

Field Methods

Regional Environmental Monitoring and Assessment Program

Physical Habitat
Water Chemistry
Macroinvertebrates
Aquatic Vertebrates
Riparian Amphibians
Periphyton

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1.0 INTRODUCTION

This manual contains the field procedures used the Oregon Department of Environmental Quality (DEQ) for monitoring streams as part of the Regional Environmental Monitoring and Assessment Program. This is a United States Environmental Protection Agency (USEPA) sponsored program. The purpose of this monitoring is to document the current status, changes, and trends of aquatic natural resources in the Western Cascades, Ecoregions 4a and 4b.

The protocols have five main parts:

- Aquatic vertebrate assemblage survey that includes the number, length, and health
- Macroinvertebrate sampling to evaluate biological integrity
- Periphyton assessment
- Habitat quality evaluation
- Chemical water quality measurements.

1.1 History

These protocols have evolved from the Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP) protocols of June 1997 (*****reference*****). DEQ has conducted EPA funded Regional EMAP studies since 1994, including three years in the Coast Range (1994-1996) and two years in the Upper Deschutes River Basin (1997-1998).

1.2 1999 protocol modifications

There are only a few minor protocol changes and additions. Proposed *reference* sites will be surveyed, nine in each of the next two years, in addition to the 25 random sites each year.

Macroinvertebrate samples will be collected from both pool and riffle samples at reference sites. At random sites, macroinvertebrate samples will only be collected from riffle habitat (however, if riffle habitat is lacking, samples will be collected from pools). This approach is similar to that used by DEQ's Oregon Salmon Plan crews.

Estimation of percent fines has also changed. Instead of the macroinvertebrate collector performing four 400 intersection counts, a member of the habitat crew will do from 10-30 100 intersection counts (dependent upon a confidence interval calculation).

Periphyton sampling has also been added.

2.0 BRIEF OVERVIEW OF SITE WORK ACTIVITY

2.1 Introduction

This section is a brief overview of what a crew does at a site. This is not intended to be a complete description of the protocols. The specifics of the various activities are described in detail in the sections that follow. Typically, three people survey a site in one full day. Each person in the crew has specific tasks. It is important that all crew members have a clear idea of the overall flow of work if work at a site is to be completed efficiently.

2.2 Before Arrival

One person in the crew organizes the paperwork for the site and checks over the maps and reconnaissance logistics information to see if landowners need to be notified or if gate keys are needed. All crew members participate in locating the site by all means available: maps, land marks, GPS readings.

2.3 Arrival: Chemistry Sample

If the site is remote from the vehicle, everyone packs up the equipment, lunch, and drinking water and hikes to the site. If the site is close to the vehicle, it usually makes sense for one person to collect the water chemistry sample immediately upon arrival. It is very important that the chemistry sampler collects the sample at a location that has not been disturbed by the survey crew walking in the stream. The sample is collected from the main part of the stream flow. The sampler also records the sampling time and stream temperature.

While one crew member is collecting the chemistry samples another is back at the vehicle setting up the meters and other equipment to process the samples. Both crew members process and analyze the samples.

If the stream is remote from the vehicle then the chemistry sample is either collected upon arrival and stored in a dark bag in the stream in the shade, or collected at the upstream end of the survey reach just prior to hiking back to the vehicle. The sample needs to be collected at a location undisturbed by crew members walking in the stream. Collection time and temperature are noted.

Water chemistry protocols are described in section ****.

2.4 Macroinvertebrates and Periphyton

The third crew member not involved in the chemistry sample collects the macroinvertebrate and periphyton samples. It is important that this person gets started right away and works fairly quickly. These samples are to be collected from locations not disturbed by crews walking in the stream. The other two crew members will be conducting the habitat portion of the survey. The macroinvertebrate/periphyton sampler needs to be well under way before the habitat portion

begins so crew members are not wasting time waiting for the macroinvertebrate/periphyton sampler to finish and move upstream.

Macroinvertebrate methods are described in section ***** and the periphyton methods are described in section *****.

2.5 Habitat

This is a fairly complex and lengthy portion of the survey. It generally takes 2 people 3 hours or more to complete. The complete protocol is in section *****. One person is the forms person and the other is the in-stream person. The in-stream person makes the measurements and calls them out to the forms person for recording.

Usually a stream flow measurement is taken first at or near the randomly selected x-spot. The x-spot is marked with flagging.

The mean wet width, length of stream to be surveyed, and the transect interval are calculated and recorded on the header form. The survey reach length varies with stream size. The mean wetted width is determined by measuring the width at three representative locations within 10 meters of the x-spot. The reach length is 40 times the mean wet width, rounded to the nearest meter, and the transect interval is $\frac{1}{10}$ of the reach length. There are eleven transects. The x-spot is at transect one.

At the transects, the in-stream person makes measurements (and calls out the results to the forms person) of substrate cross-sectional information, bank measurements, and canopy cover. Also at the transects, the forms person visually estimates fish cover, visual riparian estimates, and human influences. **Between transects**, the in-stream person collects thalweg profile information and channel unit code information, while the forms person records these measurements and visually estimates large woody debris size and occurrence. When moving upstream with the hip chain, it is important to not allow the thread to cut corners as this would not accurately measure the stream length. The thread should be looped around vegetation as needed to ensure that the thread approximately follows a route parallel to the main flow of the stream.

The in-stream person then goes to the nearest riffle habitat and starts the surface fines measurements using the 10 by 10 grid and a view bucket. Fines measurements are jotted down in a field note book (or on the fines form directly) for latter recording by the forms person. The fine sediment measurements are to be spread out randomly in the riffle habitat throughout the length of the reach. A minimum of ten measurements and a **maximum of 50 (?)** are to be taken. The number of fines samples taken at a site varies with the variability of the surface fines. The recording form has a work sheet for determining this. Sample size should be evaluated after ten samples have been measured. The number of samples per riffle varies with the number of riffles in the reach. If riffles are frequent, then perhaps only one or two samples per riffle are needed. If riffles are rare then more samples per riffle would be taken.

As the habitat crew members walk back to transect one they measure the channel gradient and sinuosity. It is important to do this measurement over the main flow of the wet channel and not cut corners. Supplemental measurements can be taken around bends in the stream channel. They also collect additional fine sediment measurements, if necessary, and collect all hip chain threads. Hip chain thread is a serious threat to wildlife and the crew should collect all of it as soon as possible.

2.6 Lunch

All three crew members return to transect one for lunch.

2.7 Aquatic Vertebrate Survey

After lunch, all three crew members participate in conducting the electrofishing survey. The vertebrate survey protocol is in section ****. One person operates the electrofisher and the two others net. The crew travels from down stream to up stream in a zig-zag pattern fishing all habitat in proportion to their presence in the stream. Netters should stay slightly downstream from the electrofisher operator. The crew stops periodically to process the collected animals. All animals are identified to the species level, length measured, examined for parasites or abnormalities, and released alive back to the portion of the stream where they were collected. Vouchers are sometimes retained to verify field identifications. Information is tallied and recorded on the appropriate form.

Button time is the time in seconds that the electrofisher is turned on. The reach is fished for a total maximum of 5000 seconds. On larger streams, the crew should keep track of how much time is spent fishing the first couple of transect intervals. If more than 500 seconds is spent in each transect interval, then sampling effort will have to be spread out accordingly so the 5000-second limit is not exceeded.

At the end of the survey reach the crew fills out the electrofishing log sheet.

2.9 Departure

All crew members look over the reach for equipment, flagging, hip chain string, collect the sample bottles, and return to the vehicle. Remember to close and lock all gates that were closed when you got to them. On the ride to the evening's accommodations, the forms person proceeds to fill in all zeros, flags, etc. that were left blank during field data collection.

3.0 HABITAT

All habitat protocols follow the procedures outlined in *Field Operations and Methods for Measuring the Ecological Conditions of Wadeable and Nonwadeable Streams, June 1997* (reference).

SECTION 12

Water Chemistry

(Revised June 10, 1998, ODEQ)

12.1 Introduction

1. There are two components to collecting water chemistry information: collecting water samples for laboratory analysis and the field chemistry measurements. Sample collection and field analysis are not difficult but careful attention to the procedures is important. Following the correct procedures is essential in ensuring that the data generated from the samples you collect is of the highest quality possible. These procedures are a summary of the DEQ's Manual of Methods and the DEQ Field Sampling Reference Guide.
2. Some of the chemicals involved in the chemistry procedures are caustic acids and bases that can injure you. We are often working in remote areas many miles away from medical help. Your **safety** and the safety of your coworkers are especially important concerns. Become familiar with how to use the chemicals safely by reading the material safety data sheets (MSDS) in the black file box that is part of each crew's equipment (another copy is kept in the DEQ laboratory front office). Safety is also part of these protocols and related training. Use disposable gloves and safety glasses to prevent contact with these chemicals. If contact occurs rinse affected area with large amounts of water and seek medical attention if necessary.

12.2 Before Leaving the Vehicle for the Site

1. 5.2.1 Leave the vehicle with the following equipment:
 - 2 "P" polyethylene bottles (one can be marked for reuse)
 - 1 "R" polyethylene bottle
 - 1 "DP" polyethylene bottle (can be marked for reuse)
 - 2 BOD bottles
 - Hach kit powder pillows for dissolved oxygen
 - Toe nail clippers
 - Water seal caps for the BOD bottles
 - Black nylon bag
 - Digital thermometer

- Duct tape
 - Disposable rubber gloves
 - Zip lock bag for trash
 - Digital thermometer
 - Temperature monitor field audit form
2. Record the site name and number, bottle numbers, and site elevation on the chemistry data sheet. The BOD bottle with the lower number is used for dissolved oxygen analysis. The first column of the form is for the STORET number, a unique site code for the STORET water chemistry database. If the site has not been visited before assign an unused STORET number from the list posted on the bulletin board in DEQ Lab room L79 (the crew leader takes care of getting these numbers). Use the existing site STORET number if the site has been visited before.

5.3 Collecting the Sample

1. The water chemistry sample is collected at a location in the stream that is at or close to the X-spot and has not been disturbed by crew members walking in the stream up stream of the sample collection location.
2. The sample should be collected in a spot that is representative of the stream. Usually this means in a flowing well-mixed location towards the middle of the stream and away from slower water near the margins.
3. One P poly and one DP poly are marked for reuse at each site. Rinse these bottles three times with stream water before filling.
4. In flowing water face the open bottle up stream and allow filling. In slow moving water move the open sample container through the water. This is to reduce sample contamination from your hand.
5. The two BOD bottles should be filled with as little turbulence as possible. Fill to overflowing and stopper the bottles without any trapped air bubbles. Tap on the side of the bottle with the stopper to dislodge bubble that cling to the sides of the bottle. The bottle with the higher number is the BOD sample and the lower number is the dissolved oxygen bottle. Cap the BOD bottle with a water seal and secure the seal with duct tape.
6. **Fixing the dissolved oxygen sample.** The powder chemicals used to fix the dissolved oxygen sample are irritating if inhaled or come in contact with eyes or skin. Wear safety glasses and disposable gloves.
 - Add the contents of one manganous sulfate powder pillow and one alkaline iodine-azide powder pillow. Open the powder pillows with toenail clippers.
 - Immediately insert the stopper so air is not trapped in the bottle. Invert the bottle several times to mix. An orange flocculent will form.
 - Wait until the floc has settled to the bottom. This should take a few minutes. Again invert the bottle several times and wait until the floc has settled.

Remove the stopper and add the contents of the sulfamic acid powder pillow. Replace the stopper without trapping air in the bottle and invert the bottle several times to mix. The floc will dissolve and leave a yellow color if oxygen is present. Cap the bottle with a water seal and secure the seal with duct tape.

7. Place all the bottles in the black nylon bag. Place the bag in a shaded spot in the stream.
8. Record the **water temperature** and collection time on the temperature monitoring field audit form. If the temperature monitor is not at the same location as the chemistry collection also record the air and water temperature at that location. When measuring air temperature make sure that the sensor is dry and shaded.
9. Collect the empty powder pillows and the gloves and place them in the small bag for trash.
10. Do not forget to pack the chemistry samples when you leave the site!

5.4 Field Analysis and Preservation at the Vehicle

1. **Safety.** Concentrated sulfuric acid is used to preserve samples in R poly bottles. The dissolved oxygen sample is acidic. Care should be taken to avoid contact with skin, eyes, or clothing. Use safety glasses and disposable gloves. The sodium thiosulfate titrant for dissolved oxygen analysis, the pH buffers and conductivity standard are not dangerous or irritating. Normal personal hygiene should be used to avoid unnecessary contact.
2. Different sample bottles are preserved and processed differently.
 1. P poly bottle (not marked for reuse): placed in the sample cooler on ice.
 2. R poly bottle: 12 drops of concentrated sulfuric acid and placed in the sample cooler on ice.
 3. BOD sample: placed in the sample cooler on ice.
 4. Reuse P poly bottle:
 - A. Filter (with a 0.45um pore Gelman membrane filter) about 250 milliliters into a DP poly and transfer to an R poly. Add 6 drops of sulfuric acid and place on ice in the sample cooler.
 - B. Filter about 250 milliliters into a DP poly and place on ice in the sample cooler. Record the DP poly number on the chemistry data sheet.
 - C. The rest of the sample in the Reuse P poly bottle is used for conductivity testing and pH, if necessary.
5. The reuse DP poly is used for pH.
3. **pH meter calibration and measurement.** The pH measurement is taken from the Reuse DP poly bottle. A meter calibration check should be done daily before sample is analyzed.
 1. Meter calibration check.
 - A. Uncap the probe, unplug the probe solution fill hole, rinse the probe and temperature sensor with deionized water and place in a pH 7 buffer.
 - B. Turn on the meter.

- C. Wait until the reading stabilizes and record the reading in the meter calibration logbook. Remember that the pH of a buffer varies with temperature.
- D. Rinse the probe and temperature sensor with deionized water and place in a pH 10 buffer.
- E. Wait until the reading stabilizes and record the reading in the meter calibration logbook.
- F. If the pH is 0.2 units or more than away from the correct value for the buffer at that temperature then recalibrate the meter and then recheck the calibration. Record the second calibration check. Recalibration procedures vary for different meters and instructions are with the meter.

2. pH sample measurement

- A. Rinse the pH probe and temperature sensor with deionized water. Place the probe and sensor into the Reuse DP poly and turn the meter on.
 - B. Compared to the buffers, the natural water samples are usually very dilute and it may take several minutes for the probe to equilibrate. Observe the pH reading every few minutes as you do other work to check for reading drift.
 - C. If the reading fails to stabilize dump out the sample and refill with fresh sample from the reuse P poly bottle. Wait and observe for several minutes.
 - D. If the reading still fails to stabilize, observe the pH reading and then add one drop of electrode storage solution. This will increase the solutes in the sample while having little influence on the pH. Record pH on chemistry data sheet.
3. Turn off the pH meter, rinse the probe, replace the probe cap and pore plug and put the meter away.
4. **Conductivity calibration and sample measurement.** The conductivity measurement is done from the sample in the Reuse P poly. Meter calibration is checked twice a week, once before the first sample of the week is analyzed and again after the last sample is analyzed.

Like the pH meters, DEQ unfortunately has a couple of different models of conductivity meters. Conductivity measurement, meter calibration and adjustments vary with the meter. Older meters require a “red line” battery strength adjustment and a separate compensation for temperature with each reading. Newer meters make these steps automatically.

1. Meter calibration check.
 - A. Rinse the probe with deionized water, shake off excess water, and place the probe in the conductivity standard.
 - B. Stir briefly. Allow the probe to sit in the container for a few minutes so that the probe comes to the temperature of the standard.
 - C. For **older meters**, turn the dial to “red line” and, if necessary, adjust the meter so that the needle lines up with the red line. When reading the meter make sure that you position yourself so that the needle covers its reflection.
 - D. Do not let the probe contact the sides and bottom of the container while taking a reading. Switch the meter to the 1X scale.

- E. For **older meters**, record this conductivity value in the meter logbook. Switch the meter to temperature and record the temperature in the logbook. In the front of the book is a table of temperature correction factors. Record the appropriate correction factor next to the conductivity reading. Multiply the correction factor with the conductivity reading and record in the logbook. The temperature compensation knob on the older meters is for salinity measurements and is not used for conductivity.
 - F. **Newer meters** make the battery check adjustment and temperature adjustments automatically. They also have a digital read out. Simply record the standard conductivity reading in the book. Remember not let the probe contact the sides and bottom of the container while taking a reading. .
 - G. Record the labeled conductivity of the standard. Compute the percent difference between the standard conductivity and the conductivity reading you obtained and record in the logbook. Record reading, date, and your initials on the quality assurance graph in the back of the logbook. Percent difference = $((\text{Standard Conductivity} - \text{Conductivity Reading}) / \text{Standard Conductivity}) * 100$.
 - H. Percent difference plus or minus 5% or less are OK, between 5 and 7% are acceptable but suspect, 7% or greater is out side the quality assurance limits and that meter cannot be used.
 - I. If the meter is reading the standard outside the 5% limit try the calibration check again and replace the standard. Unlike the pH meters, the calibration of conductivity meters usually is very constant. When there is a problem it usually is not the meter.
2. Sample conductivity measurement
 - A. Rinse the probe with deionized water, shake off excess water, and place the probe in the sample in the reusable P poly.
 - B. Stir briefly. Allow the probe to sit in the container for a few minutes so that the probe comes to the temperature of the sample.
 - C. For **older meters**, turn the dial to “red line” and, if necessary, adjust the meter so that the needle lines up with the red line. When reading the meter make sure that you position yourself so that the needle covers its reflection.
 - D. Do not let the probe contact the sides of the container. Switch the meter to the 1X scale.
 - E. For **older meters**, record this conductivity value in the meter logbook. Switch the meter to temperature and record the temperature in the logbook. In the front of the book is a table of temperature correction factors. Record the appropriate correction factor next to the conductivity reading. Multiply the correction factor with the conductivity reading and record in the logbook and the chemistry data sheet.
 - F. **Newer meters** make the battery check adjustment and temperature adjustments automatically. They also have a digital read out. Simply record the sample conductivity reading in the chemistry data sheet.
5. **Dissolved Oxygen Titration.** After the initial water sample for dissolved oxygen has been chemically preserved, it can be held for up to eight hours before titration.
 1. Remove the glass stopper and fill a 200 ml volumetric flask with sample. Transfer this sub-sample to a 250 or 500 ml Erlenmeyer flask. Add 2 ml of liquid starch solution. The starch will change the color of the sub-sample from amber to dark blue.
 2. Fill a 10 ml buret with standard sodium thiosulfate titrant. Observe the level of the titrant in the buret. Sometimes it fills 0.1 milliliters below the 10.0 milliliter mark. This difference will have to be taken into consideration when determining the titrant volume.

3. Carefully add the titrant to sub-sample drop by drop until the color of the sub-sample changes from dark blue to clear. Titrating against a white background such as a piece of blank paper makes observing the endpoint easier. Take care not to overrun the end point.
4. Check the end point by adding two drops of back titrant. The solution should turn blue. The volume of titrant used is the oxygen concentration in the sample in milligrams per liter. Record the oxygen concentration on the chemistry data sheet.
5. You overran the end point if it does not turn blue with two drops of back titrant. Add additional back titrant two drops at a time until the solution turns blue. For each additional two drops of back titrant after the first two subtract 0.1 mg/L from the dissolved concentration. Record the corrected oxygen concentration on the chemistry data sheet.
6. Record the water temperature and time when you collected the sample on the chemistry data sheet.
7. Determine the percent saturation of dissolved oxygen using table in the chemistry clipboard. Round the percent saturation to the nearest whole number and record on the chemistry data sheet.

Section 13.0 Stream Macroinvertebrates Protocol

13.1 Field Sampling Methods

13.1.1 Overview

The goal of the field sampling technique is to collect an unbiased, representative sample of benthic macroinvertebrates. At each stream reach, samples are collected by compositing D-Frame Net kick samples from a selected habitat unit (e.g. pools, riffles). Sample processing occurs either in the field or the lab.

13.1.2 Equipment

- 500 um mesh D-Frame kick net
- Three gallon sieve bucket
- Scrub brush
- Long-sleeved rubber gloves
- Zip-loc bags or Nalgene containers
- Sample label

13.1.3 Procedure

Note: CSRI and REMAP protocols differ by the following methods:

CSRI

- 4 kick composite
- random number grid to select sites
- collect riffles at all sites (if no riffle collect a pool sample)
- collect riffles AND pools at reference sites only

REMAP

- 5 kick composite
- 9 cell grid overlay to select sites
- collect riffles at all sites (if no riffle collect a pool sample)
- collect riffles AND pools at reference sites only

1. Beginning at the downstream end of the reach, select a habitat unit (riffle or pool). EXCLUDE margin habitats (edges along channel margins).

CSRI- Random numbers in the table used by DEQ have four digits. The first two identify the percent up from the bottom of the riffle or pool, and the second two are the percent of stream width across the channel. For example, a random number of 3225 would place the sample at 32 percent up from the bottom and one quarter across the stream width. These percentages are determined by visual estimates.

REMAP. Visually divide the area to be sampled into 9ths (i.e. a 3 by 3 grid; see figure 13.1). For the first habitat unit, select the lower right square; for the second habitat unit, select the center; the third, the upper left; for the fourth, select the lower left; for the fifth select the upper right. Collect the kick sample in the center of each grid square.

5		3
	2	



Figure 13.1. Visual grid overlay used to select kick sites at each habitat type (riffle or pool). Each box represents one habitat unit.

2. After locating the random sample site, place the net into the stream with the flat part of the hoop resting on the bottom and perpendicular to the stream flow. Collect the macroinvertebrate sample by disturbing a 30 by 60 centimeter area (1ft x 2ft,
3. Inspect the benthos in a 1 ft X 2ft area (approximately as wide as the kick net and twice as long) of stream bottom directly in front of the net for any large organisms such as mussels. Pick these and place in the sieve bucket.
4. Carefully rub by hand, or with a small scrub brush, all substrate larger than five centimeters (golf ball size and larger) in front of the net to dislodge any clinging macroinvertebrates. After rubbing, place the substrate outside of the sample plot.
5. Thoroughly disturb the remaining substrate in the 1ft X 2ft area with your hands or feet for 1 min to a depth of five to ten centimeters.
 - **NOTE:** Collecting a sample in slow moving water is a little more difficult. It may involve pulling the net through the water as the substrate is disturbed to capture suspended organisms.
6. After the sample is collected and the net removed, return the large substrate to the sample plot.
7. The contents of the net are placed in a sieve bucket and the sampling procedure is repeated for that habitat type. **Always sample downstream to upstream.**
8. All kick samples for the same habitat type are composited in the sieve bucket. Large organic material and rocks are rinsed, carefully inspected for clinging macroinvertebrates, and removed. As much fine sediment as possible should be washed away. Leaf packs from pool samples may require considerable rinsing and removal of debris before preserving the composite sample.
9. For lab sorting and analysis the composite sample is placed in a labeled jar or double zip-lock bag and preserved with 95% ethanol (ETOH) for sorting and subsampling in the lab. Pour enough ETOH in the container to equal the volume of sample. It is necessary to replace the alcohol in the sample with fresh alcohol within one week to ensure adequate preservation. Place a label (Rite in the Rain paper) written in pencil containing site and habitat unit information inside the container. Label the outside container with a marker written in labeling tape.
10. For field sorting, do not preserve the specimens. Keep them alive and follow the subsorting procedures described in Section 8.4.

13.2 Sample Storage

1. After samples have been collected in the field, several steps should be taken before they can be put away. Samples that are not sorted need to have the ETOH changed upon returning to the lab. This is especially important if the sample contains a lot of organic material (leaves, moss, etc.). If bags are used, check for leaky bags and replace with new ones. We recycle the used ethanol (ETOH) so do not dump it down the drain.
2. The filter apparatus is located in the fume hood. It consists of a 2000 ml Erlenmeyer flask connected to a low-pressure vacuum and a glass funnel with a removable metal sieve on top. The funnel should always contain the fiberglass filter, which needs to be discarded when it appears very soiled. In order to increase the filtering capacity, never pour the sample directly into the funnel- always use the metal sieve. Slowly drain the sample

into the metal sieve. Be absolutely sure that filtered ETOH level does not reach the vacuum tube, or the glass flask will break.

13.3 Sample logging

1. Samples should be promptly recorded in the bug logbook and assigned a bug log number (BLN). The BLN should be assigned based on the order the samples were logged, and do not necessarily correspond to the site visit number (SVN), the site ID number (e.g. ORDS97-03) or the STORET number. Information to record in the bug logbook includes: collection date, site name, site ID, collection method (e.g. 'D-frame kick net, 5 kick composite'), habitat type, and number of containers (if more than 1). Each habitat sample should be assigned a BLN. If a sample has more than one bag/container it is assigned the same number (be sure to indicate how many containers there are in the bug log book). See figure 8.3.1 for log sheet and example (if you are still unclear, please ask- this step is very important).
2. New tags with the BLN number and all other information should be placed inside the container and also attached to the outside (staple or tape). Samples that are field sorted are also assigned a BLN and should be labeled as in figure 13.2.
3. Unsorted logged samples should be placed in the rubber bins near the fume hood. Label the outside of the bin with the project name and BLN numbers. Sorted logged samples (vials) should be placed on the appropriate shelf of the bookcase next to the sink in the lab. Field ID sheets (if any) should be filed.

13.4 Subsampling

13.4.2 Supplies

- 500 um metal sieve
- three gallon sieve bucket
- 30 cm X 36 cm metal mesh tray with rectangular plastic pan (Caton Subsampling Tray)
- "cookie- cutter" 6cm X 6 cm metal frame
- 6 cm metal flat scoop
- paintbrush
- forceps
- glass specimen vials with caps
- sample labels (Rite in the Rain paper)
- counter
- 75% ETOH for macroinvertebrate preservation
- overhead light with magnifying glass

13.4.2 Procedure

Subsampling is a cost effective way to reduce time and effort required for sample processing. It involves sorting only a portion of the sample collected. On average, expect to spend at least 3 hours sorting a sample. Please do not start a sample if you cannot finish it.

1. Select a sample that needs subsampling and sorting. Drain the ETOH from the sample under the fume hood. Rinse out as much fine sediment, big, rocks, etc., as practical. The metal sieves and sieve bucket are useful for this as well as the screen for the Caton Subsampling tray. Some samples are in more than one bag and should be composited and completely mixed in the sieve bucket.
2. Place the mixed composite sample in the 30 cm by 36 cm metal mesh tray (Caton subsampling tray). If the composite is too large to reasonably fit into the tray, divide the sample in half one or more times until a workable amount of material is obtained. Make a note of any composite divisions. Put the screen tray inside the white plastic tray and add enough water to float and completely mix the sample. This ensures a random and even distribution of the debris in the tray. Lift the screened tray out of the water.

3. Use a random numbers table (one is taped to the fume hood) to select four squares and square 'cookie cutter' to clearly define the square to be sampled. Sticks, leaves and other debris that overlie the grid should be cut with scissors or a razor blade. Use the 6 cm flat metal scoop and brush to remove the debris and invertebrates from the square. The minimum random subsample size is 4 squares. Remember that it is 4 squares in the original composite. So if a large composite was divided in two parts, you have to pick 8 squares.
4. Spread the squares in a light colored tray with a small amount of water (note: it is not necessary to pick all four squares at the same time). Pick out all the invertebrates using an illumination magnifying lens and tweezers, counting the macroinvertebrates as you remove them (counters are extremely useful). Place them in a glass vial with 70% ETOH. Sort through the entire tray thoroughly. The aim is to remove all macroinvertebrates though, in practice, this is not always achieved (better than 95% removal should be achieved at all times). A good approach to sorting is to initially keep the four squares in discrete clumps and then work through each pile gradually. Finally, give the tray a good stir and check it over until you are satisfied that no invertebrates remain. For your first few sorting efforts, have someone else check over the tray before you discard the material.
5. Continue selecting and sorting sample squares until a minimum of **300 macroinvertebrates** have been selected. If you are part way through a subsampled square and you reach 300 invertebrates you must pick all the invertebrates in the remaining sample. **The maximum number of squares to pick is 16.** Do not exceed 16 squares, even if 300 macroinvertebrates have not been reached.
6. Pick every invertebrate you can see. **BE CAREFUL!** There are many invertebrates that can be easily overlooked. Among the most commonly missed are invertebrates that are in some type of case. Caddisflies can be found in cases made of stone, sand, leaves and twigs. When caddis pupate they often close off their case, so a case that is blocked at one end may still contain a caddis. Some species of Chironimidae and Oligochaeta can be found in cases made of fine sediment and sand. Another frequently missed organism is *Cricotopus nostocladius* - a chironomid often found encased in an algal sheath. Also, watch out for plant material that may resemble an organism. Check each mollusk shell for an organism. If you are unsure about whether or not something is actually an organism, the best thing to do is put it in the vial, but do not count it.
7. Ten percent of picked samples will be checked by a second person before discarding sorted material. If greater than 5% of the total is found the original sorter must continue to pick and the sample must be checked again. After the sample is checked again and less than 5% invertebrates are found, the remaining debris can be discarded.
8. When you are finished, label the vial with the site name, sample date, habitat type, number of squares sorted, number of invertebrates (either "300+" or the actual number if below 300 organisms), and the BLN (fig. 2). Also place a paper label on the inside (written in pencil on Rite in the Rain paper) with the same information. Place the vial on the appropriate shelf and record your name, date, number of invertebrates and squares picked, in the bug logbook. Mark in the 'sorted' column of the bug log book that the sample has been picked (your initials, date, number of squares and number of insects).
- 9. Lastly, clean up your equipment and preparation area.**

13.5 Macroinvertebrate Identification

13.5.1 Equipment

- Dissecting microscope (10X-60X zoom)
- Light source
- Forceps
- Macroinvertebrate taxonomic keys. See references for recommended keys
- Data recording form

13.5.2 Procedure

1. If the sample was not sorted in the field then lab sort according to the procedure described in section 8.4.
2. Identify the macroinvertebrates to the taxonomic level desired. Table 8.1 lists the level of taxonomic identification for different macroinvertebrate taxa.
3. Experienced entomologists using current taxonomic keys (see “Taxonomic References” under the supervision of a senior aquatic entomologist should perform identification to genus/species. Family level identification is possible by less experienced staff, but sufficient taxonomic training is still critical.
4. The number of each taxon is noted on a tally sheet along with other site identifier information.
5. Quality control procedures described in the Quality Assurance section should be completed to evaluate the quality of the sample identification.

Table 13.1.1. Suggested identification level of effort for aquatic macroinvertebrates

EPHEMEROPTERA - genus apart from:

Baetidae - genus only except for *Baetis* (*tricaudatus* or *bicaudatus*)

Ephemerellidae - species in almost all cases

Heptageniidae - genus only except for *Epeorus* (*albertae*, *longimanous*, *grandis*, etc.)

Leptophlebiidae - species for *Paraleptophlebia bicornuta*

PLECOPTERA

Capniidae - family only

Chloroperlidae – genus in late instars

Leuctridae - genus

Nemouridae - genus except for *Zapada* (*cinctipes*, *frigida* etc.)

Peltoperlidae - genus

Perlidae - genus

Perlodidae - genus except for *Isoperla* (*mormona*, *fusca* etc.)

Pteronarcidae – genus

Taeniopterygidae- genus

TRICHOPTERA - genus for all families apart from:

Dicosmoecus- species in late instars (*gilvipes* or *atripes*)

Neophylax- species in late instars

Parapsyche- species (*almota* or *elsis*)

Rhyacophila- to group except:

Betteni gr.- *R. malkini*

Lieftinchi gr. - *R. arnaudi*

Sibirica gr. - *R. blarina* and *R. narvae*

COLEOPTERA - Generally keep everything at family level apart from Psephenidae, Hydrophilidae, Haliplidae and Elmidae (genus in all three cases)

DIPTERA - genus level for all families apart from:

Chironomidae - sub family

Ceratopogonidae - sub family

Tabanidae, Dolichopodidae, Ephydriidae, Sciomyzidae, Syrphidae - family level only

GASTROPODA - genus level where possible (generally not possible for Physidae)

HEMIPTERA - genus level apart from Corixidae. (In many cases we should not be counting Hemipterans e.g. Gerridae)

ODONATA - genus level

CRUSTACEANS - usually genus level

PELECYPODA - family level only

LEPIDOPTERA/MEGALOPTERA - genus level

13.6 Quality Assurance

13.6.1 Overview

Quality assurance procedures (QA) assess the environmental variability, sampling procedure validity, repeatability of the sample methods, and identification quality. The quality assurance procedures involve a system of following standard methods and protocols, duplicate sampling, and identification reviews.

13.6.2 Field QA sample

Ten percent of all stream sites sampled, or one sample per survey, whichever is greater, should have a duplicate set of field samples collected. The duplicate sample is from the same sample reach.

Field QA samples look at the natural variability within a riffle and insure that the field sampling method is repeatable. This sample is sorted and identified the same as any other sample.

13.6.3 Laboratory QA Samples

Ten percent of all composite samples collected, or one sample per survey, whichever is greater, is resorted for an additional 300 specimen subsample from the original preserved composite sample. The result is a duplicate sample from the same composite.

Lab QA samples look at the variability inherent in the subsampling procedure and insure that the subsampling method is repeatable and within an acceptable range of variability.

13.6.4 Type collection

It is useful to maintain a macroinvertebrate type collection for each major basin, watershed, or ecoregion studied. This collection has a representative of each taxon identified and serves as a basin record, and as a reference for checking identifications.

13.6.5 Identification review

Data should be reviewed by an experienced taxonomist for anomalous identifications. Randomly selected samples should also be identified by an experienced entomologist independently of the first identification. Finally, specimens entered into the type collection should be checked by an experienced entomologist for accurate identification.

13.7 Taxonomic References

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