Macroinvertebrate Collection and Physical Habitat Assessment Instructions

Overview – Must Read

1. The primary objective for collecting macroinvertebrate data is to compile a species list over time and space to identify missing, additional and indicator species that might signify changes in community structure or function. One macroinvertebrate sample will be collected at a minimum of ONE station per group within a contract year. Your responsibility is for collection only. A Colorado Department of Public Health and Environment accepted taxonomist will complete identification to a genus/species level.

2. RW staff will identify the station you are to sample, whether to use Rocky or Sandy Substrate Datasheets and notify you if you are to sample this year. You will receive sample bottles and alcohol. Ten percent of participating groups will be chosen to provide a quality control sample.

3. There are three, yes three, physical habitats and analyses are associated with a macroinvertebrate sample. A depth profile in representative habitat, a micro-habitat stream bottom composition and macro-reach scale assessment. Of these three, the micro-habitat, where you collect bugs, must be completed with each macroinvertebrate sample. The micro-habitat assessment describes the bug's habitat or environment and will document changes in aquatic environment over time. The macroinvertebrate and physical habitat data sheets must be submitted with each collection. Complete all three assessments if possible and safe. A depth profile and macro-habitat assessment **can** be completed without a macroinvertebrate sample.

4. **ALWAYS** collect a water quality sample the same time/day the macroinvertebrate sample is collected. This tells us the "condition" of the river for the bugs at the time of collection. A water sample should include pH, Temperature, dissolved oxygen, alkalinity, hardness and both total/dissolved metals. If possible collect a nutrient sample as well (analyzed for total nitrogen, ammonia, total phosphorus, chloride, sulfate and total suspended solids).

5. Full instructions for both macroinvertebrate collection and physical habitat assessment are in this manual. A video/picture training is available that illustrates many of the steps and definitions (October 2006).

6. Each bug collection and/or habitat assessment is a sampling event, given a unique sample identification that is a combination of station number, date and time. If water quality samples are collected at the same time, all these samples will have the same sample identification.

7. Ship macroinvertebrates, data sheets and chain of custody within two weeks after collection. This will help to insure we can have the bugs identified prior to the end of each contract/school year.

Equipment for Macro Collection

Equipment provided by River Watch:

- A modified D-net (18" x 8"); the net is a 500-micron mesh net.
- Two forceps to pick organisms from net
- A 600-micron sieve (#36)
- One small brush
- Two 0.5 to 1.0 liter containers with alcohol preservative (four jars if you are to collect a QA/QC sample).

Additional equipment provided by you:

- A clean sample bucket – white is good (**not** your River Watch water sample bucket)
- A squirt bottle (can be any water bottle with a squirt nozzle)
- A timing device that can time 60 seconds (a second hand on a watch)
- Waders
- A ruler to measure substrate
- A broom, pole or pipe with inch and foot marks on it to measure depths
- A tape measure (can be marked string or twine)
- Rubber gloves (optional) and magnifying glasses (optional)
- A large **white** enamel or plastic tray – or white trash bag over dark trays

Field Preparation Overview

1. Retrieve blank data sheets and complete information about the sample above Part 1. Check **all** appropriate boxes. If you have been chosen to collect a QA/QC sample, check that box also. Check what you collected or assessed that day, water quality, metals, nutrients, depth profile, micro and macro habitat? Circle how many kicks our equivalent you conducted. Be sure to check box for either Rocky or Sandy Substrate.

2. Using a permanent marker, label each macroinvertebrate sample bottle with river name, station name, station number, time and date. Complete the label for inside the sample jar with the same information.

3. Gather gear from list above, including water quality sampling gear.

4. If you are collecting water quality samples, collect water quality sample **BEFORE** any macroinvertebrate sampling as this method involves disturbing the substrate and could contaminate a surface water sample.

NOTE: not following these instructions means your data is not as useable. It cannot be compiled with other data and used by the Health Department in the Clean Water Act process. That means if you kick for more/less than 60 seconds, use a rocky protocol in a sandy substrate, don't composite, sample a larger area, etc. Help us make the most of your data by following the instructions and completing all the items on each datasheet.

Choosing Bug Sites and Recording Sampling Event

The first step is to determine if your segment is one classified as rocky or sandy. RW staff will tell you, but you can determine yourself. This is important because you want to sample where the bugs live. The delineation is based on whether your stream or river is dominated by hard bottom substrate (rocky). A rocky bottom means more than 80% of the reach is boulder, rubble, cobble and gravel thus you should use the "Rocky Substrate" datasheet and protocols. If more than 80% of substrate is covered by sand/silt or fines, even if you see rocks, cobble and gravel, then you will use the "Sandy Substrate" Datasheet and protocols.

1. **Determine the general area to collect the sample by surveying a stream reach based on the relevant protocol. Some steps apply to both protocols.**

    a. **Identify your sample segment: Identify and measure a 200 foot segment:**
    - For ROCKY substrate samples, look for a segment that you can kick in two fast and two slow riffles. Riffles are the shallow fast moving sections of the river with velocities between 1.5-2.5 feet/second. Slow riffle areas have velocities between 0.5 and 1.5 feet/second, still moving but much slower, sometimes deeper. More information below.
    - For SANDY substrate sampling, look for a segment you can sample in multiple habitats within the reach (i.e. submerged vegetation, large woody debris, vegetated banks, water column and substrate) for an equivalent of 4 full minutes. More information below.

    b. **If possible,** you want to be at least 100 feet upstream from any road or bridge structure and away from any major tributaries, discharges or return flows.

    c. **Choose** reaches with habitats that are representative of the entire stream (i.e. riffles that looks like all the other riffles in the area).

    d. **Explore**. You may have to walk along the entire area of your 200 foot segment to find habitats to sample; they do not need to be right next to each other.

2. **Macroinvertebrate Collection Data Sheet Part 1:**
   Draw a map of the 200 foot section, scanning 100 feet above and below the area sampled, including the riparian zone. Draw boulders, snags, riffles, pools, dams, pipes, ditches, tributaries, bridges, wetlands, riprap and any landmarks that help identify your spot.
    - ROCKY substrate folks: draw a square for each kick sample and the number of the kick (1-4) inside the square of where you will be sampling.
    - SANDY substrate folks: include riparian and in stream vegetation, sandbars, submerged vegetation, woody debris piles, etc. Identify where you plan to sample.

3. **Macroinvertebrate Collection Data Sheet Part 2:** There is a location for recording a depth profile of a representative habitat transect. A depth profile provides a visual of the habitat, if done at the same place year to year, you and see changes in the profile or habitat (times of year too). If you can take the tape measure and hold or stake it across the river at a 90 degree angle from the bank, at a height 1-2 feet off the river surface. Take the depth measuring device and measure depths at about 1 foot or step intervals from bank to bank. Start on one bank where the water reaches the side measure depth, move a step measure and record depth, continue until across transect. Please record what unit of measure you are using. We prefer that you use feet and inches. Use another piece of paper or the back if there are not enough slots. Conduct this only if it is safe to wade in the river.

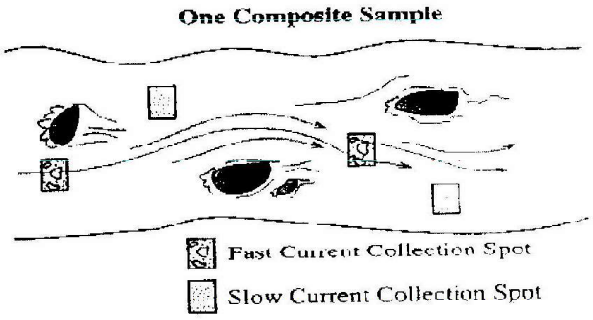
Rocky Substrate Collection

(If you are a Sandy Substrate, Go to the Sandy Substrate Collection Instructions)

Minimize tromping in the river before actual sampling. Have Rocky Substrate Datasheets ready. A team approach here can be very effective, if all teams understand their role prior to arriving in the field. Assign one team to collect water quality samples, one to draw the map, one to collect bugs, one to time and record bug collectors information, one to conduct physical habitat assessment, and one to conduct the depth transect profile.

1. **Collect Water Quality Sample**

2. **Determine four specific sites** (two fast riffles and two slow riffles) where you will collect a kick net sample. Your sample is a composite of four separate kick sub-samples collected into the net. You must identify four locations where you will collect these sub-samples. With minimum disturbance to the stream substrate, find two riffle areas where the water is flowing fast (1.5-2.5 feet per second) and two riffle areas that are flowing slower (0.5 to 1.5 feet per second), but still flowing. Use the floating device, timing and tape measure to estimate flows if you need too. See diagram below:



3. **Estimate kick area**. Approach the most downstream riffle spot for the first kick. Visualize an area on the stream bottom that is equivalent to about a 5.5 x3 feet (or 1 x 1 .7 meter) square area. Another way to measure this kick area is to lay the net down and make a mental map of the area that roughly covers from the length of the handle to the width of the net. This will be the kick area.

4. **Net placement.** Place the net in the riffle making sure the net is on the stream bottom and if possible water does not flow over the top of the net. It is best if you can see water flowing through the net. Eddies, dead flow areas or areas water is flowing back upstream behind large rocks will not work as flowing water is needed to carry the bugs into your net as you disturb the substrate. Once your kick area is defined, the net is set and the water is flowing through the net, you are ready to conduct your first kick.

5. **Conduct kick #1:**
    a. One person will hold the net open downstream.
    b. Second person will kick and disturb from upstream to down.
    c. A third person will time for 60 seconds.
    d. A fourth person is the recorder.
    e. Begin at the downstream end of your rectangle with the net close enough to your feet so that dislodged organisms will go into the net and not around it (not more than one foot away).

f. The timer starts timing 60 seconds.
g. The kicker uses their toe and heels to disturb, dislodge, uproot the upper layer of substrate and dig into the river bottom sediment. Do not kick the larger substrate out of the way, larger rocks or debris (logs, vegetation, and trash) should be picked up and brushed while immediately upstream of the net, so bugs will flow into the net. The goal is to get all bugs no matter where they are in that rectangle to flow into the net. Smaller debris like twigs and leaves should be kicked into the net and examined later for clinging bugs.
h. Have the data recorder label this kick #1 and identify it as a fast or slow riffle collection.

Micro-Habitat Assessment Rocky

6. **Data Sheet Part 3: Substrate Rocky Composition, Kick #1:** Kickers and recorders will work together on this step.
    a. Document time (60 seconds if following instructions).
    b. Circle fast or slow riffle for this kick.
    c. Record the average depth of the rectangle you sampled (in inches if can).
    d. Complete Column 1, substrate composition, using the size guidelines provided. KICKER estimates the percent of each substrate size and the RECORDER records the estimate in the appropriate shaded box. THE TOTAL SHOULD ADD up to 100%.
    e. Complete Column 2, organic components. Within the rectangle, which is your 100% sample area, how much detritus, muck/mud or marl is present? THIS WILL LIKELY **NOT** ADD up to 100%.

7. **Repeat steps 5 and 6 for kicks #2, #3 and #4:** Raise the net out of the water between each kick so that no organisms are lost. Carry the net to each riffle location; do not remove the bugs in-between kicks. After all sites are sampled, process the sample as described below.

8. **Data Sheet Part 4: Macro-habitat Assessment:**
Next complete Part 4 of the data sheet. Step back and look at the entire 200 foot reach and banks for a macro view. All habitat terms and descriptions are provided after the data sheet instructions in the sample plan. Here is a check list of all information to provide:
    a. % of cobble, snags, vegetated banks and sand in 200 foot reach
    b. Predominant land use on left and right banks
    c. % of exposed bare soil on both banks added up (if 200 feet = 100%)
    d. Amount of erosion you can see within 200 foot reach
    e. Amount of bank movement you can see within 200 foot reach
    f. Predominant vegetation type on left and right banks, name species if can
    g. Width of right and left riparian zones in feet
    h. Dominant aquatic vegetation “in” the river, not on banks, in 200 foot reach
    i. Portion or percent of reach with aquatic vegetation
    j. Canopy cover, average percent of water that is covered by bank vegetation
    k. Percent of 200 foot reach that is riffle, pool or run/glide
    l. Estimated wet water width (measured or estimated if not safe)
    m. Estimated bank full width (measured or estimated if not safe)
    n. Estimated average stream depth (can get from depth profile)
    o. Channelization: Can you see evidence of old or current channel straightening?

Sandy Substrate Collection

(If you are a Rocky Substrate, Go to the Rocky Substrate Collection Instructions)

Minimize tromping in the river before actual sampling. Have Sandy Substrate Datasheets ready. A team approach here can be very effective, if all teams understand their role prior to arriving in the field. Assign one team to collect water quality samples, one to draw the map, one to collect bugs, one to time and record bug collectors information, one to conduct physical habitat assessment, and one to conduct the depth transect profile.

1. **Collect Water Quality Sample.**
2. **Determine specific areas you will "kick" or "dip" your net**. Sandy substrate tends to shift and doesn't have the large interstitial space in rocks that many bugs prefer. In this habitat, the bugs will be in the water just above the substrate versus in the sand, or in aquatic vegetation in the stream amongst debris or along the banks. Identify **ALL** potential habitat types within the 200 foot reach (definitions of habitats are listed below), you may need to walk along the bank to do this:
    a. vegetated banks
    b. submerged vegetation
    c. snags/debris
    d. water column
    e. sandy substrate

    You may not have all of these habitats, and that is fine. Roughly estimate the amount or percentage of each habitat exists in the reach. Divide 240 seconds (equivalent of four, 60 second kicks) by the percentage of habitat. For example, you have equal occurrence of 3 habitats on the list, 33.33% of each. You will kick or dip into these habitats for 240 x .33 = 79 or 80 seconds each.

    To sample each habitat, minimize deep dipping into the sand. You are really ***dip*** netting more than kick netting. Below is a description of each habitat type and discussion on how best to sample each type. Remember you are compositing all collections into one sample. Move in a downstream to upstream direction with minimal or no wading if possible, not disturbing habitats you have not sampled yet. This protocol requires a little planning.

- Snags and other woody debris: Fallen branches, washed out or inundated shrubs/trees and small logs, which have been submerged in the water for a long time (not just fallen), provide excellent colonization habitat. Accumulated woody material in pools (deeper slower water) is considered snag habitat. To sample this habitat you would jab into the snag (with the net) and kicking around the snag with a net held downstream.

- Overhanging and Vegetated banks: Occur when lower banks are submerged and have roots and emergent plants associated with them. Submerged areas of undercut banks are good habitats. They are sampled in a fashion similar to snags by jabbing and disturbing the area upstream of the net. Bank habitat can be kicked first (with larger net) to dislodge organisms with net placed downstream to retrieve any bugs.

- Aquatic submerged macrophytes (large plants): Seasonal in their occurrence and may not be a common feature of many streams, particularly those that are of high gradient. These plants live submerged in the water and bank and can be seen with the unaided eye. Collect sample from aquatic plants that are rooted on the bottom of the stream or in the bank, and are submerged in the water by drawing the net through the vegetation from the bottom to the surface of the water. In shallow water, sample by bumping or jabbing the net along the bottom in the rooted area, avoiding sediments if possible.

- Sand and other fine sediment: Usually the least productive macroinvertebrate habitat in streams; this habitat may be the most prevalent in some streams. Collect sample from banks with no vegetation or soft soil by bumping the net along the surface of the bottom rather than dragging the net through the soft substrates, this reduces the amount of debris in the sample.

3. **Net placement in each habitat**. Approach the most downstream location. Visualize an area in the habitat you have selected that is equivalent to about a 1 x 1 .7 meter area. Another way to measure this area is to lay the net down over the habitat type and make a mental map of the area that it covers from the length of the handle to the width of the net. This will be the area that you will probe, dip and collect your sample in. This is the area you will sample for your estimated time, spreading out the time in each habitat for that habitat. You do not have to sample every occurrence of a habitat type, just a representative portion for the % of its occurrence in the reach- translated to time. In the end you should only **sample a total of 240 seconds**, regardless of where or habitat type.

4. **Micro-Habitat Assessment Sandy:**

    a. One person operates the net
    b. Second person assists with larger debris and substrate
    c. A third person can time
    d. A fourth person fourth is needed to record
    e. Sample the 1X1.7.m area using the techniques described above. Record the amount of time spent sampling the habitat in part 3, column 1 of the data sheet.
    f. Move to the next location; be careful to not lose organisms in switching habitats or dipping into the water. Repeat for all habitats selected.

Micro-Habitat Assessment:

5. **Data Sheet Part 3: Sandy Substrate Composition, Kick #1: Conduct dip or kick #1:** Kickers and recorders will work together on this step.
    a. Complete Column 1 on Sandy Substrate Dip #1, listing the time sampled for each habitat. Put 0% in habitats not sampled.
    b. Describe habitat more if need too.
    c. Record the % this habitat was present in the reach. This percent should correlate with the time sampled in this habitat.
    d. Total the time sampled and percent. Time should total 240 seconds and % 100.
    e. Record the average depth of the rectangle if sampled the water column in inches if can.
    f. Complete Column 2, organic components for each habitat sampled. WHERE appropriate (not for all types), within the sample rectangle, which is your 100% sample area, how much detritus, muck/mud or marl is present. THIS WILL LIKELY **NOT** ADD up to 100%.

6. **Repeat steps 4 and 5 for all remaining habitat dips or kicks.** Raise the net all together out of each habitat type between each kick so that no organisms are lost from the net. Carry the net to the next habitat location. Be careful not to lose any bugs when placing net in the next habitat type. After all sites are sampled, process the sample as described below.

7. **Data Sheet Part 4: Macro-habitat Assessment:** The same team or another team then completes Part 4 of the data sheet. Step back and look at the entire 200 foot reach and banks for a macro view. All habitat terms and descriptions are provided after the data sheet instructions in the sample plan. Here is a check list of all information to provide:
    a. % of cobble, snags, vegetated banks and sand in 200 foot reach
    b. Predominant land use on left and right banks

c. % of exposed bare soil on both banks added up (if 200 feet = 100%)
d. Amount of erosion you can see within 200 foot reach
e. Amount of bank movement you can see within 200 foot reach
f. Predominant vegetation type on left and right banks, name species if can
g. Width of right and left riparian zones in feet
h. Dominant aquatic vegetation “in” the river, not on banks, in 200 foot reach
i. Portion or percent of reach with aquatic vegetation
j. Canopy cover, average percent of water that is covered by bank vegetation
k. Percent of 200 foot reach that is riffle, pool or run/glide
l. Estimated wet water width (measured or estimated if not safe)
m. Estimated bank full width (measured or estimated if not safe)
n. Estimated average stream depth (can get from depth profile)
o. Channelization: Can you see evidence of old or current channel straightening?

Sample Processing

The goal for processing is to get into the sample jar all the macroinvertebrates within the kick net, but with as little water and large debris as possible. This allows the preservative to work on the organisms effectively. If the sample is too watery, the preservative is diluted and organisms will become mushy and difficult to identify. If you process (wash and scrub) large material correctly, the lab can focus on identifying bugs versus processing material. Small bunches of organic material such as algal mats need to be left in the sample.

1. Once the sample is collected from all sample habitats or locations, carry the net to the shore. Fill the bucket 1/2 to 2/3 full of stream water. Gather the sample material into one corner of the net. Grab the corner of the net from the bottom outside, holding the clump in your hand(s) and turn the net inside out into the clean sample bucket. Knock or wash any obvious macroinvertebrates, debris, algae clumps or masses into the bucket. Rinse the net from the OUTSIDE into the bucket if necessary. Examine the net closely for organisms that may want to stay behind. Pluck these organisms off with forceps and place directly into the sample jar with half the alcohol.
2. Look in bucket for large rocks or debris you can handle, bare twigs or leaves (not algae masses). Pick them up one at a time. Hold them over the sieve and look for organisms. Rinse the object with squirt bottle over the sieve; pluck the organisms off with forceps and place in the sample jar. **Do not rinse over the alcohol filled sample jar.**
3. Separate the organisms from the debris by “swirling” the sample in the bucket. Add more water if you need to and really swirl! The lighter organisms and debris will rise to the top of the water and the heavier sediment will not. Look for bugs to float to the top. Pour off the top water and floating material into the sieve, leaving the sand and gravel in the bucket.
4. Repeat the swirling until lighter material and bugs no longer rise to the top. Swirling needs to be aggressive enough to dislodge clinging organisms. This will take a MINIMUM of 15 swirls; maybe 20 to make sure all organisms are dislodged. Use more water if you need to. Limit scraping or any movement that would smash the bugs. Pick up a handful of gravel or substrate and look closely to see if it moves or you can see any bugs. If so, swirl again.
5. If you have algal masses, place them on the sieve and let as much water as possible drain out of the mass. Do not smash the mass as you will smash the bugs. Water from these masses will dilute the alcohol. The bugs in these masses are hard to see with the naked eye and require further sorting. When drained, place mass into sample jar.
6. After the last swirl and all floating bugs have been GENTLY placed into the preserved sample jar with forceps, pour the contents of the bucket onto the sieve in ***manageable*** batches. Spread the material and look for bugs. Then place sieve material onto a white tray looking for organisms one last time. Pluck bugs from sieve or tray and place in preservative. If you are collecting a QA/QC sample, place any remaining algae mats or clumps of debris in a second sample jar, draining as much water as possible. If you are not collecting a QA/QC sample, dump thoroughly processed debris from the pan. Repeat in batches until all debris has been processed.

7. If you have been selected to collect a QA/QC sample, you will have received 4 jars partially filled with alcohol. Process the sample as described below placing all your picked bugs in one jar. All processed debris throughout the process (leaves, sticks, rocks, sand and twigs) is placed in the other jar rather than being disposed of. This debris will be processed for bugs you might miss thus serving as a QA sample. Check the QA sample line on the macroinvertebrate label (your "normal" bug sample will have a similar label without the QA line checked).

8. Once all debris from the net is processed, rinse the net, sieve and pan thoroughly in the river, until no debris is visible. It is best to let net dry as soon as possible to avoid mold growth.

9. Label your sample and any QA sample you may have accordingly with a magic marker on the outside of the jar. Include sample #, station #, date and time. Place the other label inside the macroinvertebrate samples. The inside label is smaller and requests collector's name. These labels are located on page 243 of this sampling protocol.

10. **The evening of sampling, carefully decant the initial alcohol used and then pour in rest of fresh alcohol. If you have collected a QA/QC sample, decant that one also and replace with fresh alcohol.** Decanting will lessen the amount of water in the bottle that would hasten the degeneration of the bugs. Place sample label(s) from Step 2 of Laboratory Preparation in jar(s) and cap snugly.

11. Ship or deliver your sample(s) within **two** weeks of collection. Include all data sheets and chain of custody (there is a spot to label "bugs", also downloadable on our website). Complete a Field Data sheet to go with the macroinvertebrate and physical habitat data sheets. Include water quality data if it was collected (preferred). Keep a copy of data sheets. A macroinvertebrate sample, with or without a chemical sample is a sampling event.

Macroinvertebrate Collection Data Sheet Instructions

Top Section of the Data Sheet:

- Organization/school name, river and station name
- Date and time
- Check box for Rocky or Sandy Substrate
- Sample Method: Circle Modified D-net or describe other if another River Watch approved method was used.
- Record the number of "kicks" performed. This should total four for rocky substrate.

Part 1 of the Data Sheet

1a Draw a picture of the reach in which you sampled. You want to diagram from a birds eye view of the 200 foot segment and 5 to 10 feet of bank on each side for the segment you have chosen. To orient the diagram, pretend you are a bird looking down at your site. Look upstream and identify left and right bank, circle the appropriate bank on the drawing, on the top of the box circle left or right bank and do the same at the bottom of the box. If this space is too small to draw in, provide your own drawing and write in this space: "See enclosed drawing".

1b Circle the direction the flow is going in the diagram.

1c Sketch the stream banks and major objects such as boulders, debris, pools, dams, tributaries, ditches, pipes, riprap, etc. Label items you feel need labeling to understand.

1d Draw a square resembling each kick or sample area and put a number in the box to represent the order of the kicks.

1e Describe where the station is relative to your water quality station **only IF** this is not that same station.

Part 2: Average Depth Profile of Representative Sample Transect

What this seeks to identify is a cross sectional measurement from wet water width (waters edge to water's edge) that consists of a series of depth measurements. Using a marked rod (PVC pipe, broom handle) that is marked off in feet and inches, record the depth of the stream at increments of every one foot. If you need more than 35 spaces, use the back of the data sheet or an additional piece of paper. This measurement should be taken in an area representative of where you will be collecting your macro invertebrate sample. As recording this data may disturb the very habitat you are sampling, this measurement should be taken after the macroinvertebrate sampling has been completed. Over time this data will illustrate channel movement within that reach.

Note: You will need to measure bank full width as well. Both bank full and wet water measurements can be done as part of this step and the bank full measurement can be recorded in **Part 4, Section F**. Measure bank full width by noting the area from the end of the high water mark on one bank, to the edge of the high water mark on the opposite bank.

Part 3: Habitat Description for Rocky and Sandy Substrate

This is a microphysical habitat description of each rectangular kick area that was sampled. RECORDER and KICKER need to work together on this. You will be recording organic and inorganic substrate type and composition, riffle speed (for rocky habitats) and average depth of the kick area. Columns 1 address inorganic substrate composition of the **rocky** habitat sampled, and column 2 addresses organic composition of the habitat sampled **be it rocky or sandy**. Column 3 address inorganic substrate composition of the habitat sampled for **sandy** habitats.

Column 1 Inorganic Habitat Composition for Rocky Substrate: This column address inorganic substrate composition of the habitat sampled. You will first need to circle whether the area sampled was a fast or slow riffle. You need to quantitatively describe what the stream substrate is comprised of. To do this, use a ruler to measure various substrate sizes. There is a range of size that correlates to how the substrate is classified (be it boulder, gravel, sand etc.) in column 1. Once this is determined, estimate the amount of each specific type of substrate that is represented in your kick area. Check that total percent of inorganic material adds up to 100%. An example of this would be a kick area with 25% cobble, 50%pebble and 25%silt.

Column 2-Organic Habitat Composition for Rocky/Sandy Substrate: This column addresses the organic substrate components of the kick area. Different organic components are described below. This value may not add up to 100% as it is a **% of the amount of organic in the entire kick area**. The value may be anywhere from 0% to 100%, it is dependant upon how much organic material is covering the substrate (i.e. the kick area described above containing 10% detritus/leaf litter).

Detritus is any sticks, leaves, floating plant material or algae. Basically anything organic you could pick up with your hands is coarse organic material (CPOM). Look for fine slippery algae on large rocks, this is Periphyton. This is food for the bugs.
Muck-mud is very fine, yucky, black, slimy material and will sometimes have an odor of sulfur like in a wetland soil. This is fine organic material (FPOM). This is another form of food for bugs.
Marl is gray, finely broken shell like fragments. It is unlikely you will find this in most Colorado streams.

Record the average depth of the water sampled in row 1 column 2.

Part 3 Inorganic Habitat Composition for Sandy Substrate: This column address inorganic substrate composition of the habitat sampled. In Column 3 you will need to describe each habitat that was sampled. Recorder can identify the habitat type as vegetated bank, submerged vegetation, snags/debris, water column or sand/substrate. Record the amount of time that each habitat was sampled and note any worthy characteristics of each site like size, community structure (one or multiple species of plant) etc. Also record the % composition of each habitat type for each area sampled. An example: If you sampled 4 separate areas and you sampled in this 1st area that had 90% vegetated banks and 10% substrate, record as such.

Part 4: Entire Segment Physical Habitat Description

This section evaluates Habitat Features, Watershed Features, Localized Erosion, Riparian Vegetation, Aquatic Vegetation and Instream Features **for the entire 200 foot segment you have mapped in Part 1**. There are 6 sections in this description and definitions to all terms found in these sections are provided below (as discussed in the USEPA Rapid Bioassessment+6 Protocols). Observe the entire reach and be as objective and consistent as possible. Don't forget recorder's signature and the date at the bottom.

Section A Habitat Feature Descriptions

This item describes all the different habitat types that could be sampled for macroinvertebrates. In rocky substrate streams we are only sampling one habitat type, the riffle, which in theory is cobble. In sandy substrate streams we are sampling several habitats—snags, debris, vegetated banks and sand. It is helpful to know how much of the other habitat types are present for future sampling, especially if the riffles are not that numerous or large. Estimate the percentage within the 200 foot reach of cobble, snags, vegetated banks and sand present.

- Snags and other woody debris are fallen branches, washed out or inundated shrubs/trees and small logs, which have been submerged in the water for a long time (not just fallen), provide excellent colonization habitat. Accumulated woody material in pools (deeper slower water) is considered snag habitat.

- Overhanging and Vegetated banks occur when lower banks are submerged and have roots and emergent plants associated with them. Submerged areas of undercut banks are good habitats.

- Aquatic submerged macrophytes (large plants) are seasonal in their occurrence and may not be a common feature of many streams, particularly those that are high gradient. These plants live submerged in the water and bank and can be seen with the unaided eye.

- Sand and other fine sediment are usually the least productive macroinvertebrate habitat in streams; this habitat may be the most prevalent in some streams.

Section B Watershed Features of Overall Area

This is a description of the land adjacent to *both* left and right stream banks. These are always determined by looking upstream from kick site. For each bank check the predominant (top 1 or 2 most prevalent types) land uses 300 ft adjacent to the reach.

- Forests: trees, pine or deciduous in a fairly undisturbed tract

- Field/Pastures: fields of grass, left undisturbed or used for grazing even if irrigated, not cropland, etc.

- Irrigated: irrigated land for any crop

- RR/Hwy: a railroad, highway or road

- Dense housing: like a suburban or urban area
- Sparse housing:10 acres or more per house/unit
- Commercial: commingled buildings or business as on a main street in town
- Industrial: refinery, brewing company, power plants etc.
- Other: anything that doesn't fit above (please describe)

Section C Localized Erosion of Overall Area

This section evaluates local erosion and potential sources of sediment in the stream reach. A river carries a certain amount of sediment either in the water column (suspended) or moving along the bottom (bed load). How much sediment and what size particles in the sediment load are a function of the stream volume (discharge) and velocity (flow). A river is designed to carry sediment from it's headwaters to the mouth. Sediment in unnatural amounts, from sources outside the flood plain or delivered at an unnatural rate becomes a pollutant, smothering habitat and causing other effects. Natural and accelerated erosion of land causes sediment to end up in the river. Many sources of sediment to a river come from a diffuse non point source like an unchecked construction site versus a direct source like a pipe. You are evaluating evidence of diffuse or non-point sources of sediment and the amount of visual erosion. Some things to look for while assessing localized erosion include looking for extensive reaches of non-vegetated banks, traveled foot/tire paths next to, down to or even crossing the stream, culverts/bridges, any bare dirt proximate to the bank, etc. Asses what percent of the stream reach you are surveying has any of this evidence using the guide below. There are three parameters to note:

1. % Bare Bank Soil: make an estimate of the area of bare soil (80%, 10%, etc.) in the riparian zone that is not bound by plants and their root structures or covered in concrete or rocks. These bare areas can be caused by wildlife, livestock or human access, roads and crossings, clearings or undercut banks.

2. Erosion Amount: estimates the amount of erosion that is present on the banks within the reach. Choose the category that best describers your estimate.

3. Bank Movement and Stability: due to lack of vegetation, roots or other mechanisms to keep the soil and bank from entering the water, the banks may have become unstable and show signs of degradation. Choose the category that best describers your estimate of bank stability or degradation.

Section D Riparian Vegetation of Overall Area

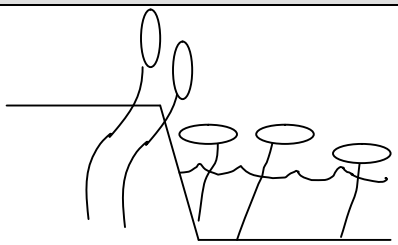
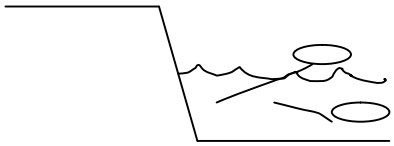
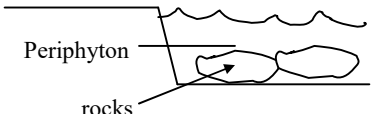
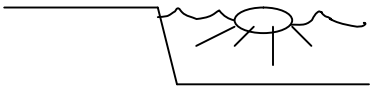
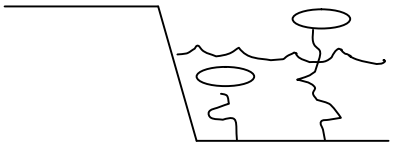
This section describes the vegetation type on each bank. Riparian vegetation along the bank and transitioning into the upland ecosystem provide food and habitat for a variety of animals in some aspect of their life cycles. It can also provide a migration corridor, soil stability, water quality filter and buffer for the water body.

In this section, please describe predominate vegetation type for each bank. You don't need to know the species, just the type (tree, grass, or shrub/bush) but if you do you know species, please document. If there is no vegetation along the riparian zone, note *other* and please describe in the space provided (i.e. pavement, dirt, etc.) Evaluate for both left and right banks. Estimate the width of each bank riparian zone; the width from the water to another vegetation type is the riparian zone width, record on data sheet.

Section E Instream Aquatic Vegetation of Overall Area

This section focuses on vegetation *in the stream* only, not on the banks. These species need water; need to be submerged or associated water for some part of their life cycle. Typical examples range from cattails to liverworts, blue, green and brown algae and periphyton. These also vary in that they are macrophytes, vascular and non-vascular and angiosperms. Aquatic vegetation is an important component of water bodies because they can provide food, oxygen and habitats for aquatic animals, supply food and habitat for birds, stabilize banks and beds, and take from the water some of the potential pollutants in runoff. Factors that affect the type and distribution of water plants include climate, flow, velocity, light, temperature and water quality. Look in the water and estimate the dominant vegetation type in the entire 200 foot stream reach using the guide below. Then estimate the percentage within the reach that is populated with vegetation.

- Rooted emergent or submerging is an aquatic plant rooted in wetland, lake or river substrate. Usually grow at the water's edge or in shallow water. Most of the plant is above water. These include common plants such as rushes and some grasses. Some are broad-leafed and some are have narrow leaves.
- Rooted floating is a rooted aquatic plant that has come loose and is now floating in the stream.
- Submerged / Floating leaf varieties These have root systems attached to the bottom of the water body and in some cases have leaves that float on the surface and / or flowers parts that emerge from the water. These include plants such as water lilies, milfoils, watercress and ribbon weed.
- Attached Algae is like periphyton, and is common in Rocky Mountain streams.
- Free floating is a plant that prefers to grow as it floats. They are not attached at any time and occur in relatively still water. The whole plant is floating with roots suspended in the water. These include common plants like azolla and exotic plants like water hyacinth.

	Visual		
Rooted emergent or submerging		Rooted Floating	
Attached Algae		Free Floating	
Submerged / Floating Leafed			

Section F Instream Features of Overall Area

This section evaluates instream features that provide quality habitat for macroinvertebrates. This is to be evaluated for the entire 200' stream reach.

Canopy Cover: Trees and large shrubs provide shade and minimize temperature changes in the stream. It also provides food and habitat for emerged macroinvertebrates and food for fish as the insects fall into the river. Look up and down the stream, if the tree canopy covers the entire width of the open water, the canopy cover would be 100%. If any coverage occurs, estimate how much of the cover is generated from both the right and left banks and record

Stream Reach Description: Pools (slow, deep water), riffles (fast, shallow water), or runs (long, deep, slow, gliding pool) are the descriptors used to identify how the stream is moving through space. Identify the percentage of each type in your reach and note.

Wet Water Width: The width of the water in the stream, from one wet edge to the opposite wet edge. If you have a measuring tape and can wade in the stream, measure this.

Bank Full Water Width: The highest level that water could reach without flowing out of the banks onto adjacent land. Usually you can tell this by old wet watermarks or vegetation changes. If you have a measuring tape and can wade the stream, measure this.

Average Stream Depth: The estimate or measurement of stream depth in several places along a transect the in the stream. A PVC pipe or stick (bug net) with measured tick marks works for this. You can get this from averaging results of Part 2.

Channelized: When you look within the stream reach up and downstream, can you see the stream meander (bend) at all? Answer this with a yes or no answer. If you cannot see any meanders, then stream may be channelized due to a road, railroad or other reasons.

Macroinvertebrate Collection Data Sheets

(Sample Site Information and Depth Profile - Page 1 of 4)

Station Name_____ Date of sample ___/___/___ Time ___:___

River_____ Station number:_____

Group (School)_____ **Substrate** : **ROCKY** ☐ **SANDY** ☐

RW Net and method ☐ or Other_____ **# of Kicks/ dips conducted?** ☐

☐ Field Data Collected	☐ Depth Profile Completed
☐ Metals Collected	☐ Micro-habitat (4 kicks/dips) completed
☐ Nutrients Collected	☐ Macro-habitat reach assessment
☐ QA Macro Sample Collected	☐ Yes or No -Is this your normal water quality station?

Part 1

a. Draw a picture of the sample site (from bank to bank, 200 feet above/below sample area):

Left bank or **right** bank-looking upstream (circle one)

Left bank or **right** bank looking upstream (circle one)

b. Flow direction on diagram ⟶ OR ⟵
(circle one)

c. Draw in stream attributes such as riffle, dams, fallen trees, pools, roads, tributaries, bridges, wetlands, riprap, pipes, and other landmarks to identify reach, Label appropriately, include larger sheet if desire.

d. Draw a square representing bug sample location and a number in each square representing each 1 of 4 kicks.

Part 2 -Average Depth Profile of representative sample transect

Select a spot typical of the sample area. Measure depths at 1-step intervals from bank to bank across the river and record below. Please use inches or feet if can, state UNIT=_____. Place transect on diagram above. Use back or another sheet if needed and record.

1 _____	6_____	11_____	16_____	21_____	26_____	31_____
2_____	7_____	12_____	17_____	22_____	27_____	32_____
3_____	8_____	13_____	18_____	23_____	28_____	33_____
4_____	9_____	14_____	19_____	24_____	29_____	34_____
5_____	10_____	15_____	20_____	25_____	30_____	35_____

ROCKY Substrate Composition for Each Kick (page 2 of 4)
DO NOT USE IF SAMPLING SANDY SUBSTRATE

Part 3 Substrate Composition kick #1:

a. Total Time Sampled Rocky Substrate habitat _____ seconds
b. Average Depth of rectangle sampled = _____inches or _____unit?_____

c. Circle: Fast Riffle (1.5-2.5 ft/sec) OR Slow Riffle (0.5-1.5 ft/sec)
1 2

Inorganic Substrate Components			**Organic Substrate Components**		
Should ALWAYS add to 100%			**May NOT add up to 100%**		
Substrate Type	Diameter	% Composition in sample	Substrate Type	Describe Characteristics	% Composition in sample
Bedrock	>11 inches		Detritus	Sticks, wood, coarse plant material, CPOM	
Boulder	>256mm, 10 inches				
Cobble	64-256mm, 2.5-10"		Muck-Mud	Black, very fine organic material, FPOM	
Gravel	2-64 mm, 0.1-2.5"				
Sand	0.06-2 mm, Gritty				
Silt	0.004-0.06mm		Marl	Grey, shell fragments	
Clay	<0.004, slick/slimy				
	TOTAL %				

Part 3 Substrate Composition kick #2:

a. Total Time Sampled Rocky Substrate habitat _____ seconds
b. Average Depth of rectangle sampled = _____inches or _____unit?_____

c. Circle: Fast Riffle (1.5-2.5 ft/sec) OR Slow Riffle (0.5-1.5 ft/sec)
1 2

Inorganic Substrate Components			**Organic Substrate Components**		
Should ALWAYS add to 100%			**May NOT add up to 100%**		
Substrate Type	Diameter	% Composition in sample	Substrate Type	Describe Characteristics	% Composition in sample
Bedrock	>11 inches		Detritus	Sticks, wood, coarse plant material, CPOM	
Boulder	>256mm, 10 inches				
Cobble	64-256mm, 2.5-10"		Muck-Mud	Black, very fine organic material, FPOM	
Gravel	2-64 mm, 0.1-2.5"				
Sand	0.06-2 mm, Gritty				
Silt	0.004-0.06mm		Marl	Grey, shell fragments	
Clay	<0.004, slick/slimy				
	TOTAL %				

ROCKY Substrate Composition for Each Kick (page 3 of 4)
DO NOT USE IF SAMPLING SANDY SUBSTRATE

Part 3 Substrate Composition kick #3:

d. Total Time Sampled Rocky Substrate habitat _____ seconds
e. Average Depth of rectangle sampled = _____inches or _____unit?_____

f. Circle: Fast Riffle (1.5-2.5 ft/sec) OR Slow Riffle (0.5-1.5 ft/sec)

1 2

Inorganic Substrate Components			**Organic Substrate Components**		
Should ALWAYS add to 100%			**May NOT add up to 100%**		
Substrate Type	Diameter	% Composition in sample	Substrate Type	Describe Characteristics	% Composition in sample
Bedrock	>11 inches		Detritus	Sticks, wood, coarse plant material, CPOM	
Boulder	>256mm, 10 inches				
Cobble	64-256mm, 2.5-10”		Muck-Mud	Black, very fine organic material, FPOM	
Gravel	2-64 mm, 0.1-2.5“				
Sand	0.06-2 mm, Gritty				
Silt	0.004-0.06mm		Marl	Grey, shell fragments	
Clay	<0.004, slick/slimy				
	TOTAL %				

Part 3 Substrate Composition kick #4:

d. Total Time Sampled Rocky Substrate habitat _____ seconds
e. Average Depth of rectangle sampled = _____inches or _____unit?_____

f. Circle: Fast Riffle (1.5-2.5 ft/sec) OR Slow Riffle (0.5-1.5 ft/sec)

1 2

Inorganic Substrate Components			**Organic Substrate Components**		
Should ALWAYS add to 100%			**May NOT add up to 100%**		
Substrate Type	Diameter	% Composition in sample	Substrate Type	Describe Characteristics	% Composition in sample
Bedrock	>11 inches		Detritus	Sticks, wood, coarse plant material, CPOM	
Boulder	>256mm, 10 inches				
Cobble	64-256mm, 2.5-10”		Muck-Mud	Black, very fine organic material, FPOM	
Gravel	2-64 mm, 0.1-2.5“				
Sand	0.06-2 mm, Gritty				
Silt	0.004-0.06mm		Marl	Grey, shell fragments	
Clay	<0.004, slick/slimy				

SANDY Substrate Composition for Each Kick (page 2 of 4)
DO NOT USE IF SAMPLING ROCKY SUBSTRATE

Part 3 Substrate Composition kick or dip #1:

a. Total Time Sampled Sandy Substrate habitat _____ seconds
b. Average Depth of rectangle, IF sampled water column = _____inches _____unit?_____

1 2

Type of Habitat sampled				Organic Substrate Components		
Time should Add to 240 Seconds % Composition Should Always add to 100%				**Will Likely NOT add up to 100% Complete if Sample in Water Column**		
Habitat Type	% Composition in Reach	Describe Characteristics	Time sampled (seconds)	Substrate Type	Describe Characteristics	% Composition of sample
Vegetated Banks				Detritus	Sticks, wood, coarse plant material, CPOM	
Submerged Vegetation				Muck-Mud	Black, very fine organic material, FPOM	
Snags/Debris				Marl	Grey, shell fragments	
Water Column						
Sand/Subs						
TOTAL %		**TOTAL TIME**			**TOTAL %**	

Part 3 Substrate Composition kick or dip #2:

a. Total Time Sampled Sandy Substrate habitat _____ seconds
b. Average Depth of rectangle, IF sampled water column = _____inches _____unit?_____

1 2

Type of Habitat sampled				Organic Substrate Components		
Time should Add to 240 Seconds % Composition Should Always add to 100%				**Will Likely NOT add up to 100% Complete if Sample in Water Column**		
Habitat Type	% Composition in Reach	Describe Characteristics	Time sampled (seconds)	Substrate Type	Describe Characteristics	% Composition of sample
Vegetated Banks				Detritus	Sticks, wood, coarse plant material, CPOM	
Submerged Vegetation				Muck-Mud	Black, very fine organic material, FPOM	
Snags/Debris				Marl	Grey, shell fragments	
Water Column						
Sand/Subs						
TOTAL %		**TOTAL TIME**			**TOTAL %**	

SANDY Substrate Composition for Each Kick (page 3 of 4)
DO NOT USE IF SAMPLING ROCKY SUBSTRATE

Part 3 Substrate Composition kick or dip #3:

c. Total Time Sampled Sandy Substrate habitat _____ seconds

d. Average Depth of rectangle, IF sampled water column = _____inches _____unit?_____

1 2

Type of Habitat sampled				Organic Substrate Components		
Time should Add to 240 Seconds % Composition Should Always add to 100%				**Will Likely NOT add up to 100% Complete if Sample in Water Column**		
Habitat Type	% Composition in Reach	Describe Characteristics	Time sampled (seconds)	Substrate Type	Describe Characteristics	% Composition of sample
Vegetated Banks				Detritus	Sticks, wood, coarse plant material, CPOM	
Submerged Vegetation				Muck-Mud	Black, very fine organic material, FPOM	
Snags/Debris				Marl	Grey, shell fragments	
Water Column						
Sand/Subs						
TOTAL %		**TOTAL TIME**			**TOTAL %**	

Part 3 Substrate Composition kick or dip #4:

c. Total Time Sampled Sandy Substrate habitat _____ seconds

d. Average Depth of rectangle, IF sampled water column = _____inches _____unit?_____

1 2

Type of Habitat sampled				Organic Substrate Components		
Time should Add to 240 Seconds % Composition Should Always add to 100%				**Will Likely NOT add up to 100% Complete if Sample in Water Column**		
Habitat Type	% Composition in Reach	Describe Characteristics	Time sampled (seconds)	Substrate Type	Describe Characteristics	% Composition of sample
Vegetated Banks				Detritus	Sticks, wood, coarse plant material, CPOM	
Submerged Vegetation				Muck-Mud	Black, very fine organic material, FPOM	
Snags/Debris				Marl	Grey, shell fragments	
Water Column						
Sand/Subs						
TOTAL %		**TOTAL TIME**			**TOTAL %**	

Part 4 – Macro - Stream Reach Physical Habitat (page 4 of 4)

Part 4 Overall area physical habitat assessment (complete whether Rocky or Sandy)

Habitat Features	**A**	Indicate % of each habitat type in reach (50 ft above/below sample): ☐ Cobble____% ☐ Snags_____% ☐ Vegetated Banks_____% ☐ Sand_____%			
Watershed Features	**B**	Predominant Surrounding Land Use **R**ight **B**ank: ☐ Forest ☐ Dense housing ☐ Field/pasture ☐ Sparse housing ☐ Irrigated ☐ Commercial ☐ RR/hwy ☐ Industrial ☐ Park/Bike Path ☐ Other_____		**L**eft **B**ank: ☐ Forest ☐ Dense housing ☐ Field/pasture ☐ Sparse housing ☐ Irrigated ☐ Commercial ☐ RR/hwy ☐ Industrial ☐ Park/Bike Path ☐ Other_____	
Localized Erosion	**C**	**% Bare Bank Soil** ☐ 80-100% ☐ 10-39% ☐ 40-79% ☐ 0-9%	**Erosion Amount** ☐ extensive ☐ localized ☐ some evidence ☐ no evidence	**Bank Movement** ☐ bank failures ☐ slight ☐ mod collapses ☐ none	
Riparian Vegetation	**D**	Indicate the dominant riparian zone vegetation type and record dominant species: **R**ight **B**ank: ☐ Trees ☐ shrubs ☐ grasses ☐ herbaceous ☐ other_____ ☐ dominant species_____		**L**eft **B**ank: ☐ Trees ☐ shrubs ☐ grasses ☐ herbaceous ☐ other_____ ☐ dominant species_____	**Riparian Zone** Right Bank ____ ft Wide Left Bank ____ ft Wide
Aquatic Vegetation	**E**	Indicate the **dominant vegetation** type instream (not on banks): ☐ Rooted emergent ☐ Submerging floating leaf ☐ Rooted floating ☐ Free Floating ☐ Attached Algae			**Portion of reach with aquatic Vegetation**: _____%
Instream Features	**F**	**Canopy Cover:** _____% of stream bank covered with Canopy/other	**% of Reach Stream:** ☐ Riffle_____% ☐ Pool_____% ☐☐ Run_____%		Estimated **Wet** Water Width _____Ft Estimated **Bank** Full Width _____Ft Estimated average stream **depth** _____Ft **Channelized** ☐ YES ☐ NO

Macroinvertebrate Sample Labels For Inside/Outside Sample Bottle

River Name ______________________________
Station Name ______________________________
Station Number ___________________________
Date _______________ Time _______________
Sample Collector _________________________

Preserved with 95% ethanol / method 1

River Name ______________________________
Station Name ______________________________
Station Number ___________________________
Date _______________ Time _______________
Sample Collector _________________________

Preserved with 95% ethanol / method 1

River Name ______________________________
Station Name ______________________________
Station Number ___________________________
Date _______________ Time _______________
Sample Collector _________________________

Preserved with 95% ethanol / method 1

River Name ______________________________
Station Name ______________________________
Station Number ___________________________
Date _______________ Time _______________
Sample Collector _________________________

Preserved with 95% ethanol / method 1

River Name ______________________________
Station Name ______________________________
Station Number ___________________________
Date _______________ Time _______________
Sample Collector _________________________

Preserved with 95% ethanol / method 1

River Name ______________________________
Station Name ______________________________
Station Number ___________________________
Date _______________ Time _______________
Sample Collector _________________________

Preserved with 95% ethanol / method 1

River Name ______________________________
Station Name ______________________________
Station Number ___________________________
Date _______________ Time _______________
Sample Collector _________________________

Preserved with 95% ethanol / method 1

River Name ______________________________
Station Name ______________________________
Station Number ___________________________
Date _______________ Time _______________
Sample Collector _________________________

Preserved with 95% ethanol / method 1

River Name ______________________________
Station Name ______________________________
Station Number ___________________________
Date _______________ Time _______________
Sample Collector _________________________

Preserved with 95% ethanol / method 1

River Name ______________________________
Station Name ______________________________
Station Number ___________________________
Date _______________ Time _______________
Sample Collector _________________________

Preserved with 95% ethanol / method 1

Optional Macroinvertebrate Collection

Method for equipment limitation (no required RW equipment) or data objectives other than RW

If your data objectives differ from the RW required macroinvertebrate protocol, (for example simple education is the only objective or if you do not have a RW set of bug equipment), you can conduct the following macroinvertebrate studies.

Collection Designs

Ask your monitoring objective, what questions are you trying to answer with this bug collection? What are you trying to learn? For example:

- If you intend to demonstrate the influence of an impoundment on benthic diversity, then collect benthic macroinvertebrates above and below the impounded reach.
- If you are interested in testing the River Continuum Concept, then collect benthic macroinvertebrates along the headwaters, midreach, and lower reach of a river system.
- You could also compare what the Sequential Comparison Index (SCI), a qualitative index, indicates about water quality with the Water Quality Index.

Choose the time of year, station location and sample frequency that will best answer your questions.

Optional RW Macroinvertebrate Method Supplies

1. A three-foot-long net made of screen-door-mesh should be used. This provides a consistent mesh size since there is only one size mesh for screen door. You may use the metal or nylon version. Nylon is more “user-friendly.” If you wish to capture a “different” size macroinvertebrate, make a second net with a mesh size less than 0.5 mm.

2. Place the net in the water riffle, not pool habitat. Depending on flow, start about 4-5 feet upstream from the net, kick and disturb the substrate (bottom of the river) moving downstream to the net. If debris is floating past net, start closer. The kicking should last one minute for consistency. DO NOT sample if the river is deeper than 24 inches for safety reasons.

3. Pull the net out of the water in such a manner that the bugs on the net are not swept downstream from the current. Put the net on the bank and pick all sizes and colors of bugs from the net for 30 person minutes—two people picking for 15 minutes equals 30 person minutes. This is also for consistency.

4. Complete the sequential index. If you are making a reference collection, store the bugs in 70 percent ethyl alcohol (ethanol) or Everclear alcohol diluted with river water. Try not to use isopropyl alcohol, the bugs become rubbery over time.

5. As a rule of thumb, you should complete at least three “kicks” or “nets” per station (transect) in riffle habitat to collect a representative sample. The more you do the more representative your sample will be. If the stream is wide enough you can do three kicks across the river. If the river is not wide enough, do your kicks in an upstream fashion: kick one, move upstream; kick two, move upstream; and kick the third.

6. Macroinvertebrates vary with season. They emerge at different times of the year filling unique niches. Because of seasonal variation you should try to sample three times a year. Refer to the biological calendar for those times of year. The calendar considers the school schedule.

7. If you want to identify the macroinvertebrates further than the sequential index use the data sheets provided. These data sheets can be transferred to the computer.

8. Identification can be taken a step further by completing the trophic level (functional feeding analysis). If the bugs are identified past the family level the tropic level (functional feeding group) can be determined. Functional feeding group or trophic level refers to how a bug "captures" its food, for example shredding detritus, filtering the water, gathering detritus, or preying on other bugs. This information can be related to the River Continuum Concept, physical habitat, and the riparian zone. There is a percent functional feeding group summary table included for you.

9. The percent composition of each family can also be computed and provide valuable information. Does one family dominate? Are the three sensitive families (mayfly, stonefly, and caddisfly) well represented? If not, why?

It is suggested that you use *An Introduction to the Aquatic Insects of North America* by Merritt and Cummins [1] for insect identification. It teaches the students how to use a key and has interesting information about bugs (life cycles, habitats, unique features, etc.) in addition to the identification key.

[1] Merritt R.W. and K.W. Cummins, 1984. *An Introduction To The Aquatic Insects of North America*, Second Edition. Kendall/Hunt Pub. Comp. Dubuque, Iowa. (Available in soft or hard back).

Sequential Comparison Index

"The Sequential Comparison Index" is a simple method for non-biologists to estimate relative differences in biological diversity (Cairns, et al. 1968)." This Index, like other diversity measures, assumes that reduced diversity is an indication of pollution. Reduced diversity may also be related to land uses such as impoundments and urbanization, or to stream order. When estimating relative differences in benthic diversity it is important to match approximately such physical variables as flow, bottom substrate, and amount of shading.

a. Empty the bugs from the net, or pick from a net and place in a white pan.

b. Ice cube trays help a lot here, but are not necessary; a white trash bag demarcated will work. Randomly pick an organism, place it in the first ice cube tray or delineated square. Pick the next organism and compare each organism with the preceding one. If the second organism is like the first organism, place it with it, if it is different place it in another cube or square. Pick the third organism and compare with the previous two, if it is like either of them, place it there or place it in a different cube or square, repeat until all organisms are placed.

c. Calculate the diversity index (DI):

DI = number of runs (number of ice cube squares = 8 = 0.57
number of organisms (total individuals) 14

Ind Org	x	x	x	y	z	z	a	b	f	f	h	h	r	r
Run			1	2		3	4	5		6		7		8

d. The greater this diversity index value, the greater the diversity and the better the water quality. The SCI runs from 0 to 1.0, with a value of 1 representing the greatest diversity. (General Water Quality Rating: 0-0.30= Poor; 0.31-0.60= Fair; 0.61-1.0 = Good.)

e. Each group in class can calculate a diversity index and these may be averaged for a particular station. The higher the ability the more sophisticated the lumping should be. For example HS students can lump species of mayflies, stoneflies, caddisflies whereas elementary students might just lump major families.

f. 15 organisms is a good number to work with, groups may continue until they have "processed" 50 organisms if they have 50 organisms in their sample and if there is enough time or complete the entire pan. For comparison purposes the same collection method, time of kick and number of organisms needs to be consistent between samples.

Suggested Equipment:

a. White enameled pans, white trash bags or steel vegetable dishes.
b. Quart Mason® type jars for collecting live material to be placed in aquariums.
c. Turkey basters (used to pick up small aquatic organisms).
d. Forceps
e. Meter stick (used for depth measurements)
f. Wire cloth or hardware cloth can be fashioned into a hand screen and various items or even window screen stretched between two pieces of wood.
g. Vials for collecting aquatic organisms.
h. Dissecting scopes.
I. Vegetable brush or soft toothbrush.
j. Small paint brush.
k. Buckets.
l. Hip waders (not essential, but a good idea).
m. Nets, dip or 3 x 3 foot screen (can be homemade).

Sugaring for Burrowing Aquatic Organisms

Swirling a large bucket 20 times or so as demonstrated in the RW macroinvertebrate method brings bugs to top. If you use a method that employs a different net that you need to empty into a bucket, sugaring can help find the organisms. To determine the concentration and distribution of aquatic organisms in stream sediments, a technique that could be used is called, “sugaring”.

Samples of stream sediments are placed in separate pans and water is added to cover the material by several inches. Saturating the water with sugar changes the density of the water and the lighter aquatic organisms will float to the surface. The organisms can then be identified into general categories and returned to the stream.

If specific identification is needed, a preservation technique is to add formalin to the water covering the sediments in the pan (10 percent formalin solution) to replace the body fluids of the aquatic organisms with formalin. After several hours pour off all liquids in the pan and replace with water. The lighter preserved organisms will float to the surface of the water as the sediments are stirred and can be removed and identified.

Sequential Comparison Index for Macroinvertebrates

Station Name_____ Date of survey ___/___/___ Time: _____

River_____ School_____

Station Description _____

Total number of samples_____ Page _____ of _____

1. Sample____ of____ A. Number of runs____ B. Number of organisms____ Diversity Index (DI) = A/B DI=____/____=_____	2. Sample____ of____ A. Number of runs____ B. Number of organisms____ Diversity Index (DI) = A/B DI=____/____=_____
3. Sample____ of____ A. Number of runs____ B. Number of organisms____ Diversity Index (DI) = A/B DI=____/____=_____	4. Sample____ of____ A. Number of runs____ B. Number of organisms____ Diversity Index (DI) = A/B DI=____/____=_____
5. Sample____ of____ A. Number of runs____ B. Number of organisms____ Diversity Index (DI) = A/B DI=____/____=_____	6. Sample____ of____ A. Number of runs____ B. Number of organisms____ Diversity Index (DI) = A/B DI=____/____=_____

Station Average DI = Sum of DIs divided by number of DIs = _____
(This average is for pages _____ of _____, Samples _____ through _____)'

Data recorded by_____ Date recorded _____

Optional Macroinvertebrate Data Sheets

Introduction

Macroinvertebrate data collected from the required RW method (modified D kit net) can be further analyzed using these data sheets. The optional RW macroinvertebrate collection method (3 x 3 foot screen door net) can also use the following data sheets. Comparison from following data sheets require the same collection and laboratory procedures. These data sheets provide additional means of displaying, viewing and analyzing your bug data. The following is an overview of what is offered.

Macroinvertebrate Summary Data Sheet

Once a species lists is completed, the functional feeding group (trophic level) of each species or taxa (depending on how far you identified your collection) can be recorded on the Functional Feeding Group Analysis data sheet. You can determine each species functional feeding group from Merritt and Cummins2 (1984) identification book. This book is recommended but others may work as well. If you do not have this book and identify your collection further than order, notify Barb Horn at the DOW to send you the functional feeding descriptions from Merritt and Cummins. This book is available from Barb Horn to "check out."

The Functional Feeding Group Data Sheet provides a visual summary of the functional feeding group analysis. It is a table you complete based on your identification and research. Do the functional feeding groups you found, fit the River Continuum Concept for the stream reach they were collected? Do you have a dominance of predators? Are you in stream order 1-3 and have no shredders?

Species Composition Summary Data Sheet. This data sheet is a table that records percent composition for the major taxonomic groups. The data sheet table enclosed is an example; you can make your own list and percent composition just of the species you collected. Calculate your EPT index, the percent of mayfly, stonefly and caddisflies.

[2]Merritt R.W. and K.W. Cummins, 1984. *An Introduction To The Aquatic Insects of North America*, Second Edition. Kendall/Hunt Pub. Comp. Dubuque, Iowa. (Available in soft or hard back).

Macroinvertebrate Functional Feeding Group Analysis

Station Name _____ Station Number _____

River _____ Date of survey ___/___/___ Time: _____

School _____

Station Description _____

Functional Feeding Group	Sample				
	1	2	3	4	5
Shredder					
Collector Grer					
Collector Filterer					
Scraper					
Predator					

Comments:_____

_____

_____

Data recorded by_____ Date recorded _____

Percent Composition Macroinvertebrates by Major Taxonomic Groups

Station Name_____ Station Number _____

River_____ Date of survey ___/___/___

Station Description_____

Taxa	Sample				
	1	2	3	4	5
Ephemeroptera (May fly)					
Plecoptera (Stone fly)					
Trichoptera (Caddis fly)					
Coleoptera (Beetles)					
Diptera (Crane flies)					
Chironomidae (Midges)					
Odonata (Dragonflies)					
Hemiptera (True bugs)					
Arachnida (Water mites)					
Turbellaria (Flatworm)					
Oligochaeta (Earthworm)					
Hirudinea (Leeches)					
Gastropoda (Snails)					

Comments_____

_____

Data recorded by_____ Date recorded _____