hours is necessary to drain the battery for complete recharge. Complete recharge takes about 8 hours, and should not exceed 15 hours.
   d) Complications under winter sampling conditions may be reduced by continually running the pump to avoid frozen lines. The reduced output of the internal battery may be supplemented by a 12-volt car battery. An external back-up battery is useful under any prolonged sampling conditions.

4.3. Acceptable Sampling Devices for Various Chemical Parameters

Sampling Device		Physical	Bacteria	Dissolved Oxygen	Nutrients	Metals	Organics	General chemical	Oil and grease
Grab Samples									
	Sample Container	√	√		√	√	√	√	√
	Dip sampler	√		√	√	√		√	
Hose Sampling		√			√	√		√	
Kemmerer									
	Brass	√		√	√	√		√	
	Plexiglass	√		√	√	√		√	
	Teflon	√		√	√	√	√	√	
Peristaltic Sampling Pump		√			√	√	√	√	

4.4. SAMPLE DEVICE CLEANING TO PREVENT SAMPLE OR WATERBODY CONTAMINATION

4.4.1. Chemical Contamination

1) Clean sampling devices used in relatively clean waters by rinsing device with ambient waters at each sampling site.
2) Clean sampling devices used in known or suspected contaminated waters routinely between samples to prevent cross contamination. Treatment technique should be dictated by the analysis to be performed and type of samples collected. Generally, non-metallic samplers can be washed with Micro water wash, followed by a thorough rinsing with laboratory water. Metallic samplers and all metla parts on equipment should be washed with a non-phosphorus laboratory grade detergent and thoroughly rinsed with distilled/deionized water.
3) An alternative to field washing sampling devices is to include a sufficient number of devices so that at no time does the same equipment have to collect samples at more than one site.
4) Equipment used to collect samples for organic analyses should be washed with a non-phosphorus laboratory grade solution, followed by three or more rinses with organic-free water.

4.4.2. Biological Contamination

1) General. Special care should be taken when sampling waters containing known or suspected aquatic nuisance species. These currently include zebra mussels, alewife, eurasian watermilfoil, water chestnut. Other contamination can occur with such diseases as whirling disease (a neurological disorder of fish caused by a parasite). Please refer to Section 2 of this document for the Water Quality Division's Fieldwork Code of Practice.
2) Before moving boats between waterbodies:

i) Inspect boat, trailer, motor and other equipment for attached plant or animal material.
ii) Remove all plant and animal material.
iii) Discard removed material in a trash receptacle or on high, dry ground where there is no danger of them washing into any water body.
iv) Drain all water from boat, boat engine, and other equipment.
v) Rinse all boat and trailer parts with tap water (preferably hot, high pressure).
vi) Dry boat, trailer and equipment out of water and in sun for at least five days.

3) After leaving a waterbody:
   i) Clean all equipment, including boats and trailers, canoes, float tubes, waders, boots, and any other equipment of mud and aquatic plants before reaching the next site. Thoroughly wash and dry equipment, preferably in the sun, before using it again.
   ii) If this is not possible, use one of the following methods to destroy the organism: This section was copied from the Whirling Disease Foundation web site: http://www.whirling-disease.org/).
      a. Chlorine (regular household bleach) is a very effective disinfectant, and one of the few that can kill all stages of the parasite if used at the proper concentration. However, chlorine is a very strong chemical and can harm your equipment with prolonged exposure, so make sure you rinse the chlorine off your waders and other equipment after you disinfect, and dry in the shade.
         - To kill the TAM (larval) stage, use 1 part chlorine to 32 parts water. It must stay in contact for about 10 minutes to assure disinfection.
         - To kill the mature myxospore that may be found in the mud from an infected stream is much more difficult and hard on equipment.
            - 50% solution (1 part chlorine to 1 part water) - dip waders into a solution of the bleach or wipe or spray it on.
            - 10% solution (1 part chlorine to 9 parts water) and soak your equipment for 10 minutes. Quaternary ammonium compounds are also effective in killing both parasite stages.
      b. These disinfectants are commercially available for disinfecting fishing equipment (Bright Water TM) or for the pet/veterinary trade (Roccal-D TM, Parvosol TM).
      c. Equally effective is water heated to nearly boiling (200 °F) poured over your gear and allowed to cool.
   iii) Whenever fishing more than one stream or lake within a day, take the time to inspect and clean all equipment having had contact with infected water before moving onto the next waterbody. If possible, fish waters identified as uninfected before going to those known to be infected. For example, if you plan to fish the Batten Kill (infected) and Mettawee River (uninfected) within the same day, make your trip to the Batten Kill last.

4.5. ASSESSING AND CONTROLLING SAMPLE CONTAMINATION

4.5.1. Most environmental sampling or analyses offers numerous opportunities for contamination. Common sources for error in environmental measurements are listed in Table 4.5.2. below.

Table 4.5.2. Potential Sources of Sample Contamination

Critical Steps in the Sampling and Analytical Process	Contamination Sources
Sample Collection	-Equipment and apparatus -Handling (e.g., filtration, compositing and aliquot taking), preservatives (acids) -Ambient contamination -Sample containers
Sample Transport and Storage	-Sample containers -Cross-contamination from other samples or reagents -Sample handling
Sample Preparation	-Glassware -Reagents -Ambient contamination -Sample handling
Sample Analysis	-Syringes used for sample injections -Carry-over and memory (residual from previous samples) effects -Glassware, equipment, and apparatus -Reagents (e.g., carrier gasses and eluents)

The analytical tool most commonly used to control and/or assess sample contamination in the field and in the laboratory are blanks. They will contain negligible amounts of the contaminants of interest and are used to estimate random errors. Blanks used to control the analytical phase are part of a laboratory's quality assurance plan. A summary of laboratory and field blanks is provided in Table 4.5.3. Laboratory blanks are used by analytical chemists; field blanks are used by field staff.

Field blanks are used to assess contamination introduced during the collection process. Matched matrix blanks are standards prepared to simulate a sample matrix; however, they are rarely used. An equipment blank is generated by taking clean sampling containers to the sample collection site. After a sample is taken, the sampling equipment is cleaned according to the standard operating procedure and rinsed with laboratory distilled water. The final rinse is collected in the sample container(s) for subsequent analysis. Sample preservation procedures should be applied to the equipment blank. (VTDEC has historically called these field blanks).

Sampling Media (Trip) Blanks are used to measure contamination which occurs during transportation or collection. VTDEC has been using trip blanks samples for volatile organic chemicals. Trip blanks determine if chlorinated and/or fluorinated volatile organic chemicals are penetrating the sample container during collection or transport. A broader use of trip blanks involves taking an aliquot of laboratory water into the field and

treating it as a sample. This includes filtration and preservation activities and allowing it to be exposed to the air.

References: Principles of Environmental Sampling, Keith, L. Ed. American Chemical Society. 1988.

Table 4.5.3 Summary of Blank Types

Common Name	Other Names	Uses	Description
Laboratory Blanks			
System Blank	Instrument blank	To establish baseline response of an analytical system in the absence of a sample.	Not a simulated sample. A measure of instrument or system background response.
Solvent Blank	Calibration blank	To detect and quantify solvent impurities; the calibration standard corresponds to zero analyte concentration.	Consists only of the solvent used to dilute the sample.
Reagent Blank	Method blank	To detect and quantify contamination introduced during sample preparation and analysis.	Contains all reagents used in sample preparation and analysis and is carried through the complete analytical procedure.
Field Blanks			
Matched Matrix Blank		To detect and quantify contamination during sample collection, handling, transport, preparation and analysis.	Made to simulate the sample matrix and carried through the entire sample collection, handling, and analysis process.
Sampling Media Blank	Trip blank	To detect contamination associated with sampling media such as filters, traps, and sample bottles.	Consists of the sampling media used for sample collection
Equipment Blank	Field Blank	To determine types of contaminants that may have been introduced through contact with sampling equipment; also to verify the effectiveness of cleaning procedures.	Prepared by collecting water or solvents used to rinse sampling equipment.

5. Parameter Specific Water Collection Methods

5.1. DISSOLVED OXYGEN (D.O.) – FULL BOTTLE TECHNIQUE

5.1.1. Collect the dissolved oxygen sample using either a grab or a Kemmerer (see Table 4.3). The hose from the Kemmerer should be inserted to the bottom of the dissolved oxygen (D.O.) bottle and the sample bottle should be allowed to overflow twice the volume and then capped with the stopper. Care must be taken to prevent turbulence and the formation of air bubbles when collecting the sample and while filling the bottle.

5.1.2. Record the temperature of the water sample; the solubility of dissolved oxygen is temperature dependent.

5.1.3. Remove the stopper from the D.O. bottle and immediately add 2 ml of manganous sulfate solution (labeled "#1"), followed by 2 ml of alkaline iodide-azide solution (labeled "#2"), below the surface of the liquid. Re-stopper the bottle with care to exclude air bubbles and mix well by inverting the bottle 8 to 10 times.

5.1.4. When the precipitate has settled leaving a clear liquid on top, shake again by inverting the bottle 8 to 10 times.

5.1.5. Store the sample in the dark for up to 8 hours.

5.2. BACTERIOLOGICAL SAMPLES

5.2.1. Sample Scheduling and Bottles

1) The bacteriological laboratory operates only on specific days. Notify the laboratory at least one day in advanced of sample arrival in order to allow proper preparation of the media. Samples for coliform bacteria are accepted Monday through Thursday, although this is subject to change. Tests requiring 48 hours incubation are accepted Monday through Wednesday (e.g. *E. coli*). Refer to section 4.1.3 for additional information.
2) Collect samples in sterilized 100 ml or 250 ml plastic bacteriological bottles. Do not remove the caps until sampling begins. Do not allow contamination of the inside of the bottle or cap.
3) Use special sample bottles containing sodium thiosulfate ($Na_2S_20_3$) for chlorinated samples. These bottles are sealed 250 ml IDEXX bottles.
4) Do not rinse bottles prior to sample-collection.

5.2.2. Sample Collection

1) Remove cap from sterile bacteria bottle and grasp at base with one hand. Do not rinse bottle.
2) Where possible, go to a depth of three feet of water, face upstream, and allow any disturbance to flow downstream. Plunge bottle mouth down into the water to avoid introducing surface scum to a depth of one foot below the surface. If this is not possible, the sampling depth should be a minimum of 15 to 30 cm (6 to 13 inches) below the water surface, or at the depth relevant to the swimwater under evaluation.
3) If the water body is static, an artificial current can be created by moving the bottle horizontally in the direction it is pointed and away from the sampler.
4) Position the mouth of the bottle into the current away from the hand of the collector and away from the side of the sampling platform or boat.
5) Tip the bottle slightly upwards to allow air to exit and the bottle to fill.
6) After removal of the bottle from the water, pour out a small portion of the sample to allow an air space of 2.5 to 5.0 cm (1 to 2 inches) for proper mixing of the sample before analysis.
7) Replace cap on bottle then ice samples until analysis.

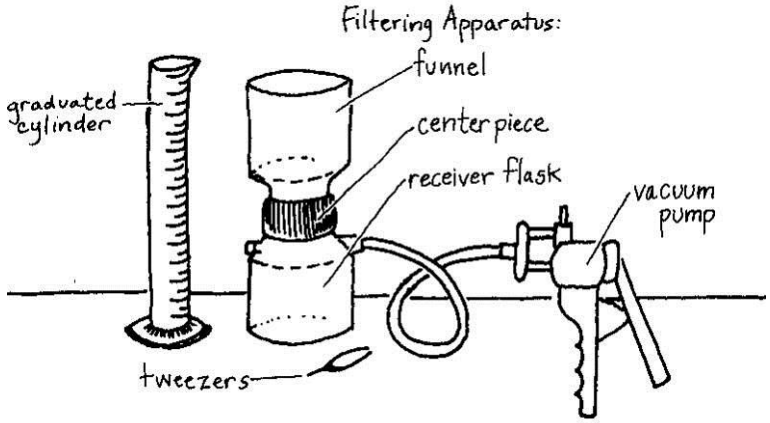
5.3. CHLOROPHYLL-A SAMPLES

5.3.1. General

Collect chlorophyll-a samples using an appropriate sampling method and keep samples on ice until filtered. Appropriate sampling methods are described in Section 4.2. Refer to the Lay Monitoring Program Manual (VTDEC 2000) for specific instructions related to that Program.

5.3.2. Filtering Procedure [excerpted from Lay Monitoring Program Manual (VTDEC 2000)].

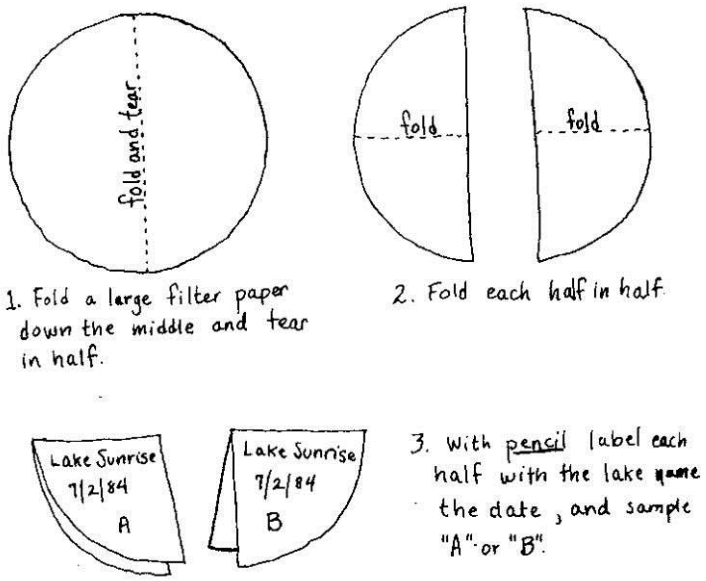
1) Equipment and supplies (see figure below)
   a) Graduated cylinder
   b) Filtering apparatus: funnel, center piece, receiver flask
   c) Vacuum pump
   d) Tweezers
   e) Large filter paper jackets (Whatman 3 GF/A Glass Microfibre Filter Papers, 9 cm)
   f) Small filters (Whatman Grade GF/A Glass Microfibre, 4.7cm, diameter)
   g) Paper clips
   h) pencil



LABORATORY EQUIPMENT

2) Check laboratory equipment and wash hands to prevent contamination of the filter papers.
3) Prepare filter jackets. Cut or tear a large filter paper in half. Fold each half in half. Label clearly, in pencil, on the outside: (See figure below)

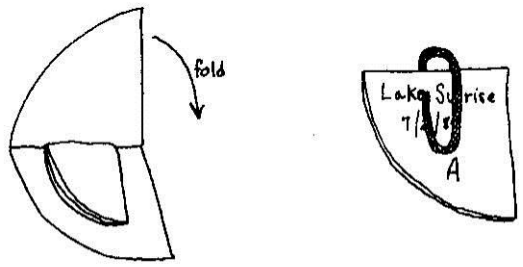
- Lake Name (if Lake Champlain use station #)
- Date (mm/dd/yy)
- If submitting duplicates: "A" on one half, "B" on the other half



4) Rinse and assemble apparatus.
   a) Unscrew the funnel from the center piece. Shake bottle "A" vigorously and use a small amount to rinse the funnel and center piece twice. Using tweezers to avoid contamination, carefully center a small filter paper on the top of the center piece.
   b) Check the placement of the red rubber ring (proper positioning ensures a tight seal). Screw the funnel back onto the center piece as tightly as possible without ripping the filter paper or cracking the plastic.
   c) Attach the vacuum pump to one of the nozzles on the side of the receiving flask. A tan rubber cap must be attached to the other nozzle.
5) Filter 100 mls of sample water.
   a) Shake bottle "A" vigorously again and use a small amount to rinse the graduated cylinder twice.
   b) Using the graduated cylinder, measure 100 mls of sample duplicate "A" water and pour it into the funnel of the filtering apparatus.
   c) Squeeze the vacuum pump until all the water in the funnel has been drawn through the filter paper into the receiving flask. Squeeze the pump several extra times to remove as much water as possible from the filter paper.

d) Release the pressure by squeezing the small trigger on the pump or by disconnecting the pump. This helps prevent the filter form being torn when the funnel is removed. All of the algae (and other particles) in the 100 mls of sample water will now be trapped on top of the filter paper.

6) Fold and remove the filter paper.
   a) Carefully unscrew the funnel from the center piece. Using the tweezers, gently lift one edge of the filter paper and fold it in half right on top of the center piece. Be sure to fold it so the top side of the filter is on the inside. Fold the filter paper again (into quarters).
   b) Place the small, folded, chlorophyll filter inside the pre-prepared, properly labeled, filter paper jacket ("A" filter should be placed in the "A" jacket). Use a paper clip to hold the small filter paper tightly inside the filter jacket.



1. Place the folded chlorophyll filter inside the folded filter jacket.

2. Paper clip the filter jacket shut.

7) Repeat the filtering procedure with sample "B." NOTE: It is not necessary to rinse the filtering apparatus a second time.
8) Rinse and store equipment.
   a) Discard extra sample water. Rinse the graduated cylinder and funnel with tap water. Store all equipment in a box (or inside the sample bucket) where it will not get dirty between sampling events.
   b) Sample storage: Filters should be frozen immediately after the filtering procedure in an airtight, dark jar or dark sealed baggie, such as Ziploc. Freezing filters and removing them from light sources prevents degradation of the chlorophyll and yields more accurate test results. Black electrical tape works well for completely covering jars and baggies. If it is necessary to transport samples, please do so in a cooler containing ice.

5.4. OIL AND GREASE

5.4.1. Collect the sample for oil and grease in a clean 1 L wide mouth glass bottle, such as a quart jar which has been rinsed with freon. Never use plastic bottles or transfer sample to another container.

5.4.2. Use a bottle cover with a Teflon coating, or place a piece of aluminum foil over the mouth of the bottle between the glass and the metal cover to prevent contamination from the lid. Add 5 ml of 1:1 HCl per liter and close the bottle tightly but do not rip the aluminum foil.

5.4.3. All samples must be cooled to 4°C for transportation to the Laboratory.

5.5. METALS

5.5.1. Earth Metals and Target Analytes (Priority Pollutants)

1) General
   a) Collect samples for metal analysis in 250 ml or 500 ml wide mouth plastic bottles (see section 4.1.8 for specifics). The sample bottles provided by the Laboratory are cleaned and acid rinsed by standardized procedures and are the only containers acceptable for metals analysis.
   b) All samples must be cooled to 4°C for transportation to the Laboratory.
   c) All water samples for metals analysis (except Hexavalent Chromium) must be preserved by acidification with HNO_3. Samplers preserve samples at the VTDEC Laboratory in the sample prep room (Room 142). Samplers must wear safety glasses while using the automatic acid dispenser located in the fume hood. Add one ml of concentrated HNO_3 to a 500 ml sample bottle.
   d) Filter blanks must be submitted with each batch of filtered samples. Obtain a clean metals container, fill with Laboratory deionized water, filter through filtration unit and log sample in as "filter blank". Generally speaking, ground water and surface water samples taken for monitoring should be filtered.
   e) Table 5.6.2 identifies commonly requested groups of metals. These can be requested as a group at the time of log in. Refer to the sample code reference notebook located next to the log in computers.

Table 5.6.2. Commonly requested metals

VTDEC Laboratory LIMS Code	Metals, Earth	Metals, Target Analyte (Priority Pollutants)	Metals, AsSeZn
Parameters	Aluminum	Antimony	Arsenic
	Calcium	Barium	Selenium
	Magnesium	Beryllium	Zinc
	Potassium	Cadmium	
	Sodium	Chromium	
	Total Calculated Hardness	Cobalt	
		Copper	
		Iron	
		Lead	
		Manganese	
		Molybdenum	
		Nickel	
		Silver	
		Thallium	
		Vanadium	

5.6.3. Mercury

1) General. This method describes the collection technique for trace-metal clean sampling for mercury or other sensitive parameters.
2) Sampling Position. Samples should be collected upstream or beyond contact with the boat to avoid contamination by metal equipment.
3) Equipment.
   - Tyvek suits
   - Teflon Kemmerer
   - Cooler
   - Shoulder-length gloves
   - Waterproof sample labels
   - Latex gloves
   - Trace grade HNO_3
   - Pipet (5 ml capacity)
   - Disposable pipet tips
   - Teflon sample bottles (double bagged in zippered plastic bags, e.g. Ziploc bags)

   a) Surface Grab: Using a clean 500 ml teflon bottle, collect a water sample using a surface grab. Do not rinse.
   b) Kemmerer: Use an all-Teflon Kemmerer sampler to collect a sample one meter above the sediment water interface. The sampler should be rinsed three times in the lake water prior to collecting the sample.
4) Procedure
   a) Collection of aqueous mercury samples will conform to the EPA method 1669 'clean hands-dirty hands' techniques (USEPA, 1996).
   b) In brief, sampling staff will wear clean Tyvek windsuits and gloves. 'Clean hands' will be assisted by 'dirty hands' in all aspects of sampling to ensure that clean hands does not touch anything but the Teflon Kemmerer while sampling. It is especially important to avoid all metal, such as the side of an aluminum boat, pens, jewelry, clipboard, etc. Mercury has an affinity for metals, especially gold, and will potentially contaminate the sample if touched.
   c) 'Clean hands' will wear shoulder-length gloves. Gloves will be new from the box at the time they are put on. Aqueous mercury samples will be stored in a separate cooler from other samples collected.
   d) Preserve samples in situ with 3.6 mls concentrated trace-metals grade HNO_3, using a new pipet tip rinsed twice in mercury-clean 10% HCl, and once in trace metal grade HNO_3.
   e) Epilimnetic Grab Sampling
      i. Waterproof sample labels are prepared using waterproof ink. See section 4.1.1. for field sampling information.
      ii. 'Dirty hands' opens the 'clean box,' gloves, and dons a Tyvek suit.
      iii. 'Dirty hands' removes shoulder gloves, and assists 'Clean hands' in donning shoulder-gloves and shorter gloves if necessary. From this point forward, 'Clean hands' handles nothing but the sample bottle, or the inner Ziploc bag which contains the sample bottle.

iv. ‘Dirty hands’ opens the ‘clean cooler,’ and removes one 1000ml double bagged bottle. ‘Dirty hands’ opens the outer bag. ‘Clean hands’ reaches into the outer bag, opens the inner bag, removes the bottle, and folds the inner bag over. ‘Dirty hands’ seals the outer bag, and replaces it into the ‘clean cooler.’

v. ‘Dirty hands’ removes the autopipet from the clean cooler, and affixes a new pipet tip. ‘Dirty hands’ rinses the pipet tip two times in reagent-water dilute 10% HCl, and one time in HNO_3. Rinsates are evacuated into a waste-acid container.

vi. ‘Clean hands’ opens the sample bottle, evacuates the contents, and closes the bottle.

vii. ‘Clean hands’ submerses the bottle to a 0.5 meter minimum depth, opens the bottle, and fills it ⅓ rd full. The bottle is then surfaced, shaken, opened, and the rinsate evacuated away from the immediate sampling point. The bottle is resealed. This is repeated twice.

viii. ‘Clean hands’ re-submerses the bottle, and allows the bottle to fill entirely. The bottle is recapped underwater. ‘Clean hands’ surfaces the bottle, and opens the cap slightly.

ix. ‘Dirty hands’ draws 3.6 mls trace-metal grade HNO_3, and pipets this into the sample bottle. ‘Clean hands’ then tightly caps the bottle. ‘Dirty hands’ opens the clean cooler, withdraws, then opens the outer bag.

x. ‘Clean hands’ unfolds the inner bag, replaces the bottle, and seals the inner bag. ‘Dirty hands’ then seals the outer bag, affixes the label, and replaces the double-bagged sample in the clean cooler.

f) Hypolimnetic Kemmerer Sampling

i. ‘Dirty hands’ un-bags the double-bagged teflon Kemmerer, affixes the line, and rinses the sampler three times in lake water by submersing the sampler, forcefully retrieving it, and allowing it to drip off. The sampler is then lowered 2 meters below the boat, and tied off.

ii. ‘Dirty hands’ opens the ‘clean cooler,’ and removes a 500ml double bagged bottle. ‘Dirty hands’ opens the outer bag.

iii. ‘Clean hands’ reaches into the outer bag, opens the inner bag, removes the bottle, and folds the inner bag over. ‘Dirty hands’ seals the outer bag, and replaces it into the ‘clean cooler.’

iv. ‘Dirty hands’ lowers the Kemmerer sampler to 1 meter from the sediment-water interface, and trips the closure mechanism with the non-metallic messenger. The sampler is retrieved.

v. ‘Clean hands’ opens and evacuates the bottle. ‘Dirty hands’ directs the sample stream from the Kemmerer sampler to fill the bottle ⅓ rd full. ‘Clean hands’ caps the bottle, shakes vigorously, and evacuates the rinsate. This is repeated two times.

vi. ‘Dirty hands’ directs the sample stream to fill the bottle entirely. ‘Clean hands’ caps the bottle, and ‘Dirty hands’ re-submerses and ties the Kemmerer sampler to hang in the water at 2 meters of depth.

vii. ‘Dirty hands’ draws 1.8 mls trace-metal grade HNO_3 and pipets this into the sample bottle which was opened by ‘Clean hands’. ‘Clean hands’ tightly caps the bottle.
viii. ‘Dirty hands’ opens the clean cooler, withdraws the bag and then opens it.
ix. ‘Clean hands’ unfolds the inner bag, replaces the bottle, and seals the inner bag. ‘Dirty hands’ then seals the outer bottle, affixes the label, and replaces the bottle in the clean cooler.
x. ‘Dirty hands’ bags the Kemmerer sampler with new bags, using ‘’Clean hands’’ assistance.

5.7. VOLATILE ORGANIC SAMPLES

5.7.1. Sample Scheduling and Bottles (See also section 4.1)

It is imperative that all samples for organics analysis are scheduled with the laboratory in advance and be collected in the proper containers. These arrangements can be made with Laboratory personnel at 244-4522.

1) Residual chlorine: If the samples will contain residual chlorine, please notify the laboratory. Sample bottles containing sodium thiosulfate (0.008% $Na_2S_2O_3$) to neutralize the residual chlorine will be provided. Samples containing unneutralized chlorine cannot be accepted as by the time of analysis the chlorine will have reacted with other substances giving new compounds not present in the original sample.
2) The sample bottles are 40 ml vials which are sealed with a Teflon faced silicone septum secured by a screw top. Care must be taken to insure that the Teflon surface faces the sample. Samples submitted in bottles other than those provided by the laboratory will not be accepted for analysis.
3) The Laboratory will provide enough bottles to take two “trip blanks" and triplicates of each sample. Trip blanks are vials of water found by the lab to be free of organic compounds. These vials are carried through all phases of the sampling procedure without being opened. If sodium thiosulfate is used in the sampling vials, it should also be used in the trip blanks.

5.7.2. Procedure

1) Collect all samples in duplicate using 2 vials; both vials are acidified.
2) Fill the sample bottles such that no air bubbles pass through the sample as the bottle is filled.
   a) Sampling from an open tap: Turn on the water and allow the system to flush until the temperature has stabilized. If using a sampling pump, adjust the flow to about 500 ml per minute and collect duplicate samples from the flowing stream.
   b) Sampling from an open body of water. See Table 4.3 for proper sampling devices. Fill a one quart wide mouth jar with sample from a representative area. Carefully fill duplicate sample bottles from the one quart bottle. Do not allow materials other than glass and Teflon to touch the sample.
3) Fill the sample bottles to overflowing and place on a level surface. Add 5 drops of 1:1 HCl to all vials prior to sealing. This prevents biodegradation of the aromatics. Position the Teflon side of the septum seal upon the convex meniscus and seal the bottle by screwing the cap on tightly.
4) Invert the sample and lightly tap the cap on a solid surface. The absence of air indicates a successful seal. If bubbles are present open the bottle, add a few drops of sample and reseal the bottle as above.
5) Ice samples immediately after collecting.
6) Analysis must take place within 14 days of collection. Return samples and trip blanks to the laboratory as soon as possible after collection.

5.8. SEMI-VOLATILE ORGANIC SAMPLES

5.7.1. Sample Scheduling and Bottles (see section 4.1)

1) It is imperative that all samples for organics analysis be scheduled with the VTDEC Laboratory in advance and be collected in the proper containers. These arrangements can be made with laboratory personnel at 244-4522.
2) Residual chlorine: If the samples contain residual chlorine, please notify the laboratory. Sample bottles containing sodium thiosulfate (0.008% $Na_2S_2O_3$) to neutralize the residual chlorine will be provided. Samples containing unneutralized chlorine cannot be accepted. By the time of analysis the chlorine will have reacted with other substances giving new compounds not present in the original sample.
3) Water sample bottles are 2.6 liter amber glass bottles which are capped and sealed with a teflon faced screw top. Samples submitted in bottles other than those provided by the laboratory will not be accepted for analysis.
4) Solid sample bottles are 500 ml wide-mouth glass bottles with teflon-lined caps. Submit them to the laboratory as soon as possible.

5.7.2. Sample Collection

1) A complete semi-volatile analysis on water requires 1 liter of sample. Fill sample bottles completely.
2) Do not pre-rinse sample containers; compositing must be performed in the sample container.
3) Avoid plastic sampling materials. They will introduce artifacts into the analysis. Sample removal equipment and/or funnels must be made of glass or Teflon.
4) Project plans calling for the use of automatic sampling devices should be discussed with the laboratory. The use of Tygon tubing must be minimized or eliminated if possible.
5) Sample must be extracted within seven days of collection. They should be kept at 4ºC and returned to the laboratory as soon as possible.
6) Semi-volatile analyses requests on water samples are logged in as Method 8270-Water.

5.8. POLYCHLORINATED BIPHENYLS (PCBs), PESTICIDES

5.8.1. Sample Scheduling and Bottles (also see section 4.1)

1) It is imperative to schedule all samples for organics analysis with the VTDEC Laboratory in advance and that they are collected in the proper containers. These arrangements can be made with Laboratory personnel at 244-4522.
2) Residual chlorine: If the samples will contain residual chlorine, please notify the laboratory. Sample bottles containing sodium thiosulfate to neutralize the residual chlorine will be provided. Samples containing unneutralized chlorine cannot be accepted as by the time of analysis the chlorine will have reacted with other substances giving new compounds not present in the original sample.
3) Water sample bottles are 2.6 liter amber bottles which are capped and sealed with a Teflon faced screw top. Samples submitted in bottles other than those provided by the laboratory will not be accepted for analysis.
4) Solid sample bottles are 500 ml wide-mouth glass bottles with Teflon-lined caps. Submit them to the laboratory as soon as possible.

5.8.2. Sample Collection

1) Completely fill container using an acceptable method outlined in section 4.3.
2) Do not pre-rinse sample containers; compositing must be performed in the sample container.
3) Do not use plastic sampling materials. Plastic will introduce artifacts into the analysis. Sample removal equipment and/or funnels must be made of glass or Teflon.
4) Project plans calling for the use of automatic sampling devices should be discussed with the laboratory. The use of Tygon tubing must be minimized or eliminated if possible.
5) Sample must be extracted within seven days of collection. They should be kept at 4ºC and returned to the laboratory as soon as possible.
6) Both semi-volatiles and PCBs/Pesticide samples can be collected in the same container.
7) PCB/Pesticide analysis requests on water samples are logged in as Method 8081-Water.
8) If the sample is not between a pH of 5.0 and 9.0, extraction must take place within 72 hours.

6. Biological Collection Methods

6.1. PHYTOPLANKTON

6.1.1. Vertical Qualitative Sampling-Plankton Net

1) Equipment
   - Wisconsin plankton net (63 μm mesh) with calibrated line
   - Amber glass bottles with labels (long-term storage) <u>or</u> plastic 50 ml centrifuge tubes (short-term storage)
   - Lugol's iodine preservative
   - Secchi disk
   - Depth tape
   - Squirt bottle
   - cooler
2) Procedure
   a) Determine the depth at the sampling site. Vertical qualitative samples can be obtained in waters greater than 3 m in depth.
   b) Determine the Secchi disk transparency.
   c) Rinse out the plankton net with ambient surface water and close stopcock.
   d) Lower the net to the desired depth (i.e. twice the Secchi and at least one meter from the bottom).
   e) Retrieve slowly and rinse out excess water. Drain the sample through the stopcock opening into the amber bottle or centrifuge tube.
   f) Rinse the inside of the net with a squirt bottle of lake water between hauls, collecting rinse water into the sample bottle. Rinse from the outside of the net to avoid contamination, especially if using ambient water to rinse.
   g) Repeat the vertical hauls until an adequate amount has been collected. This will vary with project needs.
   h) Record information on bottle label: date, time, location, depth of haul, type of sample (i.e. vertical-qualitative).
   i) Add Lugol's preservative (10 ml/L), secure container tightly and invert to mix thoroughly.
   j) Store sample in a cool, dark place, such as a cooler.

6.1.2. Horizontal Qualitative Sampling

1) Equipment
   - Wisconsin plankton net (63 μm mesh) with calibrated line
   - 250 ml amber glass bottle with label (long-term storage) <u>or</u> plastic 50 ml centrifuge tube (short-term storage)
   - Lugol's iodine preservative
   - Secchi disk
   - Depth tape
   - Collection bucket (>4 liter)
   - Squirt bottle
   - Cooler

2) Procedure
   a) Rinse out the plankton net and close stopcock.
   b) Rinse collection bucket with ambient water.
   c) Throw the net from a stationary point and slowly retrieve until an adequate sample has been collected or tow the net slowly behind a boat until an adequate sample has been collected. Prevent the net from coming in contact with the bottom, particularly when sampling from shore.
   d) Shake out excess water and drain the sample through the stopcock opening in the collection bucket into the amber bottle.
   e) Record information on bottle label: date, time, location, type of sample (e.g. horizontal-qualitative), and length of haul.
   f) Add Lugol's preservative (10 ml/L) and invert container to mix thoroughly.
   g) Store sample in a cool, dark place, such as a cooler.

6.1.3. Composite Quantitative Sampling-Hose

1) Equipment
- Sampling hose
- 250 ml amber glass bottle with labels (long-term storage) <u>or</u> plastic 50 ml centrifuge tube (short-term storage)
- Lugol's iodine preservative
- Secchi disk
- Depth tape
- Cooler
- Collection bucket (>4 liters)

2) Procedure
   a) Determine the Secchi disk transparency and the depth at the sampling site.
   b) Rinse collection bucket with ambient water.
   c) Collect a hose sample at twice the Secchi transparency depth or to within one meter of the bottom, whichever is less.
   d) Empty the entire contents of the hose into a clean container and swirl to mix.
   e) Pour a 200 ml subsample into the amber bottle.
   f) Record information on bottle label: date, time, location, depth of composite, and type of sample (i.e. composite - quantitative).
   g) Add Lugol's preservative (10 ml/L) and invert container to mix thoroughly.
   h) Store sample in a cool, dark place, such as a cooler.

6.1.4. Discrete Quantitative Sampling-Kemmerer

1) Equipment
   - Kemmerer sampler, if necessary
   - Amber glass bottles with label (long-term storage) <u>or</u> plastic 50 ml centrifuge tubes (short-term storage)
   - Lugol's iodine preservative
   - Cooler

   a) Collect sample from a discrete depth using a Kemmerer or from the surface by taking a grab sample. A minimum of 200 ml is necessary.

b) Pour sample into amber bottle. Record information on label: date, time, location, and type of sample (i.e., discrete sample - quantitative), depth of haul.
c) Add Lugol's preservative (10 ml/L) and invert container to mix thoroughly.
d) Store sample in a cool, dark place, such as a cooler.

6.1.5. Discrete Quantitative Sampling- Plankton Net

Note: This method is used primarily by the Lake Champlain Monitoring Program to collect blue-green algae

1) Equipment
   - Wisconsin plankton net (63 µm mesh) with calibrated line
   - 250 ml amber glass bottle with label (long-term storage) <u>or</u> plastic 50 ml centrifuge tube (short-term storage)
   - Ethyl alcohol (ethanol or ETOH) (preservative)
   - Secchi disk
   - Collection bucket with stopcock (e.g. 2 gallon water cooler)
   - Cooler
2) Procedure
   a) Rinse the collection bucket in ambient water
   b) Rinse the plankton net with surface water and close stopcock.
   c) Throw the net from a stationary point and slowly retrieve or tow the net slowly behind a boat until an adequate sample has been collected. Prevent the net from coming in contact with the bottom, particularly when sampling from shore.
   d) Shake out excess water and drain the sample through the stopcock opening in the collection bucket into the amber bottle.
   e) Record information on bottle label: date, time, location, type of sample (e.g. horizontal - qualitative) and length of haul.
   f) Add Lugol’s preservative (10 ml/L) and invert container to mix thoroughly.
   g) Store sample in a cool, dark place, such as a cooler.

6.2. ZOOPLANKTON

6.2.1. Vertical Qualitative Sampling-Plankton Net

1) Equipment
   - Wisconsin plankton net (63 µm mesh) with calibrated line
   - 250 ml amber glass bottle with label (long-term storage) <u>or</u> plastic 50 ml centrifuge tube (short-term storage)
   - CO_2 water (seltzer)
   - Ethyl alcohol (ethanol or ETOH) or formalin sugar (preservative)
   - Secchi disk
   - Depth tape
   - Collection bucket with stopcock (e.g. 2 gallon water cooler)
   - Cooler
2) Procedure
   a) Determine the depth at the sampling site. Vertical qualitative samples can be obtained in waters greater than 3 meters in depth.
   b) Determine the Secchi disk transparency.
   c) Rinse the collection bucket in ambient surface water.
   d) Rinse out the plankton net and close stopcock.
   e) Lower the net to the desired depth (i.e. twice the Secchi and at least one meter above the bottom).
   f) Retrieve slowly (0.5-1.0 m/sec) and shake out excess water. Drain the sample through the stopcock opening in the collection bucket into the amber bottle or centrifuge tube.
   g) Repeat the vertical hauls until an adequate sample has been collected.
   h) Record information on bottle label: date, time, location, depth of haul, type of sample (i.e. vertical qualitative) and mesh size.
   i) Add a small amount of seltzer (carbonated water) to reduce distortion observed under a microscope.
   j) Add enough preservative to create a 70% ethanol final concentration. Invert container to mix thoroughly.
   k) Store sample in a cool, dark place, such as a cooler.

6.2.2. Vertical Quantitative Sampling for trace metal burdens in zooplankton

1) Equipment:
   - Metal-free Wisconsin-style net with “cod” end or equivalent (with appropriate mesh size, see sections below)
   - 200 ml lot-certified PETE ‘compositing vessel’
   - 500ml acid cleaned squirt bottle (Clean after every tenth sampling event).
   - 500 ml squirt bottle for CO_2 water (seltzer)
   - CO_2 water (seltzer)
   - 1 pre-weighed, pre-coded, lot-certified 50 ml polycarbonate sample vessel
   - 2 non-weighed 50 ml polycarbonate vessels
   - powder-free vinyl gloves

- protective plastic sheet, 4'x 4' or larger
- field sheet
- 200 ml lot-certified PETE ‘compositing vessel’
- formalin solution (preservative)
- Depth tape
- Dolphin bucket (201 μ mesh)
- Cooler

2) General Procedure
   a) Prior to going out into the field, a pre-coded 50 ml sample vessel is weighed to the nearest 0.001 g, and the weight and code recorded.
   b) In the field, after the vessel has arrived at station and has been securely anchored, ‘Clean hands’ and ‘Dirty hands’ are designated. ‘Clean hands’ and ‘dirty hands’ don regular-length powder-free vinyl gloves.
   c) A plastic sheet is draped over the gunwale of the sampling boat, such that the net will not have the opportunity to contact the boat.
   d) ‘Dirty hands’ removes and assembles the non-metallic net, and ‘clean hands’ and ‘dirty hands’ jointly backflush the net three times in lake surface water. The dolphin bucket is similarly rinsed.
3) Procedure for Tows of Total Mercury (HgT) and Biomass Determination:
   a) ‘Dirty hands’ lowers the net to within 1 meter of the lake bottom, and rests the net 30 seconds to allow the water column to recolonize.
   b) ‘Dirty hands’ records the depth of this tow on the field sheet.
   c) ‘Dirty hands’ retrieves the net at a rate of < 1 m/second.
   d) When the net-hoop breaches the surface, ‘dirty hands’ lifts the net, and rinses the contents down along the net-sides using lake water and an acid cleaned squeeze bottle.
   e) Once the sample is condensed into the dolphin bucket, ‘clean hands’ removes the bucket, further reduces the sample, and decants it into the 200 ml ‘compositing vessel’.
   f) This tow collection procedure is repeated until a minimum of 5 tows are collected. The field coordinator will determine if additional tows are necessary to obtain sufficient material for biomass and HgT analyses.
   g) The contents of the compositing vessel are decanted to the dolphin bucket, and the contents reduced to < 50 ml volume.
   h) ‘Clean hands’ opens the 50 ml sample vessel, rinses it three times with lake water, and decants the reduced composite plankton material into the vessel. The vessel is then filled to 50 ml with lake water, and capped tightly.
   i) ‘Dirty hands’ opens a zippered bag, and ‘clean hands’ gently drops the filled 50 ml vessel into the bag.
   j) ‘Dirty hands’ closes the bag and places it into the designated cooler for submission to the laboratory for analysis.
4) Procedure for tows for Taxonomic Analyses 201 μ mesh
   a) Two additional tows are composited, using the 201 μ mesh, into the compositing vessel using the procedure outlined above.
   b) The contents of the compositing vessel are then covered with seltzer water, capped, and allowed to sit 1 minute. At this time, the contents are returned to

the dolphin bucket, reduced to the maximum extent possible, rinsed using the seltzer-squeeze bottle into a labeled 50 ml sample vessel, to approximately 25 ml volume.

c) The sample is capped and allowed to sit 5 minutes. The sample is then opened, and filled to 50 ml with formalin-solution. Invert container to mix thoroughly

d) Store sample in a cool, dark place, such as a cooler.

5) Tows for Taxonomic Analyses - 45-200 μ mesh:

a) Two tows are composited using the 45 μ mesh net, following the procedure outlined directly above.

b) While the dolphin bucket is held above the assembled 45 μ net, the contents of the 45 μ composite are passed through the dolphin bucket, and allowed to run out into the 45 μ net. This step removes plankton in the 201 μ fraction from the 45 -200 μ fraction.

c) The 45 μ sample is then recondensed, and transferred back to the compositing vessel.

d) The contents of the compositing vessel are then covered with seltzer water, capped, and allowed to sit 1 minute. At this time, the contents are returned to the dolphin bucket, reduced to the maximum extent possible, rinsed using the seltzer-squeeze bottle into a labeled 50 ml sample vessel, to approximately 25 ml volume.

e) The sample is capped and allowed to sit 5 minutes. The sample is then opened, and filled to 50 ml with formalin-solution.

f) Store sample in a cool, dark place, such as a cooler.

6.2.3. Horizontal Qualitative Sampling-Plankton Net

1) Equipment

- Wisconsin plankton net (63 μm mesh) with calibrated line
- 250 ml amber glass bottle with label (long-term storage) <u>or</u> plastic 50 ml centrifuge tube (short-term storage)
- CO_2 water (seltzer)
- Ethyl alcohol (ethanol or ETOH) or formalin sugar (preservative)
- Secchi disk
- Collection bucket with stopcock (e.g. 2 gallon water cooler)
- Cooler

2) Rinse the collection bucket in ambient water.

3) Rinse the plankton net with surface water and close stopcock.

4) Throw the net from a stationary point and slowly retrieve or tow the net slowly behind a boat until an adequate sample has been collected. Prevent the net from coming in contact with the bottom, particularly when sampling from shore.

5) Shake out excess water and drain the sample through the stopcock opening in the collection bucket into the amber bottle.

6) Record information on bottle label: date, time, location, type of sample (e.g. horizontal - qualitative) and length of haul.

7) Add a small amount of seltzer (carbonated water) to reduce distortion observed under a microscope.
8) Add enough preservative to create a 70% ethanol final concentration. Invert container to mix thoroughly.
9) Store sample in a cool, dark place, such as a cooler.

6.2.4. Composite Quantitative Sampling-Plankton Net

1) Equipment
   - Wisconsin plankton net (63 μm mesh) with calibrated line
   - Amber glass bottles with labels <u>or</u> plastic 50 ml centrifuge tubes (short-term storage)
   - CO_2 water (seltzer)
   - Ethyl alcohol (ethanol or ETOH) or formalin sugar (preservative)
   - Depth tape
   - Cooler
2) Procedure
   a) Determine the depth at the sampling site.
   b) Rinse out the plankton net and close the stopcock.
   c) Lower the net until it is one meter above the bottom or other appropriate depth.
   d) Pull the net to the surface using a slow steady retrieve (0.5-1.0 m/sec). Keep the net vertical.
   e) Shake out excess water and drain the sample into an amber glass bottle or centrifuge tube.
   f) Rinse the net before attempting the next haul, collecting rinse water into the sample. This prevents loss of organisms back into the surface water as it descends the water column.
   g) Collect a minimum of three hauls, all emptied into the sample amber bottle (composite) or in separate bottles for discrete replicates).
   h) Record information on bottle label: date, time, location, depth of composite, number and length of hauls, diameter of net opening, and type of sample (i.e. composite-quantitative) and mesh size.
   i) Add a small amount of seltzer (carbonated water) to reduce distortion observed under a microscope.
   j) Add enough preservative to create a 70% ethanol final concentration. Invert container to mix thoroughly.
   k) Store sample in a cool, dark place, such as a cooler.

6.2.5. Discrete Quantitative Sampling-Schindler or Juday Trap

1) Equipment
   - Schindler or Juday plankton trap (63 μm mesh net) with calibrated line
   - Amber glass bottles with labels <u>or</u> plastic 50 ml centrifuge tubes (short-term storage)
   - CO_2 water (seltzer)
   - Ethyl alcohol (ethanol or ETOH) or formalin sugar (preservative)
   - Cooler

2) Rinse out the net of the sampling device with surface water and close the stopcock.
3) Lower the sampling device to the desired depth, close device if required, and retrieve the sample.
4) Shake the excess water from the collection net and drain the sample into an amber glass bottle.
5) Record information on bottle label: date, time, location, type of sampling device (e.g. 25 L Schindler), depth of sample and type of sample (e.g. discrete - quantitative). Add a small amount of seltzer (carbonated water) to reduce distortion observed under a microscope.
6) Add enough preservative to create a 70% ethanol final concentration. Invert container to mix thoroughly.

6.3. MACROPHYTES

6.3.1. Qualitative Aquatic Plant Surveys

1) Background.
   Aquatic plant populations on selected lakes are identified, surveyed, mapped and summarized. Purposes of the surveys are to: assess current plant densities, abundance and distribution; create a species list for each surveyed lake; document nuisance conditions or the degree of use impairment; monitor changes in plant populations over time; monitor plant response to control programs; evaluate effectiveness of control on selected species; and monitor populations of rare, threatened or endangered plants. A computer record of each lake's species list is maintained in the Lakes and Ponds Inventory, and field notes and map are kept on file. An aquatic plant herbarium is also maintained. (This information provided by Aquatic Plant Surveys, Work/QA Project Plan, VTDEC Water Quality Division, Warren 1995.)
2) Types. Plant surveys may be one of several possible types:
   a) Plant Survey: A survey of the portion of the littoral zone visible from the water's surface, noting as many plant species as feasible (based primarily on visibility) and providing information on plant abundance, distribution, condition, depths (of certain species) and bottom type.
   b) Eurasian Watermilfoil or Water Chestnut Survey: A survey primarily of the non-native species in question, noting abundance, location, and depths of growth. Although areas of native species' growth are noted where they are mixed with the non-native species, this type of survey does not provide a thorough list of all species present or their abundance.
   c) Non-native Species Search: Lakes thought to be especially susceptible to a watermilfoil, water chestnut or other non-native species introductions are checked on a rotating basis to identify new introductions as soon as possible. Field notes contain general descriptions of the amount of plant growth and nuisance conditions, but this type of survey does not provide a thorough list of all species present or their abundance.
3) Equipment
   - Boat and associated equipment
   - Light anchor
   - Garden rake
   - Depth tape
   - Water scope
   - Bucket
   - Ziploc bags
   - Detailed lake map
   - Paper, pencil and clipboard
   - Aquatic plant key (see reference list)
4) General Sampling Procedures
   a) Slowly circle the entire perimeter of the lake, observing all areas less than 20 feet in depth.

b) Record the plant species seen and describe their relative abundance or density. According to the categories listed below. Note the depth of water that the plants are growing in. Sketch their location on a lake map. Each time a plant community changes, a new area should be delineated on the map. Be sure to map areas devoid of plants as well as areas with plants.

Density Category	Cover
Very abundant	75 - 100%
Abundant	50 - 75%
Common	25 - 50%
Occasional	5 - 25%
Scattered	1 - 5%
Uncommon	1%
Solitary	single plant

c) If waves or glare limit visibility, view plants through a water-scope or drag up plants with an anchor or rake to examine more closely.

d) When field identification is not possible, plants are placed in plastic bags, labeled, and transported to the Lakes and Pond Management and Protection Section for future identification. If kept overnight, the specimens are stored in a cool location to avoid deterioration. Dissecting microscopes, taxonomic keys and a reference collection are available at Lakes and Ponds to assist with identification.

e) See the attached example for the final report format. The completed report should include a description of each area, a species list for the lake and a brief general statement on the plant growth in the lake. This statement should be a general "feeling" concerning what was found, referring to dominant plants, areas of growth, possible problem areas and the necessity for plant control procedures. The more descriptive the statement, the more useful it will be in the future.

An example of a completed plant survey is presented in Table 6.3.2 below.

Table 6.3.2. LAKE MOREY, AQUATIC PLANT SURVEY RESULTS, July 7, 1981

Area	Identification	Abundance
1	*Ceratophyllum demersum*	sparse
	Vallisneria americana	occasional patches
	Potamogeton amplifolius	occasional patches
	Megalodonta Beckii	occasional patches
	Nymphaea odorata	small patch east of Aloha Manor boathouse
	Nuphar sp.	small patch west of Aloha Manor boathouse
	Typha sp.	along shoreline
2	*Ceratophyllum demersum*	very dense monoculture
	Potamogeton Praelonqus	small moderate patches
	P. amplifolius	occasional plants off inlet - patch of sand, no plants in shallow water 100% plant coverage in deep water in this area plants do not reach the surface of the water
3	*Ceratophyllum demersum*	sparse dense patches, no longer a monoculture
	Potamogeton praelongus	dense
	P. amplifolius	sparse moderate patches
4	*Ceratophyllum demersum*	moderate
	Potamogeton praelongus	noticeably less dense than area 3
	P. amplifolius	noticeably less dense than area 3
	Megalodonta beckii	noticeably less dense than area
5	*Elodea canadensis*	abundant
	Vallisneria americana	near shore at tip of Gilmore's Point
	Megalodonta beckii	abundant
	Eriocaulon septangulare	abundant
	Heteranthera sp.	moderate
	Unidentified plant	single plant

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6.4.AQUATIC BIOTA – MACROINVERTEBRATES

6.4.1. Lotic Semi-Quantitative Benthic Survey

1) Riffle Habitat. Bottom Kick Net Sampling.
   a) General. This is a four point composite sample representing the macroinvertebrate community of the riffle habitat within a stream reach. The sampler spends 30 seconds per sampling point for a total of two minutes sampling effort.
   b) Equipment
      - Bottom Kick net - mesh size 500 microns, rectangular 18" wide x 12" high
      - Quart size jars (wide mouth preferred)
      - 80% ethyl alcohol (ethanol or ETOH)
      - field sheets
      - Waders
   c) Sampling Procedure. This method is for use in a stream- riffle habitat, where velocities are greater than 0.2 feet per second (fps), and the depth is less than 1 meter.
      i) Record physical characteristics of sampling site on the Lotic Benthos Field Sheet (Refer to section 6.4.3 Physical Characteristics Lotic and Lentic Habitats).
      ii) Place the net in the riffle at a representative location on the stream bottom, perpendicular to the flow. A representative location is identified based on overall riffle habitat present at the site including depth, flow velocity, and substrate composition. Avoid artificial habitat such as riprap and culverts.

      iii) Collected sample from an 18" x 18" square area immediately upstream of the net. First, move all cobble sized substrate to the mouth of the net and rub clean of attached organisms. Discarded to the side of the sample area.
      iv) Second, disturb all remaining substrate by hand down to a 5– 10 cm depth. This entire procedure takes about 30 seconds per section, and should take no more then 1minute. Move in a general up-stream direction and repeat at 4 different points within the riffle. Sample collection points should represent a range of velocity and substrate characteristic of the riffle habitat within the stream reach being sampled. The four point composite sample will equal approximately 1 square meter of bottom and 2 minutes of active sampling (30 seconds per section).
      v) Use a stopwatch to record time spent actively disturbing the substrate. Time spent relocating to a new location within the riffle is not counted as part of the two minute sampling effort.
      vi) Transfer contents of the net from each sample point into a wide mouth quart jar and preserve with 80% ethanol.

vii) Collect a replicate at each site. This will result in 2 four point composite samples per site. Repeat steps ii-vi, being careful to avoid areas previously disturbed. This composite sample represents the macroinvertebrate community of that riffle.

Note: This sampling protocol is most comparable to the riffle-run sampling portion of EPA Rapid Bioassessment Protocol III (RBPIII) as described in Plafkin et al. (1989).

2) Low gradient stream habitat. Sweep Bottom Kick Net Sampling
   a) General. This is a four point composite sample representing the macroinvertebrate community of the run habitat within a stream reach. This method is for use in wadeable low gradient streams with substrates dominated by silt or sand and velocities, where velocity is less than 0.2 fps and the depth is less than 1 meter.
   b) Equipment
      - Bottom Kick Net - mesh size 500 microns, 18" wide x 9" high
      - 5-gallon bucket
      - No. 30 Sieve bucket
      - Squirt bottle
      - Quart size jars (wide mouth preferred)
      - 80% ethyl alcohol (ethanol or ETOH)
      - Field sheets
      - Waders

   c) Sampling Procedures
      i) Record Physical Characteristics of sampling site (Section 6.4.3 Physical Characteristics–Lotic and Lentic Habitats)
      ii) In slow moving, low gradient streams, macroinvertebrates generally attach to overhanging plants, roots, logs, submerged vegetation and stream substrate. The Kick Net is used to collect a total of four jabs from representative habitats as described below. A jab is performed by jabbing the net into debris dams, vegetation, or root wads, pulling back rapidly to dislodge animals, then sweeping forward again into the same area to scoop up dislodged animals. This jabbing and sweeping motion should be repeated several times at the same point and considered one of four jabs. Move in a general upstream direction for each subsequent point. All four jabs (from different points in reach) are then combined into a single composite sample or replicate. Two replicates should be collected at each site.
      iii) The following habitats should be sampled if present:
         - Vegetated Margins – consist of overhanging bank vegetation and submerged root mats. Sample by jabbing vigorously, with an

upward motion, brushing the net against the vegetation and roots along the bank. The entire jab should take place underwater to avoid collecting terrestrial organisms.

- Snags and logs – consist of submerged wood, primarily dead trees, logs, branches, roots and leaf packs lodged between rocks or logs. Sample by holding the net under the submerged wood, rubbing the area above the net and scooping organisms, bark, twigs and other dislodged organic matter into the net.
- Aquatic Vegetation beds and decaying organic matter – consist of beds of submerged, green/leafy plants that are attached to the stream bottom. Sample by jabbing vigorously, with an upward motion, against or through the plant bed. The entire jab motion should occur underwater.

Note: Silt, sand or gravel substrate should not be sampled. This includes sandy, silty or muddy stream bottoms.

6.4.2. Lotic Quantitative Benthic Survey

1) Surber Sampler- stream, riffle habitat, natural substrate. This is used in streams where the velocity is greater than 0.2 fps, the depth is less than 0.5 meters and the substrate is sand, gravel, and cobble < 20 cm. diameter.
   a) Equipment
      - Square foot Surber sampler - mesh size 500 microns, base encloses area of 12” x 12”
      - Quart size jars (wide-mouth preferred)
      - 80% ethyl alcohol (ethanol or ETOH)
      - Field sheets
      - Waders

   b) Sampling Procedure
      i) Record Physical Characteristics of sampling site (see Section 6.4.3 Physical Characteristics – Lotic and Lentic Habitats)
      ii) Place frame of Surber sampler quickly and firmly on to the substrate with the net opening facing directly into the current.
      iii) Carefully lift and clean the substrate lying within the sampler frame so that all organisms associated with the substrate 5 - 10 cm down are washed into the net.
      iv) When the substrate within the frame is completely cleaned of organisms, carefully lift the net from the water.
      v) Remove the contents from the net and place in a properly labeled quart jar and cover with 80% ethanol.
2) Artificial Substrate – Rock filled baskets or bags -Quantitative stream/river. This method is employed within a riffle or run habitat where the velocity is greater than 0.2 fps and the dominant natural stream substrate is gravel, cobble, and/ or boulder but not muck.

a) Equipment
   - Rock baskets –use either
     - "Lo-Boy" basket 20 cm x 30 cm x 5 cm (7.5" x 12" x 2"), filled with 10 lbs of 1 ¾" (4.5 cm) graded granite rock

     <u>or</u>
     - "High-Boy" basket 20 cm wide, 30 cm long, 10 cm height (7.5x 12 x 4"), filled with 25 lbs of 4" (10cm) graded granite rocks.

     <u>or</u>
     - "Barbecue Basket Commercial" 6 ½ diameter x 11" length

     <u>or</u>
     - Rock bags filled with 10 lbs of 1 ¾" (4.5 cm) graded granite rock
   - Quart size jars (wide-mouth preferred)
   - No. 30 sieve bucket
   - Flow meter
   - 5 gallon plastic buckets (2)
   - 80% ethyl alcohol (ethanol or ETOH)
   - Meter stick or depth tape
   - Hand scrub brush
   - Field sheets
   - Waders

b) Procedure
   i) Record Physical characteristics of sampling site (Section 6.4.3 Physical Characteristics – Lotic and Lentic Habitats)
   ii) Deployment of Substrates
      - Expose substrates to the stream or river for 4-6 weeks. Exposure time must be equal between control and assessment areas.
      - Water velocity differences between substrates should be less than 0.5 ft/sec when this method is being used to bracket a discharge or impact stress.
      - Record velocity and depth above each substrate on field sheets.
      - Set substrates in midstream.
   iii) Substrate Retrieval
      - Record Physical characteristics of sampling site (6.4.3 Physical Characteristics – Lotic and Lentic Habitats)
      - Record velocity, depth and condition of each rock basket.
      - Retrieve each rock basket using a No. 30 mesh sieve bucket. Retrieve the furthest downstream basket first and work upstream.
      - Open baskets and pour rocks into a clean 5 gallon bucket.
      - Give rocks several initial swirling rinses, decanting the water into the retrieval sieve bucket each time.
      - Clean all rocks of invertebrates using a brush and hands into a 5 gallon bucket half full of water. Discard rocks after cleaning.
      - Swirl-rinse remaining sand and gravel several times into the retrieval sieve basket.
      - Place animals from sieve bucket into quart jar and label. Cover sample with 80% ethanol.

- Pour remaining sand and gravel into sieve bucket and rinse.
- Place remaining sand and gravel into quart jar, preserve with 80% ethanol and properly label.
- Record on field sheets amount of sand/gravel in second half of sample.

6.4.3. Physical Characteristics – Lotic and Lentic Habitats

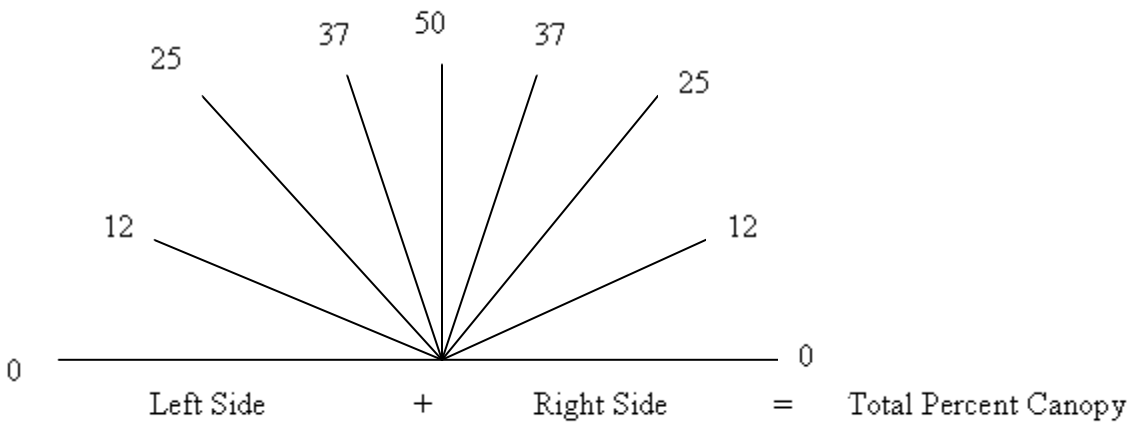
Both physical characteristics and water quality chemical parameters are pertinent to characterization of the aquatic habitat. An example of the data sheet used to characterize the physical characteristics and water quality of a lotic site is shown in Figure 6.4.2. The following physical characteristics should be recorded on the field sheet from Lotic and Lentic Habitats:

1) Header Information – The header information is identical on all data sheets and requires sufficient information to identify the station and location where the survey was conducted, date and time of survey, and the investigators.
2) Weather Conditions – Note the present weather conditions on the day of the survey and those immediately proceeding the day of the survey. This information is important to interpret the effects of storm events on the sampling effort.
3) Site Location/Map – A photograph may be helpful in identifying station location and documenting habitat conditions. A hand drawn map is useful to illustrate major landmarks or features of the channel morphology, vegetation zones, buildings etc. that might be used to aid in data interpretation. GPS units can be used to obtain latitude and longitude coordinates.
4) Watershed Features
   a) Describe predominant surrounding land use type
   b) Note local watershed nonpoint source pollution
   c) Note if stream is channelized, or if there is an upstream dam
   d) Note if the water level is regulated
5) Riparian Vegetation – An acceptable riparian zone includes a buffer strip of a minimum of 50 feet from the stream on either side, and a minimum of 100 feet from the lake shore. The width of the riparian zone and the vegetation within the riparian zone should be documented.
6) Estimate Water Depth – Estimate the vertical distance from the water surface to the bottom at a representative depth to obtain an average depth.
7) Velocity (Lotic only) – Measure the bottom velocity at the points in the stream where macroinvertebrate samples are taken. Use a calibrated velocity meter and record type of meter used. If a direct measurement is not done, estimate the velocity within the sampling reach as slow (<0.4ft/sec), moderate (0.4-2 ft/sec) or fast (>2ft/sec).
8) Stream Width (Lotic)
   a) Bankfull Width estimate Channel width at bankfull discharge. Measure or pace off the distance from bank to bank at a transect representative of the average stream width in the reach.
   b) Wetted Width estimate – Measure or pace off the wetted width of the stream at time of sampling.

9) Percent Canopy Measurement (Lotic) – Note the general proportion of open to shaded area which best describes the amount of cover at the sampling reach or station.

a) Visual Estimate Method - Stand in the center of the stream or river, extend both of your arms straight out creating a 180-degree angle. Observing the overhead canopy cover, start to lift your arms up from the straight out position slowly towards your head. Stop when each arm is in alignment with the overhead canopy. Then estimate the angle of your left and right arm. Combine the percent canopy values from your left and right side to obtain the total percent canopy (Figure 6.4.1).

Figure 6.4.1. Percent Canopy Angle Calculator



b) Densiometer Measurements using spherical convex densiometer

i) Stand near the sampling site at mid-channel facing upstream. Hold the densiometer 0.3 meters (~1 ft) above the water surface. Hold the densiometer so that it is level using the level bubble indicator. Count the number of points covered by vegetation. Percent canopy cover is estimated as number of points with vegetation divided by the total number of points, multiplied by 100. Record this value:

% canopy = (# points with vegetation/total # points) x 100

ii) Repeat the procedure above by sampling site facing downstream. Stand on the transect with the densitometer halfway from the sampling site to the left bank. Repeat again for the right bank.

iii) At this point you will have four measurements for the transect: two from the center and one halfway to each bank. Canopy cover is estimated as the mean for the four measurements.

10) Chemistry. Measure and record values for target water quality parameters (i.e. temperature, conductivity, dissolved oxygen, pH and turbidity) using the appropriate calibrated water quality instruments. Note the type of instrument and unit number used.

11) Embeddedness (Lotic) – Refers to the extent to coarse gravel and cobble sized substrate are buried in silt, sand, or mud. As more coarse substrates become embedded, the amount of surface area available as habitat to macroinvertebrates and fish decreases, due in part to decreasing interstitial space.

12) Substrate Composition

a) Visual Estimate Method – Percent Composition

i) Locate a representative section of stream reach sampled. For macroinvertebrates, this section is defined as a “riffle”, “run” or “pool”.

ii) Estimate the percentage of materials that make up the river bottom within the targeted habitat being sampled for macroinvertebrates at the collection site along a diagonal transect. Estimate percentage of the following size categories: Sand <2 mm (0.08"), Gravel 2-16 mm (0.08-2.5"), Coarse Gravel 16-64 mm (0.63-2.5"), Cobble 64-256 mm (2.5-10.1"), Boulder >256 mm (>10.1") and ledge (exposed bedrock).

b) Gravelometer -Modified Pebble Count

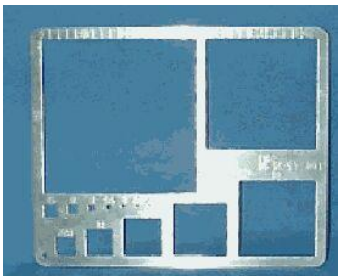
i) General: This method is used to describe the substrate particle size classes within the “riffle” habitat for macroinvertebrate community assessments. The method is based on the more rigorous technique developed by Wolmen (1954) to describe coarse river bed materials. Modifications of this technique developed by the Forest Service describe the channel bed materials within stream reaches (Bevenger and King, 1995 and Harrelson et al, 1994).

ii) Procedure: Pace off diagonal zigzag transects across the stream to cover the full length of the stream reach (riffles) being assessed. This includes dry gravel bars and scoured but dry stream edges. Begin at the lower end of the stream bed within the macroinvertebrate sampling section or riffle. Select a pebble as described below every two paces in larger streams (those greater than 20 m or 65 ft across), or every pace in smaller streams (those smaller than 20 m or 65 ft across). Examine a minimum of 100 particles over the course of the reach.

iii) Avert the eyes away from the streambed to reduce visual bias on substrate selected. Extend an index finger until it touches the first piece of substrate encountered in the streambed. Place the substrate in the gravelometer to determine particle size category. Size categories were initially based on the Wentworth's size classes. Currently, the size classes used by the Vermont Water Quality Division are grouped into larger biologically

based size classes to describe substrate composition. The size categories are: Sand <2 mm (0.08"), Gravel 2-16 mm (0.08-2.5"), Coarse Gravel 16-64 mm (0.63-2.5"), Cobble 64-256 mm (2.5-10.1"), Boulder >256 mm (>10.1").

iv) Size categories are determined by using a gravelometer. This is essentially a metal plate with square openings ranging from 4 mm to 128 mm while notches on the top edge measure 180, 256 and 362 mm. Bottom notches provide additional measurements. The particle must be placed through the smallest opening so that the intermediate axis is perpendicular to the sides (not diagonally across) of the opening. The smallest size category that the particle falls through is called out to a recorder, who keeps track of the tally until the minimum of 100 particles is reached. Complete the transect if this occurs in the middle of the stream.



13) Periphyton Cover Site Measurements (Lotic)

This method can be done in concert with the Gravelometer (Modified Pebble Count) described in Section 12b of Section 6.4.3

a) Procedure:

i) Measure 100 pieces of substrate and classify the moss and algae cover on each substrate within the stream reach sampled. This will generally be the reach in which a macroinvertebrate or fish survey was conducted. Riffle and/or run sections are the preferred habitat to perform periphyton cover measurements.

ii) Diagonal zigzag transects begin at the lower end of the stream bed within the macroinvertebrate sampling section or riffle. A pebble is selected as described below every two paces in larger streams > 20 m (65 ft) across, or every pace in smaller streams <20 m (65 ft) across.

iii) Substrate size will be measured using the gravelometer method described in Section 12 b of 6.4.3. Moss and algae cover observations will be measured on each piece of substrate larger than 2 cm in diameter.

iv) Avert the eyes away from the streambed. Extend an index finger until it touches the first piece of substrate encountered in the streambed (See Figure in section 13, iv). The randomly selected pebble is then placed in a particle size category, and the amount of moss, macro-algae, and micro-algae cover is recorded separately. If substrates are less than 2 cm in diameter, do not tally an entry, but measure the substrate size with the gravelometer. Do not perform periphyton measurement on dry substrate. Record moss and macro-algae cover using a scale from 0-3 with separate estimates for each, where:

0 = no moss or macro-algae present;
1 = some (but < 5% coverage) moss or macro-algae present;
2 = 5-25% cover of substratum by moss or macro-algae; and
3 = > than 25% cover of substratum by moss or macro-algae.

v) Estimate average thickness of micro-algae (periphyton) on the rock with a 0-5 thickness scale, where:
   0 = substrate is rough with no apparent growth;
   1 = substrate is slimy, but biofilm is not visible (tracks cannot be drawn in the film with the back of your fingernail; endolithic algae can appear green but will not scratch easily from the substratum);
   2 = a thin layer of microalgae is visible (tracks can be drawn in the film with the back of your fingernail);
   3 = accumulation of microalgae to a thickness of 0.5-1 mm;
   4 = accumulation of microalgae from 1 mm to 5 mm thick;
   5 = accumulation of microalgae from 5 mm to 20 mm;
   6 = layer of microalgae is greater than 2 cm.

Note that if substrate is too large to pick-up, algal growth should still be characterized.

6.4.4. Lentic Qualitative Littoral Zone Benthic Survey

1) General. This procedure can be used to target a specific macroinvertebrate taxonomic group or groups such as Gastropoda or Amphipoda or to get a general inventory of the whole macroinvertebrate fauna of a lake's littoral zone. The lake littoral zone is made up of many microhabitat types that have a strong influence on the macroinvertebrate species present. In order to qualitatively inventory the macroinvertebrate species of a lake the following microhabitats should be sampled in a lake:
   - Rocky/Cobble/Large Woody Debris -
   - Macrophyte Beds-
   - Fine organic material and leaves

a) Equipment
   - Bottom Kick Net - mesh size 500 microns, 18" wide x 9" high
   - Forceps, strainer
   - No. 30 Sieve bucket
   - Quart size jars (wide mouth preferred)
   - 80% ethyl alcohol (ethanol or ETOH)
   - Field sheets
   - Waders

b) Procedure:
   i) Record Physical Characteristics of sampling site (Section 6.4.3 Physical Characteristics – Lotic and Lentic Habitats)
   ii) Collect animals from all habitat types by sweeping with net thru macrophytes, mud and leaf litter, rinsing off woody debris, macrophytes

and rocks thru a bottom kick net or #30 sieve, and picking animals off substrate of large wood and cobble.

iii) Place collected animals and rinsed material in wide mouth jar and cover with 80% ethanol

iv) Place collected animals and rinsed material in wide mouth jar and cover with 80% ethanol.

v) Label jar with lake name and date.

vi) Record location of habitat sampled on field sheet or map.

vii) Abundance Rating – Benthos (optional). Assign an observed abundance rating to recognizable taxa in the field. This may also be done after the samples are processed in the laboratory. Use the following categories:

Code	Rating	Total # individuals
A	Abundant	10
C	Common	5-10
UN	Uncommon	3-4
R	Rare	1-2

6.4.5. Lentic Semi-Quantitative Benthic Survey

1) General. For the purpose of evaluating the overall macroinvertebrate biological integrity of Vermont Lakes when collecting samples from the littoral and sublittoral zones, the lake will be trisected into equal areas of shoreline. Samples will then be collected from within each trisection of the lake and composited so as to represent the overall condition of the lake.

2) Lake Littoral Zone Habitats. The lake littoral zone is made up of many microhabitat types that have a strong influence on the macroinvertebrate species composition present. Therefore, the littoral zone macroinvertebrate assessment protocols will stratify sample collection based on specific habitat types. Qualitative sampling of each habitat type will take place at three random sites on a lake. Samples of each habitat type will be composites from the three sites to represent the macroinvertebrate assemblage of the lake littoral zone for each microhabitat type. The following microhabitats will be assessed separately:
   - Rocky/Cobble/Large Woody Debris -
   - Macrophyte Beds-
   - Fine organic material and leaves

   a) Equipment
      - Ekman dredge, 6"x 6" square, (15 x 15 cm)
      - Quart size jars (wide mouth preferred)
      - 80% ethyl alcohol (ethanol or ETOH)
      - No. 30 sieve bucket
      - Field sheets
      - Waders

b) Procedure
   i) Record physical characteristics of each sampling site see example field sheet (Section 6.4.3 Physical Characteristics – Lotic and Lentic Habitats).
   ii) Rocky/Cobble/Woody Debris. Composite three sites with10 minutes searched per site. Pick up and wash off rocks and woody debris through a net or sieve and pick off animals from substrate with forceps. Place representative animals in quart size jars. Cover with 80% ethanol and label jar with date, lake name and habitat sampled.
   iii) Macrophyte Beds. Composite three sites with 3 sweeps per site. Sweep net through representative macrophyte beds. Do not sample the lake bottom. Place representative animals in quart size jars. Cover with 80% ethanol. Label jar with date, lake name and habitat sampled.
   iv) Muddy habitat- fine organic material and leaves. Composite three sites with 3 sweeps per site. A sweep net is run through the top 5cm (2.0') of bottom muck. Sieve mud through net or No. 30 sieve. Place sample into quart size jar. Cover with 80% ethanol and label jar with date, lake name and habitat sampled.

6.4.6. Lentic Quantitative Benthic Survey

1) Lake Rocky/Coblle Littoral Zone
   a) Quadrate Sampling using SCUBA. General. Quantitative sampling of the benthic community is conducted by use of SCUBA areal sampling methods.
   b) Procedure. Remove all substrate within the one-quarter square meter quadrate and place into a large 500 micron mesh bag. Seal the bag and return to the surface (boat/shore) for further processing. Wash each sample through a U.S. Standard No . 30 sieve (600 micron). Place the retained sediment, debris, and organisms into labeled jars and preserve with 80% ethanol.
2) Lake Profundal Zone (muck, soft bottom)
   a) Equipment
      i) Ekman dredge usually 6" square (15cm)
      ii) Quart size jars (wide mouth preferred)
      iii) 8O% ethyl alcohol (ethanol or ETOH)
      iv) No. 30 mesh sieve bucket
      v) Squirt bottle
   b) Procedure.
      i) Prior to collecting sample
         - Record physical characteristics of sampling site (Section 6.4.3 Physical Characteristics of Lotic and Lentic Habitats).
         - Identify sample site location from 3 shoreline markers, and/or lake map.
         - Measure water depth, temperature profile. Collect dissolved oxygen sample 1 meter above bottom.

- Record the following (if applicable)
  - ♦ secchi depth
  - ♦ ice thickness, and snow cover
  - ♦ Date, time, water temperature
  - ♦ Sampling personnel
  - ♦ weather, air temperature, wind
  - ♦ Substrate composition of dredge sample
  - ♦ Equipment used, other sampling conducted

ii) Sampling

- Lower dredge to within 2 meters (6.5 ft) of bottom.
- Allow dredge to stabilize (hang straight down, stop swinging).
- Allow dredge to drop into bottom muck.
- Release messenger to dredge.
- Retrieve dredge with a smooth steady pulling action.
- Place dredge into No. 30 sieve bucket and empty contents.
- Record substrate type and composition.
- Sieve out fine muck.
- Pour remaining sample into quart jar.
- Use squirt bottle with ethanol to clean remaining sample from No. 30 sieve bucket into quart jar.
- Cover sample with ethanol.
- Label jar with lake name, lake zone, date.

Figure 6.4.2. **LOTIC BENTHOS FIELD SHEET**

Site Location:
River (site) _____ River Mile _____Site ID_____ BioLab ID _____
Town _____ Stream Order_____ Drainage Area _____Km^2 Elevation_____ft
Latitude _____ Longitude _____ Lat/Long source (GPS, USGS Map,Datum) _____
Site Description_____

Sampling Information:
Date _____ Time _____ Personnel (circle sampler)_____Gear_____
Effort time (min)_____ Area (m^2)_____ Mesh (um)_____ Quantitative: Y / N #Reps:_____ Comp/rep_____
Weather_____ Flow/Weather Previous (2 weeks/2days)_____
Baseflow_____, or Freshet flow_____ Rising / Receding _____ Present Flow: H - M - L

General observations circle those that apply: Overall Aesthetic Rating 0 (poor) – 5 (exc.) _____

A:-Debris Obvious Pollution: Sludge, Sawdust, Paper Fiber, Sand, Silt, Sewage, Oily Sheen, Trash, Iron, Scum, None

B:-Water Clarity Clear, Slightly Turbid, Moderately Turbid, Very Turbid, Secci Tube_____mm

C:-Water Color Clear, Green, Milky, Brown (Tannic) L M H , Gray, Metallic, Reddish

D:- Odors None, Musty, Fishy, Sewage, Manure, Sulfur(eggs), Oily/gas

Embeddedness: 0-5% Excel (5) 5-25% V Good (4) 25-50% Good (3) 50-75% Fair (2) > 75% Poor (1)
Estimate_____%

Silt rating (0-5) _____(0=none, 5= high) CPOM rating (leaf packs)(0-5): _____ (0= none,5=high)
Snags/debris dams #_____/_____m (reach)

Bank Stability: EX , G , F , P ; **Riparian Width**(facing upstream) L_____m, R_____m

% Canopy: 100 90 80 70 60 50 40 30 20 10 0 Overhead = Open, Partly Open, or Closed
(See back for Densiometer form)

Vegetation: (both sides) %: Overstory: Softwood _____ Hardwood _____
(Does not need to add up to 100%) %: Understory: Shrub (brush)_____Grass/ferns etc_____
Surrounding Land Use:_____

Periphyton Cover: For each type 0-100% (*See back for Periphyton Cover Form*)
Diatom _____% Filamentous green _____% and length _____(in) Blue Green _____% Moss _____%
Green_____% Other _____% General Trophic Rating (0-5) _____ (0=oligo, 5=Eutroph)

General Water Type:
Riffle _____ Winder _____ Other _____ Warm _____ Cold _____ Mixed _____
B.F.Width _____(m), Wetted Width_____(m) Riffle Depth _____(m) Pool Depth _____(m) and Obs _____
Velocity (measured): _____ ft/sec, Velocity RANGE-estimate: (circle): _____ <.4 ft/sec (S)_____ .4-2 ft/sec (M) _____ >2 ft/sec (F)
Channelized: Y / N Upstream Dam: Y ____mi / N Other modifications:_____
Water Quality Parameters: Field (**Record)** or Collected (**Circle**), **Meter** (type, #)_____**Chemistry LabId**_____
Temp Air____ **°C,°F Water**____**°C fpH**_____lab pH_____ **fCond**_____labCond _____***D.O.%***_____ **D.Omg/l**_____

Cond pH Alkalinity Color_____ TSS TP DP Cl SO4 Turb TN N02-3 TNH3 DOC, Ca Mg Na K

Hardness Metals Other____,____,____,____,____ ,_____,_____,_____,_____

Field Sheet Complete: _____(initial) **Photo's:** _____ **Fish Survey Conducted (circle):Y / N**_____

Site Sketch & Observations

Aquatic Biota Observed:

Mussels
Crayfish
Gastropods
Fish
Other

River (Site) _____ Personnel _____ Date _____

Pebble Count Field Form

Particle	Millimeters	%Observed	Transect 1 (100 pebbles)	Tot #	Item %
Clay	<.004				
Silt	.004 -0.6				
Sand	0.06 – 2.0				
Gravel	2.0 -16				
Coarse Gravel	16 – 64				
Cobble	64 – 256				
Boulder	>256				
Bedrock					
			TOTALS		

Periphyton Cover Observations

	Moss Cover Index			
Category	0	1(<5%)	2(5-25%)	3(>25%)
Tally				

	Macro-Algae Cover Index			
Category	0	1(<5%)	2(5-25%)	3(>25%)
Tally				

	Micro-Algae Cover Index						
Categor	0	1 (slimy)	2 (draw line)	3(.5-1mm)	4(1-5mm)	5(5-20mm)	6(>20
Tally							

6.5. FISH

6.5.1. Stream Sampling

1) Safety. (This brief outline is not meant as a guide to the beginner. An experienced biologist should always be present on an electrofishing crew).
   a) Never operate shocker alone.
   b) Operator and netters should always use high voltage protective gloves.
   c) At least two individuals in a crew should be certified in CPR.
   d) Never operate shocker in very deep or fast water where footing is uncertain.
   e) Crew should be supervised by an experienced fisheries biologist.
   f) Avoid operation near livestock or other people.
   g) Electrofishing should proceed at a deliberate pace avoiding erratic "fish-chasing" movements.
   h) All participating personnel should wear heavy duty hip boots or chest waders in good condition (no leaks).

2) Sampling Method General
   a) Check equipment ahead of time for problems which may hamper safety and effectiveness.
   b) Select current and pulse level which is sufficient to stun fish effectively but not so strong as to cause mortality. To determine voltage prior to sampling, consult available shocking data or conductivity data. If no data is available, measure field conductivity prior to sampling.
   c) Delineate a stream reach between 75-200 meters (245-650 ft) depending on the goals of the sampling. Describe the depth, velocity and substrate (see field sheet, Figure 6.5.1).
   d) Use block nets to delineate the sampling reach when the river is wide and/or there are no natural barriers. Fishing efficiency will be increased when escapement is minimized.
   e) Sampling should continue in a systematic fashion, sweeping the total area of the stream reach beginning at the downstream boundary. The same individual should operate the anode pole within successive runs in one reach and between reaches which are to be directly compared. This is an important step in minimizing variation in results due to fishing effort.
   f) Captured fish should be placed in a 5-gallon plastic bucket filled ⅓ with ambient water and carried by each person netting fish. At the end of a sweep or run, fish are placed in plastic buckets with holes submerged in the stream. These fish are either identified and enumerated immediately or placed in a stable stream area until all runs are completed. Care must be exercised for

holding cold water species captive during summer months by maintaining acceptable water temperatures.

g) Non-biological observations and measurements may include weather conditions, flow conditions or discharge measurements, water temperature, specific conductance, dissolved oxygen, turbidity, pH and alkalinity. See Vermont DEC Fish Section Habitat Evaluation Sheet, Figure 6.5.1.

3) Sampling Method - Index of Biotic Integrity (IBI) Determination

a) Site Selection - Present use of the IBI is restricted to wadeable-sized streams which naturally support at least two species. Selection of the sampling site is a critical step in fish community evaluation regardless of index used or program goal. The section sampled must be representative, in habitat, of the stream reach in which it is located. More specifically, the sampled section should include all forms of habitat (pool, run and riffle) in approximately the same proportion as observed in the stream reach. If possible, the section should include two pool-riffle cycles.

i) Sampling Method - The standard method of collecting fish is by electrofishing using a backpack shocker. Large Rivers or wide streams should be sampled with 2 or 3 back pack shockers to provide efficient coverage. One to three passes of the reach can be made depending on the goal of the sampling. Block nets are used in the absence of natural barriers at the upstream and downstream limits to minimize fish escapement out of the sampling section. Sites will be sampled during low flow periods of the year between August 1 and October 15.

ii) Fish data- All fish collected will be identified to species and enumerated on field sheets. Notation will be made of presence and relative abundance of young of the year fish and any abnormalities documented (e.g., black spot, yellow grub). Lengths will be measured in mm for all salmonids captured.

6.5.2. Lake Sampling

1) Gill Nets

a) An experimental (multi-mesh size) gill net is a very effective device if the sacrifice of a portion of the catch is consistent with the goals of the study. Since mortality is usually a result of extended periods of entanglement in the mesh, checking nets frequently and retaining captured fish should minimize mortality. If an overnight set is required, then the net should be checked as late in the evening and as early in the morning as possible.

b) Gill netting generally provides qualitative data but relative abundance may be estimated by calculating catch/effort.

c) Depending on the nature of the study, one to many gill nets may be set in a lake. Nets used by the Water Quality Division will be of the experimental type (multiple mesh sizes in a single net). Nets are generally set overnight on the bottom but may be just deployed during daylight. Location of sets should cover as many habitats and depths as possible.

2) Seine Nets
   a) Seines are generally used for strictly qualitative work. The use is limited to waters of less than 1 m (3.2 ft), i.e. littoral shallows, where little or no bottom obstructions exist.
   b) Operation consists of two people, one at each end, walking the net (which is parallel to shore) towards shore while maintaining net contact with the bottom and the float end of the net on the surface. The netters move the net through the water up onto the shore trapping the fish in the net.
   c) Seine nets range in size from 4.6 to 15.2 m (15 – 50 ft) in length and are 1.2 m (4 ft) in width.
3) Minnow Traps
   a) These devices are small wire mesh traps with holes at each end. When baited with dog food or bread, small fish attracted by the scent find their way into the trap and cannot escape.
   b) Minnow traps are inexpensive and provide qualitative data only.
   c) Traps are generally set on the bottom at depths less than 2 m (6.5 ft).

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Figure 6.5.1.

Vermont DEC Fish Section Habitat Evaluation Sheet

INSTREAM COVER E VG G F P COVER TYPES_

BANK VEGETATION CANOPY (%) BANK STABILITY E VG G F P DISCHARGE H M L

EMBEDDEDNESS: 0-5% Excel (5) 5-25% V Good (4) 25-50% Good (3) 50-75% Fair (2) > 75% Poor (1)

BOTTOM TYPE: hard soft mix GRADIENT: high med low flat

WATER CHEMISTRY: Temp_____ Cond_____ pH _____D.O. _____Other _____

LAB CHEMISTRY TAKEN:

LENGTH AND WIDTH MEASUREMENTS	
LENGTHS	WIDTHS

Site Map

PARTICLE	INCHES	PEBBLE COUNT FOR SECTION	TOT	%
SILT				
CLAY				
SAND	0.002 -0.08			
GRAVEL	0.08 - 0.63			
COARSE GRAVEL	0.63 - 2.5			
COBBLE	2.5 - 10.1			
BOULDER	>10.1			
BEDROCK				

General observations circle those that apply: Overall Aesthetic Rating 0 (poor) – 5 (exc.) _____

A: Debris Obvious Pollution: Sludge, Sawdust, Paper Fiber, Sand, Silt, Sewage, Oily Sheen, Trash, Iron, Scum, None

B: Water Clarity Clear, Slightly Turbid, Moderately Turbid, Very Turbid, Secci Tube_____mm

C: Water Color Clear, Green, Milky, Brown (Tannic) L M H , Gray, Metallic, Reddish

D: Odors None, Musty, Fishy, Sewage, Manure, Sulfur(eggs), Oily/gas

6.6. MACROINVERTEBRATE LABORATORY SAMPLE PROCESSING METHODS

6.6.1. General. The following method describes how all macroinvertebrate samples are processed (including standardized sub-sampling methodology) for estimates of density, and number of taxa reported per a specified sampling method. All samples are initially processed by one individual until considered completely picked. A second highly qualified technician or biologist (with at least two years experience in macroinvertebrate sample processing) then checks through the entire sample or sub-sampled portion of the sample for completeness, and does a qualitative taxa pick if applicable.

6.6.2. Macroinvertebrate Sample Density. (To evaluate the number of individuals of macroinvertebrate animals/sample unit.)

1) Equipment
   a) 80% ethyl alcohol (ethanol or ETOH)
   b) No. 30 sieve
   c) 12″ x 18″ white (enamel) tray, surface of tray delineated into a minimum of 24 equal squares
   d) 3″ - 4″ fine pointed watchmaker forceps
   e) Phloxine B red dye
   f) 2-3 gallon plastic bucket
   g) 1 inch x 1-3 inch x ¼ inch granite blocks – used to delineate sub-sampling grids (or an acceptable device to delineate grids for sub-sampling).
   h) counter
   i) quarter sectioned plastic petri dishes
   j) marker
   k) tap water
   l) 2x magnification illuminator
2) General Processing Procedure (for full sample processing and sub-sampling)
   a) Pour sample into a No. 30 sieve and wash sample thoroughly of preservative.
   b) Wash large pieces of woody debris and leaves of animals into sieve and discard.
   c) Backwash sample from sieve into delineated tray.
   d) Spread sample evenly over entire tray surface. Add enough water to aid in spreading the sample evenly Note: Water should just cover gravel and debris; too much water will cause animals to float around tray (~ ⅓-½″ deep).
   e) Pick all macroinvertebrates from tray and sort into major taxonomic groups (orders) in petri dish quadrants. Cover with 80% ethanol. If after five minutes of searching, no animals are found, the sample is considered totally picked by initial picker. Sample is then checked by qualified technician/biologist before being considered completely processed.

3) Sub-sampling Processing Procedure

a) After sample is spread evenly onto tray, choose a random square (grid) to start picking procedure from.
b) Pick all animals from each grid proceeding in a clockwise direction until ¼ of sample is picked (six grids) and at least 300 animals. If less then 300 animals are picked from first ¼ of sample (6 of 24 grids), pick additional grids until 300 animals are picked or the entire tray is picked. Record the final number of grids picked on the laboratory bench sheet. Once a square is started, all animals from that square must be picked.
c) Sample is then checked by a qualified technician/biologist , and the number of animals picked is determined. If less then 300 animals are picked from first ¼ of sample (6/24 grids) then the number of additional grids needed to reach a 300 animal minimum sub-sample size is estimated. From this estimate the number of additional grids are delineated and the procedure of initial picking and checking is continued until 300 animals are picked or the entire tray is picked. The final number of grids picked (or proportion of sample) is recorded. Once a grid or square is started, all animals from the square must be picked.
d) Total density or abundance of macroinvertebrates for a sample is calculated as follows:

$$A = 24/B * C$$

Sample Total Density	=	A
Total # grids	=	24
Total # grids picked	=	B
Total # individuals picked	=	C

4) Variations on sample processing
   a) Pre-sieving "swirling" technique
      i) If the sample contains a large amount of sand and gravel (more than ⅓ of the quart jar), it is recommended that the sample be gently "swirled" to remove much of this material before attempting to pick out the animals from the sample. It is important to gently swirl the sample to avoid damaging fragile macroinvertebrate body parts such as mayfly gills.
      ii) Pour the preserved sample into a No. 30 sieve. Transfer sample from the sieve into a 5 gallon bucket. Fill the bucket half full of water. Gently swirl the contents of the bucket. The lighter materials, including the animals, will float to the top.
      iii) Pour off the water and the material floating in it, into the sieve, leaving the sand and gravel in the bucket. Repeat these steps several times, until there is no longer lighter material floating to the surface.
      iv) When the sample has been successfully swirled, be sure to save the sand and gravel remaining in the bucket. This will need to be checked for animals when the primary sample has been completed. Stone cased caddisflies and other animals could be remaining in the separated sand and gravel. Subsample this material the same way that the primary sample is processed.
   b) Phloxine B red dye. Sample is dyed to aid in seeing and picking the smaller animals (especially small worms and dipterans).

i) Add a few grains of dye by dipping wetted glass rod or pointer into dye then stirring into the quart jar of preserved macroinvertebrates.
ii) Gently invert jar to dissolve and distribute dye.
iii) Allow sample to sit for 24 hours before processing.

5) Qualitative pick.
   a) General. If a subsampling procedure is used, conduct a qualitative pick of the remaining sample.
   b) Procedure
      i) Search for taxa not found in sub-sample procedure.
      ii) Pick animals out and place is new Petri dishes, separate from the subsampled taxa.
      iii) These taxa are reported as additional qualitative information and not included in taxa richness calculations.

6.6.3. Taxa Richness and EPT Richness.

Richness is the total number of distinct taxa identified in a sample. EPT Richness includes just those animals from Ephemeroptera, Plecoptera and Trichoptera.

1) Materials
   a) 80% ethyl alcohol (ethanol or ETOH)
   b) Binocular dissecting microscope 7x - 60x minimum range
   c) Fiber optic illuminator
   d) petri dishes – divided and quartered
   e) VTDEC listed taxonomy keys
   f) Labeling paper, permanent ink
   g) 3-4" fine pointed watchmakers forceps
   h) Fine pointed probes
   i) Scalpel - #15 blades
   j) 20 - 30 ml snap cap glass vials
   k) ¼ dram - 2 dram open glass vials
   l) Cotton
   m) 500 ml screw cap, wide mouth, glass jars
2) Method
   a) Sort animals to taxonomic groups (Orders) under low power and placed in 20 - 30 ml bottles with laboratory ID number.
   b) Identify groups to lowest possible/ recommended taxonomic unit (usually genus or species) using recommended VTDEC keys.
   c) Certain groups (i.e. Chironomidae, Oligochaeta) or individuals are then slide mounted for identification. See method 6.6.4.
   d) Animal names and number of individuals are recorded on bench sheets by lab ID number. The taxonomist initials each identification and assigns a confidence level in the identification, ranging from A (confident) to C (uncertain). Typically biologists will not use a “C” rating; instead they will use the broader taxonomic classification (i.e. genus instead of species)
   e) Animals are archived by taxonomic order in dram-sized vials, in 80% ethanol, and identified by laboratory ID number.

f) Vials are capped with cotton wads and stored by year sampled and order groupings in 500 ml screw cap jars topped with 80% ethanol.

6.6.4. Chironomidae and Oligochaeta Identification. Used to determine the genera and species of chironomid and oligochaeta animals in a sample unit.

1) Materials
   a. 80% ethyl alcohol (ethanol or ETOH)
   b. Mounting Media - Hoyers Media
      i. Chloral Hydrate 200 g
      ii. Gum Arabic 30 g
      iii. Glycerine 20 g
      iv. Distilled H_2O 50 ml
   c. Binocular Dissecting Microscope - 7x - 60 x minimum range
   d. Fiber optic illuminator
   e. Compound Microscope with phase contrast - 40 - 400x, 1000x oil immersion
   f. Divided petri dishes
   g. Microscope slides - 75 x 25 mm x 1 mm thick
   h. Cover glass slips - 22 x 22 mm, thickness 0-1
   i. Slide boxes
   j. VTDEC listed keys
   k. Labeling stickers, permanent ink
   l. 3-4″ fine pointed watchmakers forceps
   m. scalpel with #15 blade
2) Method
   a. Presort chironomids/oligochaetes into genus/species groups using the dissecting microscope.
   b. Mount individuals of each grouping on individual microscope slides. Usually 2 - 3 animals can be mounted per cover slip area. This equals 4-6 animals per slide.
   c. Orientation of animals
      i. Chironomids are mounted so the ventral side of the head capsule faces upward.
      ii. Oligochaetas entire body is mounted on the slide.
   d. Genus/species groups represented by many individuals (> 10) may be sub-sampled in sufficient amounts to insure a correct identification of the group (10-50% depending on the distinctiveness of the group).
   e. Labels are marked to identify the exact sample the animals came from using the laboratory log ID number.
   f. Genus/species names and number of individuals of each are recorded in a laboratory work sheet identified by the laboratory log ID number.

7. Miscellaneous Collection Methods

7.1. FIELD SAMPLE FILTERING

7.1.1. Filtering of an ambient water sample should be done at the time of sample withdrawal prior to field preservation. Filtering is necessary to remove solids from the ambient water collected.

7.1.2. Dissolved concentrations (dissolved phosphorus, dissolved nitrogen, metals, etc.) must be filtered for analysis.

1) Filtering Procedure
   a) Rinse filtering apparatus (filter, syringe, etc) with distilled water 3 times.
   b) Rinse syringe with ambient water 3 times.
   c) Fill syringe with ambient sample water and purge filter with ~10 mls of water.
   d) Rinse sample bottle if necessary 2 times with filtered water before filling (Do Not Rinse container for phosphorus analysis).

7.2. AUTOMATIC SAMPLING DEVICES

7.2.1. ISCO 2900 Portable Sampler

1) General: An ISCO Automatic sampler can be used to sample remote locations and/or for sampling a location frequently when manual sampling is inefficient or otherwise prohibitive. The sampler consists of a weather tight plastic body to protect the samples, the instrumentation, a solar panel and tubing which runs from the unit to the waterbody being sampled.
2) Before Use:
   a) Charge ISCO Lead-Acid Battery Pack.
   b) Clean ISCO sample bottles, lids and collection tubing.
3) Install the ISCO sampler in a flat area or use the field stand to insure the instrument is level (See image to the right).
4) Program the volume and frequency of samples to be collected. Refer to Model 2900 Sampler Instruction Manual (1992, revision E) for more detailed directions.

7.3. LAKE SEDIMENT SAMPLING FOR TRACE METALS

7.3.1. Sediment sampling for mercury analysis

1) Due to high mercury concentrations found in lake sediments, strict mercury-clean techniques will not be required, provided that cores are sectioned in the field, as soon as practical after collection. Project staff will, however, use gloves and an adapted clean hands-dirty hands protocol when collecting and sectioning sediments.
2) Equipment needed
   - Clean lot-certified PETE bottles, 125ml
   - Glew-design, modified KB corer with a 60 cm by 7 cm lexan core tube, <u>or</u> a KB corer with a 60 cm by 5cm lexan tube and a cellulose acetate butyrate liner.
   - Core rubber caps
   - large plastic bags (to store lexan tubes)
   - scraper
   - lexan sectioning tray
   - Sonar
   - Disposable powder-free gloves
   - Zip-style plastic bags

3) Preparation and storage of field equipment: Acid clean the lexan core tubes before going into the field. Core tubes will be stored in doubled, plastic bags between acquisitions. These bags will be replaced regularly. Core tubes will be acid washed every tenth sample collected, or when the field coordinator determines that cleaning is necessary. Core sectioning tools (scraper, lexan sectioning tray) will be cleaned following the same schedule as core tubes, and will be stored in plastic as well.
4) Sampling Procedure
   a) Once positioned at the lake sampling station, rinse tubes copiously in lake water prior to use.
   b) Label two sample bags. The inner bag with a grease pencil, the outer bag with an adhesive label marked with indelible ink.
   c) ‘Clean hands’ and ‘dirty hands’ are designated.
   d) ‘Dirty hands’ assists “Clean hands’ in donning gloves. ‘Clean hands’ rinses and handles the core tube, placing it into the corer head. ‘Dirty hands’ is responsible for handling the corer head and line, and for collecting the core. The core descent is tracked using SONAR.
   e) When corer is just above lake sediments, release corer until the descent stops. Release messenger, wait for trigger to release and retrieve corer slowly.
   f) ‘Clean hands’ caps the core bottom upon its arrival at the surface. Do not pull corer from water until bottom is capped. ‘Dirty hands’ removes corer head, while ‘clean hands’ holds the core. ‘Clean hands’ caps and sets the core to a rack, ‘Dirty hands’ assembles extrusion equipment.
   g) ‘Clean hands’ places the core onto the extruder. ‘Clean hands’ affixes sectioning tray onto the core tube. ‘Dirty hands’ tightens associated fasteners. ‘Clean hands’ prepares sample bottles and removes sectioning tools from their bags. While ‘dirty

hands' controls extrusion from the core bottom, 'clean hands' sections the sediment into the sample bottle. Bottles are placed singly into zip-style bags.

h) Sectioning sediment samples for dated sediment cores.
   i) Rinse a lexan sectioning tray copiously.
   ii) For those depths from which mercury samples are to be analyzed, the extruded sediments will be split on the tray, and each half moved into a new, clean Whirlpak-type bag using a plastic scraper.
   iii) Sediments will be stored in double bags, in a specially designated cooler. At no time will sediment samples be placed into the same cooler as aqueous mercury samples.

i) Rinse all field equipment (corer, tubes, scraper, and lexan sectioning tray) in lake water after sediments are removed.

j) Sample Acceptance/Rejection: Cores will be rejected and the core re-collected if: 1) sediments contact metal portions of the corer head (overflow); 2) the sediment-water interface is disturbed; 3) the field coordinator judges that a contamination may have occurred, or the core is of poor quality; or 4) gaseous ebullition caused by temperature differential causes the core to break apart before sectioning.

k) Two cores will be acquired from each sampling station. The core reflecting the least disturbance will be selected for analysis at the laboratory.

8. Chain Of Custody

8.1. Chain of custody procedures are necessary to insure the legal integrity of sample materials collected and submitted to the VTDEC Laboratory for analysis. The validity of the test results is assured if the Department can show that the samples collected were maintained safe from tampering or interaction with adulterating chemicals. This requires the complete written documentation of the security of the sample must be kept from collection to disposal.

8.2. Current information on chain of custody procedures is located in Section 7 of the VTDEC Laboratory QA Plan (2005).

8.3. Contact the VTDEC Laboratory prior to submitting samples. Laboratory staff must be present to submit Chain of Custody samples.

9. Sample Log-In

9.1. General: All samples brought to the Laboratory for analysis must be logged in to the VTDEC Laboratory Information Management System (LIMS). The log-in computers are located on the second floor of the VTDEC Laboratory in room 224. Current information regarding the sample log-in process is located in Section 7 of the VTDEC Laboratory QA Plan (September 2005).

9.2. Required fields. In order to log-in to the LIMS system, the following data fields are required:

- Date collected
- Time collected
- Matrix (water, solid, etc.)
- Collector
- Tests requested

9.3. Procedure: Detailed instructions are available next to the log-in computers in room 224.

10. Safety

10.1. GENERAL POLICY

It is the policy of the Department of Environmental Conservation to prevent and control anything that may cause personal injury or property damage. The Department equates safety in importance with work quality and productivity. It is the responsibility of the Department to provide a safe as possible working environment, and explanation of the relative hazards of all materials an employee might come in contact with and the information on how to safely handle these materials, hazardous or non-hazardous. It is the responsibility of the employee to know the nature and safe handling of the materials he or she works with, to follow the laboratory safety rules and to use common sense while working.

All employees must read and understand the Water Quality Division Employee Health and Safety Plan (VTANR, 2005) before conducting field work. This plan discusses medical monitoring, injury reporting, driving and boating on state business, operation of boats, and fish electroshocking precautions. Employees involved in underwater diving must read the Division's Policy for Underwater Work Utilizing SCUBA (March 1998).

For safety requirements at the VTDEC Laboratory, refer to the Laboratory Chemical Hygiene Plan and Safety Manual (VTANR, 1999) for further information.

10.2. BASIC RULES OF VTDEC LABORATORY

10.2.1. **No samples are allowed into the laboratory office area.**

10.2.2. All laboratory users must sign in and out with the laboratory secretary.

10.2.3. The laboratory supervisor will maintain a record of which laboratory employees and users are present on any given day. Laboratory employees should cooperate with the supervisor by calling promptly if sick and by submitting agendas when leaving the facility. This record becomes very important should a building evacuation be necessary.

10.2.4. Each new employee must receive and read a copy of the Safety Manual and take a tour of the building.

10.2.5. All employees must wear prescription or non-prescription eye glasses while handling hazardous or potentially hazardous substances.

10.2.6. All users must wear enclosed shoes, i.e., no open-toed shoes such as sandals.

10.2.7. All employees must wear protective gloves while handling hazardous materials.

10.2.8. All laboratory users will be provided lab coats and encouraged to wear them.

10.2.9. Mouth pipetting is forbidden.

10.2.10. Eating and drinking is forbidden while handling samples or reagents.

10.2.11. When hazardous liquids, such as acids or organic liquids, are moved from room to room, they must be transported in plastic safety containers. When traveling between floors with hazardous liquids, employees must use the elevator rather than the stairs.

10.2.12. Smoking is forbidden while handling flammable or potentially hazardous substances.

10.2.13. All employees must know the substances they are working with, the potential hazards of the substances and how to react in the case of an accident.

10.2.14. Accidents, regardless of severity, must be reported to one's supervisor immediately.

10.2.15. All laboratory users must inform the laboratory supervisor when working in the laboratory other than during normal working hours.

10.2.16. Temporary employees and permanent non-laboratory personnel must not work without a permanent laboratory employee present in the building.

10.2.17. All chemical containers must be adequately labeled to indicate their contents. A commercial chemical container label must include the date of receipt, opening and the initials of the person who opened it.

10.2.18. All materials, hazardous and non-hazardous must be stored and handled according to the specific rules applicable to those materials.

10.2.19. A chemical inventory must be kept current.

10.2.20. Safety equipment maintenance must be performed according to manufacturer instructions.

10.3. CHEMICAL RATINGS

10.3.1. Introduction

The chart below (10.3.6) provides a brief summary of chemicals used in sampling and ranks their degree of hazard accordingly. These ratings are no substitute for the Material Safety Data Sheets (MSDS). MSDS are available from the VTDEC Safety Officer and are located in the VTDEC Laboratory Conference Room library. Users are required to read the MSDS for each chemical used in the analyses they are assigned, and to be knowledgeable about the hazard of each chemical they are using. The rating system for Health, Flammability and Reactivity is based on Fisher's HSDS; the assigned ratings are based on regulations promulgated under CERCLA by EPA. .

1) HEALTH - The danger or toxic effect a substance presents, if it is inhaled, ingested or absorbed.
2) FLAMMABILITY - The tendency of a substance to burn. Refer to Table 10.3.2 for a description of each rating.
3) REACTIVITY - The potential of a substance to explode or react violently with air, water or other substances. Refer to Table 10.3.3 for a description of each rating.
4) CONTACT - The danger a substance presents when exposed to skin, eyes and mucous membranes. Refer to Table 10.3.4 for a description of each rating.
    a) Severity Toxicity (Rating of 3)
        i) Acute local. Materials which on single exposure lasting seconds or minutes cause injury to skin (dermal) or mucous membranes or sufficient severity to threaten life or to cause permanent physical impairment or disfigurement.
        ii) Acute systemic. Materials which can be absorbed into the body by inhalation, ingestion (oral), or through the skin and which can cause injury of sufficient severity to threaten life following a single exposure lasting seconds, minutes, or hours, or following ingestion of a single dose.
        iii) Chronic local. Materials which on continuous or repeated exposure extending over periods of days, months, or years can cause injury to skin or mucous membranes of sufficient severity to threaten life or cause permanent impairment, disfigurement, or irreversible change.
        iv) Chronic systemic. Materials which can be absorbed into the body by inhalation, ingestion or through the skin and which can cause death or serious physical impairment following continuous or repeated exposures to small amounts extending over periods of days, months or years.
5) Moderate Toxicity (Rating of 2)
    a) Acute local. Materials which on single exposure lasting seconds, minutes, or hours can cause moderate effects on the skin or mucous membranes. These effects may be the result of intense exposure for a matter of seconds or moderate exposure for a matter of hours.
    b) Acute systemic. Materials which can be absorbed into the body by inhalation, ingestion, or through the skin and which produce moderate effects following single exposures lasting seconds, minutes, or hours, or following ingestion of a single dose.

c) Chronic local. Materials which can be absorbed into the body by inhalation, ingestion, or through the skin and which produce moderate effects following continuous or repeated exposures extending over periods of days, months, or years.
d) Chronic systemic. Materials which can be absorbed into the body by inhalation, ingestion, or through the skin and which produce moderate effects following continuous or repeated exposures extending over periods of days, months, or years. Those substances classified as MOD or having "moderate toxicity" may produce irreversible as well as reversible changes in the human body. These changes are not of such severity as to threaten life or produce serious physical impairment.

6) Slight Toxicity (Rating of 1)
   a) Acute local. Materials which on single exposures lasting seconds, minutes, or hours cause only slight effects on the skin or mucous membranes regardless of the extent of the exposure.
   b) Acute systemic. Materials which can be absorbed into the body by inhalation, ingestion or through the skin and which produce only slight effects following single exposures lasting seconds, minutes, or hours, or following ingestion of a single dose, regardless of the quantity absorbed or the extent of exposure.
   c) Chronic local. Materials which on continuous or repeated exposures extending over periods of days, months, or years can cause only slight and usually reversible effects following continuous or repeated exposures extending over days, months, or years. The extent of the exposure may be great or small.
   d) Chronic systemic. Materials which can be absorbed into the body by inhalation, ingestion, or through the skin and which produce only slightly usually reversible effects following continuous or repeated exposures extending over days, months, or years. The extent of the exposure may be great or small.
7) No Toxicity (Rating of 0)
   a) Materials which cause no harm under any conditions of normal use.
   b) Materials which produce toxic effects on humans only under the most unusual conditions or by overwhelming dosage.

10.3.2. Flammability Rating

Rating	Description
3	Very flammable gasses, very volatile flammable liquids, and materials that in the form of dusts or mists readily form explosive mixtures when dispersed in air. Shut off flow of gas or liquid and keep cooling water streams on exposed tanks or containers. Use water spray carefully in the vicinity of dusts so as not to create dust clouds. <u>or</u> Liquids which can be ignited under almost all normal temperature conditions. Water may be ineffective on these liquids because of their low flash points. Solids which form coarse dusts, solids in shredded or fibrous form that create flash fires, solids that burn rapidly, usually because they contain their own oxygen, and any material that ignites spontaneously at normal temperatures in air.
2	Liquids which must be moderately heated before ignition will occur and solids that readily give off flammable vapors. Water spray may be used to extinguish the fire because the material can be cooled to below its flash point.
1	Materials that must be preheated before ignition can occur. Water may cause frothing of liquids with this flammability rating number if it gets below the surface of the liquid and turns to steam. However, water spray gently applied to the surface will cause a frothing which will extinguish the fire. Most combustible solids have a flammability rating of 1.
0	Materials that will not burn.

10.3.3. Reactivity Rating

Rating	Description
3	Materials which in themselves are readily capable of (1) detonation (2) explosive decomposition or (3) explosive reaction at normal temperatures and pressures. Includes materials which are sensitive to mechanical or localized thermal shock. If a chemical with this hazard rating is in an advanced or massive fire, the area should be evacuated. Or Materials which in themselves are capable of (1) detonation (2) explosive decomposition or (3) explosive reaction, but which require a strong initiating source or which must be heated under confinement before initiation. Includes materials which are sensitive to thermal or mechanical shock at elevated temperatures and pressures or which react explosively with water without requiring heat or confinement. Fire fighting should be done from an explosion-resistant location.
2	Materials which in themselves are normally unstable and readily undergo violent chemical change but do not detonate. Includes materials which can undergo chemical change with rapid release of energy at normal temperatures and pressures or which can undergo violent chemical change at elevated temperatures and pressures. Also includes those materials which may react violently with water or which may form potentially explosive mixtures with water. In advanced or massive fires, fire fighting should be done from a protected location.
1	Materials which in themselves are normally stable but which may become unstable at elevated temperatures and pressures or which may react with water with some release of energy but not violently. Caution must be used in approaching the fire and applying water.
0	Materials which are normally stable even under fire exposure conditions and which are not reactive with water. Normal fire fighting procedures may be used.

10.3.4. Contact Rating

Rating	Description
3	A corrosive material under the U.S. Department of Transportation regulations. It is a liquid or solid which causes visible destruction or irreversible alterations in human skin at the site of contact. This would include those chemicals which can quickly penetrate the skin and cause internal damage.
2	A liquid or solid which is an irritant or causes a burning reaction on the skin. It may sensitize some individuals to further exposure.
1	A liquid or solid which can cause a mild irritation on skin contact.
0	A liquid or solid which produces no known skin problems on contact.

10.3.5. Carcinogens. Table 10.3.6 lists chemicals commonly used by Water Quality Division staff. This table ranks these chemicals according to their hazard rating as described in the previous sections. Please refer to Section XIII of the Laboratory Chemical Hygiene Plan and Safety Manual (VTANR, 1999) for a complete listing

In reference to Table 10.3.6, a "+" symbol denotes any substance that is one or more of the following:

- a human carcinogen
- an animal carcinogen
- a suspected human or animal carcinogen
- a compound that has earned even limited tumorigenic data insufficient for definitive classification as a carcinogen.

Any compound denoted by a "+" symbol should be handled as if it were a known human carcinogen.

10.3.6. Chemical Chart.

Chemical Compound	Hazard rating				
	Health	Flammability	Reactivity	Contact	Carcinogen
acid spill kit	U	0	0	1	
ascorbic acid	0	0	0	0	
pH buffer 4	0	0	0	0	
pH buffer 7	0	0	0	0	
pH buffer 10	U	U	U	0	
chloral hydrate	3	2	0	1	+*
cupric sulfate	3	0	0	1	
ethanol	1	3	0	0	+*
formalin	2	1	0	1	+
glycerine	1	1	0	1	
gum arabic	0	0	0	1	
hydrochloric acid	3	0	0	3	
Lugol's preservative:					
glacial acetic acid	3	2	2	2	
iodine (crystalline)	1	0	1	3	
potassium iodide	2	0	0	1	
manganous sulfate	U	0	0	1	
nitric acid	3	0	0	3	
phloxine B	U	0	0	1	
phosphoric acid	2	0	1	2	
potassium chloride	1	0	0	0	
rhodamine	U	0	0	0	+
sodium azide	3	0	2	1	
sodium iodide	2	0	0	1	
sodium thiosulfate	2	0	0	1	
sulfuric acid	3	0	2	3	

U=unknown
* data in the Registry of Toxic Effects of Chemical Substances (RTECS) indicate evidence of possible tumorgenic action
+ indicates the material is a possible carcinogen. See section 10.3.5 above.

10.4. MATERIAL DATA SAFETY SHEETS

The Material Safety Data Sheet (MSDS) is a key element in identifying the hazards associated with the handling of chemical reagents. The information that appears on a MSDS goes beyond hazards and includes the following: physical and chemical properties, acute and chronic health hazards, physical hazards, exposure limits, precautionary measures, and emergency and first-aid measures. Sampling personnel are required to know the specific hazards of the substances they are working with, and how to react in case of an accident.

Material Safety Data Sheets are available for all substances in the VTDEC Laboratory. These are stored in room 225 (conference room) on the second floor of the laboratory.

11. References

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12. Appendix A.

Hydrolab Standard Operating Procedure

This standard operating procedure addresses the use and maintenance of the Hydrolab minisonde 4 with Surveyor 4 and Hydrolab minisonde 4a with Surveyor 4a.

I. HYDROLAB MINISONDE 4 AND SURVEYOR 4

IA. Parameter Sensors

Temperature
Dissolved Oxygen
PH
Conductivity
Depth

IB. Maintenance

1. MAINTENCE PERFORMED PER HYDROLAB MANUAL

2. RECHARGE SURVEYOR

a. On last run turn off Timed shutdown (Setup/Cal, Setup, Timeout:Shutdown, 0 to disable)
b. Leave unit on to discharge battery completely (usually lasts 24 hrs total)
c. Recharge 3.5 hrs (plug in and turn on)
d. Turn Timeout on to save charge (Setup/Cal, Setup, Timeout:Shutdown, 5)

3. MAINTENANCE SCHEDULE

a. Done once a month or as readings indicate need or as sensors foul
b. Change DO membrane & electrolyte, make sure no bubble under membrane
c. Change pH electrolyte
d. Clean glass pH probe with methanol
e. Clean conductivity port, removing o rings and buffing prongs

IC. Calibration

1. CALIBRATION SCHEDULE

a. Do once each day
b. Calibrate following Hydrolab manual
c. PH (7 first, then 4 or 10, then check sample); Check sample recipe:
    1. Add 1mL of .02 N H_2SO_4 to a 1,000 mL erlenmyer flask.

    2. Add 999 ml of distilled water.
    3. pH of 4.70ish +or- .10 units.
d. Conductivity (10 first, then 100)
e. Dissolved Oxygen
    1. Dissolved Oxygen Make sure that the stirrer is enabled (Setup/Cal, Setup, Sonde, Circulator, On)
    2. Fill the calibration cup with finished water, up to the DO membrane O ring, leaving the membrane dry (but stirrer may flick water onto it).
    3. Set the black cap balanced on top
    4. Read the BP, and remember it Select Setup/Cal, Calibrate, DO%, enter BP
    5. Do a zero check using Standard Methods 4500 0-G
f. Thermometer check
    1. Fill bowl (water bath) with tap water.
    2. Using laboratory thermometer and Hydrolab sonde, simultaneously measure temperature of water bath
    3. Verify agreement of temperature readings.
    4. Calibrate temperature sensor per Hydrolab instructions to match laboratory thermometer.
g. Record calibrations in calibration excel spreadsheet log like that shown in Table 1 (see section IIC.

Verify readings at the end of each sampling day. If calibrations have drifted, field data from that day's sampling will need to be qualified.

ID. Field Operation

1. CALIBRATING DISSOLVED OXYGEN

This step is done on site at first site visited for day.

a. Using Sun Company Altimeter 202 © and Barometer obtain the real world barometric pressure on site. The Hydrolab dissolved oxygen calibration uses the True, real-world barometric pressure and not the sea-level compensated barometric pressure reported on the radio. Hence, use the uncompensated barometric pressure reading shown in the orange area of the barometer when on site (Figure 1).

Figure 1. Sun Company Altimeter 202 ©, with ambient barometric pressure gauge in orange.

b. Using the pressure conversion chart in Figure 2, the barometric pressure is converted from In. Hg to mm Hg.
c. Connect Hydrolab to Surveyor (careful to pull plug off – DO NOT TWIST)
d. Remove white cap off calibration cup
e. Cover sensors with ambient water to just below O ring of DO sensor membrane
f. Set white cap over calibration cup (just to keep temperature from fluctuating)
g. Make sure stirrer is on. If it is not, use the below steps to turn it on:

- Setup/Cal on Surveyor
- Setup
- Sonde
- Select 'Circltr: Off/On
- Select 1 for On
- Done
- Hit any key
- Hit Go Back until Go Back is no longer a selection

h. Have Barometric Pressure # handy from step b
i. Hit Setup/Cal on Surveyor
j. Calibrate
k. Sonde
l. Select 'DO%'
m. Enter BP
n. Done
o. Hit any key
p. Hit Go Back until Go Back is no longer a selection
q. Dissolved Oxygen calibration is complete for today.

Figure 2. The Surveyor 4 will only accept barometric pressures in mm Hg, so this table is used to convert the barometer reading from In. Hg.

PRESSURE CONVERSION TABLE

Millibars	In. Hg	mm Hg
900	26.577	675.06
901	26.607	675.81
902	26.636	676.56
903	26.666	677.31
904	26.695	678.06
905	26.725	678.81
906	26.754	679.56
907	26.784	680.31
908	26.813	681.06
909	26.843	681.81
910	26.872	682.56
911	26.902	683.31
912	26.931	684.06
913	26.961	684.81
914	26.990	685.56
915	27.020	686.31
916	27.049	687.06
917	27.079	687.81
918	27.109	688.56
919	27.138	689.31
920	27.168	690.06
921	27.197	690.81
922	27.227	691.56
923	27.256	692.31
924	27.286	693.06
925	27.315	693.81
926	27.345	694.56
927	27.374	695.31
928	27.404	696.06
929	27.433	696.81
930	27.463	697.56
931	27.492	698.31
932	27.522	699.06
933	27.551	699.81
934	27.581	700.56
935	27.611	701.31
936	27.640	702.06
937	27.670	702.81
938	27.699	703.56
939	27.729	704.31
940	27.758	705.06
941	27.788	705.81
942	27.817	706.56
943	27.847	707.31
944	27.876	708.06
945	27.906	708.81
946	27.935	709.56
947	27.965	710.31
948	27.994	711.06
949	28.024	711.81

Millibars	In. Hg	mm Hg
950	28.053	712.56
951	28.083	713.31
952	28.113	714.06
953	28.142	714.81
954	28.172	715.56
955	28.201	716.31
956	28.231	717.06
957	28.260	717.81
958	28.290	718.56
959	28.319	719.31
960	28.349	720.06
961	28.378	720.81
962	28.408	721.56
963	28.437	722.31
964	28.467	723.06
965	28.496	723.81
966	28.526	724.56
967	28.555	725.31
968	28.585	726.06
969	28.615	726.81
970	28.644	727.56
971	28.674	728.31
972	28.703	729.06
973	28.733	729.81
974	28.762	730.56
975	28.792	731.31
976	28.821	732.06
977	28.851	732.81
978	28.880	733.56
979	28.910	734.31
980	28.939	735.06
981	28.969	735.81
982	28.998	736.56
983	29.028	737.31
984	29.058	738.06
985	29.087	738.81
986	29.117	739.56
987	29.146	740.31
988	29.176	741.06
989	29.205	741.81
990	29.235	742.56
991	29.264	743.31
992	29.294	744.06
993	29.323	744.81
994	29.353	745.56
995	29.382	746.31
996	29.412	747.06
997	29.441	747.81
998	29.471	748.56
999	29.500	749.31

Millibars	In. Hg	mm Hg
1000	29.530	750.06
1001	29.560	750.81
1002	29.589	751.56
1003	29.619	752.31
1004	29.648	753.06
1005	29.678	753.81
1006	29.707	754.56
1007	29.737	755.31
1008	29.766	756.06
1009	29.796	756.81
1010	29.825	757.56
1011	29.855	758.31
1012	29.884	759.06
1013	29.914	759.81
1014	29.943	760.56
1015	29.973	761.31
1016	30.002	762.06
1017	30.032	762.81
1018	30.062	763.56
1019	30.091	764.31
1020	30.121	765.06
1021	30.150	765.81
1022	30.180	766.56
1023	30.209	767.31
1024	30.239	768.06
1025	30.268	768.81
1026	30.298	769.56
1027	30.327	770.31
1028	30.357	771.06
1029	30.386	771.81
1030	30.416	772.56
1031	30.445	773.31
1032	30.475	774.06
1033	30.504	774.81
1034	30.534	775.56
1035	30.564	776.31
1036	30.593	777.06
1037	30.623	777.81
1038	30.652	778.56
1039	30.682	779.31
1040	30.711	780.06
1041	30.741	780.81
1042	30.770	781.56
1043	30.800	782.31
1044	30.829	783.06
1045	30.859	783.81
1046	30.888	784.56
1047	30.918	785.31
1048	30.947	786.06
1049	30.977	786.81
1050	31.006	787.56

http://www.novalynx.com/reference-bp-table.html

3. CALIBRATE DEPTH IF USING IN LAKES

a. Do at each lake
b. Setup/Cal
c. Calibrate
d. Sonde
e. Select 'Dep100: meters'
f. Hang Hydrolab sensors to 1 m depth
g. Select 1.0
h. Done
i. Hit any key
j. Hit Go Back until Go Back is no longer a selection

4. ANNOTATE FILES

a. Do at each different site
b. Files
c. Svr4a
d. Annotate
e. Select 'BASS REMAP'
f. Enter name of site
g. Done
h. Hit any key
i. Hit Go Back until Go Back is no longer a selection

5. RECORD READINGS

a. Hang or lie Hydrolab in stream or lake
b. Wait until temperature equilibrates
c. Hit 'Store'
d. Select 'BASS REMAP' or 'Lake Assessment'
e. Wait until logging active note disappears
f. Record on backup data sheet
g. If in lake, drop at 1 m intervals with last reading 1 m above bottom
h. At each depth equil DO & hit 'Store'
i. If in lake, pull up to 1 m, equil and hit 'Store' to check pH against 1st reading

6. REVIEW FILES

a. Do this to see what logged in
b. Files
c. Svr4a
d. Review
e. Select 'BASS REMAP' or Lake Assessment' depending on if working in streams of lakes
f. Choose to review from beginning or by date and time
g. Done
h. Hit 'Go Back' until Go Back is no longer a selection

IE. TRANSFERING DATA FROM SURVEYOR TO EXCEL

1. DUMPING DATA FROM SURVEYOR (important to follow steps in order listed)

a. Hook up serial port cable to computer and Surveyor
b. Open up C:\Program Files\Accessories\Hydrolab.ht
c. Turn on Surveyor
d. Send file from Surveyor: Hit 'Files' key, Choose 'Transmit', 'Bass Remap' or 'Lake Assessment', 'SS-Importable'
e. On Computer: Go to 'Transfer' tab and choose 'Receive File'
f. At 'Place received file in the following folder' prompt, enter folder name (i.e. 'K:\Bass\Hydrolab')
g. Use receiving protocol: 'Xmodem'
h. Hit 'Receive'
i. Enter file name. Use project and date download and .txt extension (i.e. Remap06-10-02.txt)
j. Close program

2. TRANSFERING DATA TO EXCEL

a. Open Excel
b. Open .txt file
c. Choose boxes for Deliminers Tab and Commas
d. Skip any blank columns or data don't want or don't trust (faulty sensor, salinity).
e. Finish (should see data imported now)
f. Check for outliers, redundancy, etc
g. Save file

3. WIPE LOG ON SURVEYOR

a. Verify data successfully transferred to Excel file before doing this
b. Select 'Files', 'Wipe', BASS REMAP' or 'Lake Assessment', Select '1' for yes, 'Done'

II. HYDROLAB MINISONDE 4A AND SURVEYOR 4A

IIA. Parameter Sensors

Temperature
Dissolved Oxygen
PH
Conductivity
Redox
Chlorophyll *a*
Depth

IIB. Maintenance

1. MAINTENCE PERFORMED PER HYDROLAB MANUAL

2. RECHARGE SURVEYOR

e. On last run turn off Timed shutdown (Setup/Cal, Setup, Timeout:Shutdown, 0 to disable)
f. Leave unit on to discharge battery completely (usually lasts 24 hrs total)
g. Recharge 3.5 hrs (plug in and turn on)
h. Turn Timeout on to save charge (Setup/Cal, Setup, Timeout:Shutdown, 5)

3. MAINTENANCE SCHEDULE

f. Done once a month or as readings indicate need or as sensors foul
g. Change DO membrane & electrolyte, make sure no bubble under membrane
h. Change pH electrolyte
i. Clean glass pH probe with methanol
j. Clean conductivity port, removing o rings and buffing prongs
k. Clean Chlorophyll *a* glass surfaces with lens paper

IIC. Calibration

1. CALIBRATION SCHEDULE

Do once each day and calibrate following Hydrolab manual

a. PH (7 first, then 4 or 10, then check sample)
b. Check sample recipe:
    1. Add 1mL of .02 N H_2SO_4 to a 1,000 mL erlenmyer flask.
    2. Add 999 ml of distilled water.
    3. pH of 4.70ish +or- .10 units.
c. First disable the stirrer (Setup/Cal, Setup, Sonde, Circulator, Off)
d. Then rinse with DI and shake the cup with DI in it.
e. Then rinse with about a half inch of buffer 7 (yellow)
f. Then fill with pH 7 until the DO membrane is just submerged
g. Lay the black cap on top of cup loosely
h. Wait 2 minutes to equilibrate
i. Record what reading for pH before calibrate
j. Select Setup/Cal, Calibrate, Sonde, pH and enter 7.00 and done and then after see calibration successful hit go back
k. Rinse the calibration cup 3 times with DI
l. Repeat calibration with pH 4 buffer
m. Record in the notes in back of white DataSonde 4 manual, pH reading of the buffer before calibrating it and after
n. Retrieve pH check sample 4.70 from the top shelf of the fridge by Jim's office. Then use the little heater/stirrer thing by Jim's office to heat the check sample to 25 degrees in a glass beaker (or run hot water over the outside of the calibration cup). Needs to be 25 degrees to read it as 4.70. Then use Hydrolab to read it and then record pH reading and temperature of the check sample.

o. Conductivity (10 first, then 100)
    1. Rinse calibration cup 3 times with DI water
    2. Then rinse with the 10 (11.24 or 9.96 or something)
    3. Fill to above the DO membrane
    4. wait 2 min
    5. Record what reading for SpCond before calibrate
p. Dissolved Oxygen, do saturated calibration via manual and do a zero check
    1. Make sure that the stirrer is enabled (Setup/Cal, Setup, Sonde, Circulator, On)
    2. Fill the calibration cup with finished water, up to the DO membrane O ring, leaving the membrane dry (but stirrer may flick water onto it).
    3. Set the black cap balanced on top
    4. Read the BP, and remember it Select Setup/Cal, Calibrate, DO%, enter BP
    5. Do a zero check using Standard Methods 4500 0-G
q. Chlorophyll *a* with calibration block and DI
    1. Use the glass lenses cleaner sheets to dry the SCUFA end
    2. Put the calibration cube on the end of the SCUFA
    3. Select Setup/Cal, Calibrate, Sonde, Chla, ug/L, enter 50.0
    4. Remove the calibration cube
    5. Wash the white bucket and fill with DI
    6. Submerge the SCUFA in it, keeping sensors 2" above the bottom
    7. Select Setup/Cal, Calibrate, Sonde, Chla, ug/L, enter 0.0
r. Redox with Zobels solution
    1. Rinse calibration cup 3 times with DI water.
    2. Pull out the Zobell's solution from inside the fridge on the bottom right hand side of the door
    3. Pour Zobell's solution to above the DO membrane
    4. Read the temperature
    5. Use the table to determine the appropriate redox number given the temperature. You will need to be quick entering the value, since the temperature warms up fast.
    6. Select Setup/Cal, Calibrate, Sonde, ORP, enter std # from table
    7. Rinse calibration cup 3 times with DI water.
s. Record calibrations in calibration excel spreadsheet log like one shown in Table 1. K:\Lakes\Assessment\Hydrolab\Calibration record.xls
t. Fill bowl (water bath) with tap water.
u. Using laboratory thermometer and Hydrolab sonde, simultaneously measure temperature of water bath
v. Verify agreement of temperature readings.
w. Calibrate temperature sensor per Hydrolab instructions to match laboratory thermometer.

Table 1. Example calibration log for Hydrolab 4a

Date	pH 7 pre	pH 7 post	pH 4 pre	pH 4 post	pH check 475 @ 25C	10 Cond pre	10 Con post	100 Cond pre	100 Cond post	0 Chl a pre	0 Chl a post	Chl a pre	Chl a post	Redox pre	Redox post	Redox temp C	0 DO pre	0 DO Post	Initials	Lab Thermo	Hydrolab Thermo	Calibration notes
5/29/2002	7.24	7	3.92	4	4.76	11.2	10.1	95.1	99.3	0	0	51.8	50.0	448	450	15	0. 1	0	KM	13.5	13.2	Used buffer used for pH

IID. Field Operation

1. CALIBRATING DISSOLVED OXYGEN

This step is done on site at first site visited for day.

a. Connect Hydrolab to Surveyor (careful to pull plug off – DO NOT TWIST)
b. Remove black cap off calibration cup
c. Cover sensors with ambient water to just below O ring of DO sensor membrane
d. Set black cap over calibration cup (just to keep temperature from fluctuating)
e. Make sure stirrer is on. If it is not, use the below steps to turn it on:
   - Setup/Cal on Surveyor
   - Setup
   - Sonde
   - Select 'Circltr: Off/On
   - Select 1 for On
   - Done
   - Hit any key
f. Hit Go Back until Go Back is no longer a selection
g. Note BP reading
h. Setup/Cal
i. Calibrate
j. Sonde
k. Select 'DO%'
l. Enter BP
m. Done
n. Hit any key
o. Hit Go Back until Go Back is no longer a selection
p. DO calibration complete

2. CALIBRATE DEPTH IF USING IN LAKES

k. Do at each lake
l. Setup/Cal

m. Calibrate
n. Sonde
o. Select 'Dep100: meters'
p. Hang Hydrolab sensors to 1 m depth
q. Select 1.0
r. Done
s. Hit any key
t. Hit Go Back until Go Back is no longer a selection

3. ANNOTATE FILES

a. Do at each different site
b. Files
c. Svr4a
d. Annotate
e. Select W.S. Reservoirs
f. Enter name of site
g. Done
h. Hit any key
i. Hit Go Back until Go Back is no longer a selection

4. REVIEW FILES

a. Do to see what logged in
b. Files
c. Svr4a
d. Review
e. Select W.S. Reservoirs
f. Choose to review fr beginning or by date and time
g. Done
h. Hit Go Back until Go Back is no longer a selection

IIE. TRANSFERING DATA FROM SURVEYOR TO EXCEL

1. Plug Surveyor into to computer
2. Turn Surveyor on
3. On Surveyor select Files, Transmit, 'LakeAssmt', SS-Importable
4. Go to Start menu on Computer, select Applications, Hydrolab.ht
5. In Hydrolab.ht program, select Transfer, Receive File, Browse to D:\MyFiles\Hydrolab Dump, select Xmodem, click Receive, enter name of file (i.e. Practice-10-27-03.txt)
6. Press any key on the Surveyor to start modem transfer
7. Close Hydrolab.ht program, Yes you are sure you want to disconnect now
8. Open Excel
9. Open file to D:\MyFiles\Hydrolab Dump\Practice-10-27-03.txt, make sure you select Files of type .txt so that you can find the file you created in step 5.
10. Text Import Wizard will open. Choose 'Delimited', 'Next', 'Tab', 'Comma', 'Next', 'General', 'Finish'
11. Save as same name, but as excel file in D:\MyFiles\Hydrolab Dump

12. Insert 2 new A&B columns 'LakeID' and 'StaNo' and fill in making sure that match with names in Inventory.
13. QC the data by
    a. Round depths off to 1 meter mark (if appropriate)
    b. Delete empty columns
    c. Remove duplicates (keep 2nd value if represents longer time to equilibrate)
    d. Compare 1st 1M depth reading to ending 1M depth reading to look for any drift in calibrations. Delete any values for any parameters when think that the calibration for that parameter is in question. Can refer to calibration record for Hydrolab.

Calibrating the SCUFA calibration cube

SETTING THE CALIBRATION CUBE/BLOCK CHLOROPHYLL CONCENTRATION

1. Load ScufaSoftware onto PC (download from Turner website or copy from CD)
2. Soak Scufa sensor in DI water for 30 minutes
2. Clean Scufa sensors with Q-tips and/or lens paper
3. Open calibration block all the way using small screw driver (turn counter-clockwise)
4. Put dry calibration block on dry SCUFA end
5. Hook Turner cable to SCUFA
6. Plug Turner cable into outlet
7. Plug Turner cable into Com1
8. Open up Scufa Soft software on PC (icon at top should show blue dot bouncing back and forth between PC and Scufa, if not see SCUFA manual for appropriate baud rate settings, etc)
9. Select tab 'Analog Output'
10. Make sure reads zero and 200
11. Select tab 'Calibration'
12. Make sure reads 300 and ug/L
13. Uncheck the Blank Subtract box
14. Calibrate
15. Average reading should be somewhere between 10 and 13
16. Let it calculate the average reading
17. Select No Secondary Standard
18. Fluorescence should be reading around 0300
19. Now use the screw driver to turn down the fluorescence on the cube to 50 (turn it clockwise), you will need to remove the calibration cup on the Hydrolab in order to get the screwdriver in.
20. Close Scufasoft. You are finished communicating with the Scufa directly and you have set the calibration block to 50 ug/L. If you suspect that the calibration block screw has moved in either direction, then you will want to repeat the above process in order to calibrate the block again. Otherwise, now the block with serve as the calibration standard for future Hydrolab/Scufa calibrations.
21. Reconnect the SCUFA to the Hydrolab minisonde 4a

CALIBRATING THE SCUFA WITH THE CALIBRATION BLOCK

1. Plug the calibration cable into the Hydrolab
2. Plug the AC adapter into an outlet
3. Plug the cable into Com1 of your PC
4. Open Hyperterminal on your PC
5. Wait a minute for data to start scrolling. If this does not happen check settings by:
    a. Go to Files
    b. Connect using 'Direct to Com1'
    c. Configure
    d. Bits per second '19200'
    e. Data bits '8'
    f. Parity 'none'
    g. Stop bits '1'
    h. Flow control 'Xon/Xoff'
    i. Hit o.k. and o.k.
    j. May hear the hydrolab issue a long beep and then should begin to see data scrolling after a minute or so.
6. Right arrow key across the highlighted menu choices to 'setup' and hit enter
7. Highlight 'sensors' and hit enter
8. Highlight 'chlorophyll' and hit enter
9. Select 'Chl: aeg/L' for units and hit enter
10. Enter 150 after the [150.0] prompt and hit enter (this sets the out of range feature at 150 ug/L)
11. Put on the calibration cube (making sure SCUFA end is clean as well as the cube
12. Highlight 'Calibrate' and hit enter
13. Highlight 'Chlorophyll' and hit enter
14. Highlight 'Chl: aeg/L' and hit enter
15. Note what the chlorophyll reading reads for the calibration block
16. Enter 50 after [50.0] prompt and hit enter, this calibrates the unit with the block to 50 ug/L
17. Will say calibration completed
18. Now submerge the Scufa in a DI water making sure that the Scufa sensors are 2 inches off the bottom of the container.
19. Now Highlight 'Calibrate' and hit enter
20. Highlight 'Chlorophyll' and hit enter
21. Highlight 'Chl: aeg/L' and hit enter
22. Note what the chlorophyll is reading the DI water as
23. Enter 0.0
24. Calibration completed!