Final Report for CHRF Project: River Watch Macroinvertebrate Sampling

Program Summary

Rivers of Colorado Water Watch Program (**River Watch**) was created in 1989 with the philosophy of training private and public school teachers and their students to collect and analyze river samples. This was an ideal fit since schools will always be in communities and teachers always need to teach concepts related to river ecology. The program began with two primary goals that remain steadfast today. First, to provide a hands-on experience for individuals to understand the value and function of the river ecosystem and second, to collect quality aquatic ecosystem data over space and time to be used for the Clean Water Act and other water quality decision-making processes.

River Watch has achieved these goals and continues to grow with the dedication and commitment from thousands of volunteers. The program started with six schools on the Yampa river and grew to cover all watersheds in Colorado and 350 schools. Since 1989 we have involved over 60,000 individuals in Colorado, provided data on 3,000 stations covering over 300 rivers. In 2009, there were approximately 119 different organizations actively participating in the program. Each volunteer group receives the training, support and supplies needed to monitor their respective rivers and provide consistent and accurate data. A QA/QC plan is in place to ensure the quality of the data collected, and a staff member visits each group once a year in order to provide one on one support and technical assistance. Thanks to funds from the Healthy Rivers grant, we have expanded our biological assessment and physical habitat portion of the program.

Our program is dependent on our volunteers. Volunteers agree to monitor on a monthly basis. Samples are collected which the volunteers analyze for hardness, alkalinity, dissolved oxygen, pH and temperature. Additional samples are collected to be analyzed for total and dissolved metals, which include Al, As, Ca, Cd, Cu, Fe, Mg, Mn, Pb, Na, K, Se and Zn. Twice a year volunteers collect nutrient samples that are analyzed by for ammonia, chloride, sulfate, total suspended solids, total phosphorous, nitrate and nitrite. **Some volunteers perform one physical habitat assessment to accompany their annual macroinvertebrate sample which sent to an outside lab for identification.**

Quality assurance is essential in the program operation and in the use of the data. Each volunteer group performs their sampling and analysis according to a uniform set of guidance documents. Quality assurance checks are performed regularly throughout the year and include an annual site visit to each volunteer group to ensure the volunteers are performing up to our standards and even our RW lab is checked

through a series of spikes, lab blanks and duplicates to ensure the highest quality data outputs.

River Watch data is stored on an internet server and can be accessed by anyone. All the data is reviewed and validated by the Division of Wildlife before it is made public. The high quality River Watch data is currently utilized by the Water Quality Control Commission, the Colorado Division of Wildlife, and many grass roots level watershed groups in the state for the management of Colorado's waters.

Currently, the **River Watch** program operated by the non profit 501©3 Colorado Watershed Assembly in partnership with the Colorado Division of Wildlife. Our mission is to work with voluntary stewards to monitor water quality and other indicators of watershed health, and utilize this high quality data to educate citizens and inform decision makers about the condition of Colorado's waters. This data is also used in the Clean Water Act decision making process.

Project Summary

River Watch applied for and received funding to improve our macroinvertebrate sampling component of the program. While we have anywhere from 80-120 active groups a year, not all groups are equipped to collect the annual macroinvertebrate sample. The River Watch program supports benthic macroinvertebrate monitoring by providing volunteer groups with standard RW sampling equipment. Monitoring macroinvertebrates is particularly beneficial to a comprehensive watershed monitoring program because it can help characterize ecosystems and identify actual aquatic life impairments that chemistry alone cannot. Macroinvertebrates are a key indicator of watershed health. Our goal is to expand and improve the macroinvertebrate portion of the program and through grant money from the CHRF, we were able to purchase 15 new collection kits and have samples analyzed.

River Watch received $18073 in funds from the CHRF to improve our macroinvertebrate portion of the program and the money was used in the following manner:

1. ***Provide equipment and training to our new River Watch volunteers on macroinvertebrate sampling techniques.***

In addition to the seasoned groups trained in River Watch macroinvertebrate collection techniques, we were able to train an additional 25 new volunteer groups this fall using equipment purchased through this grant. We plan on training even more new groups in 2010. *Attached are our 2008 protocols. These protocols are subject to change with the new methods being considered by the CDPHE.

2. ***Provide professional analysis and public access to the data.***

We used an outside lab to provide us macroinvertebrate analysis and have just received the data back. The Division of Wildlife is working on validating the data, and once this data is validated, it will be uploaded and available on our website. The

monies provided from this grant were instrumental in helping to identify a taxon not yet officially recorded in the State of Colorado. *Attached is the 2009 summary from our macroinvertebrate analyst.

3. Upload macroinvertebrate data to CDSN and STORET.

River Watch is planning to upload the new macroinvertebrate data to both the CDSN (Colorado Data Sharing Newtork) and the USEPA STORET database. Both of these uploads are planned for May 2010. We are also working to have this data accessible to all interested parties through our River Watch website as well at http://wildlife.state.co.us/LandWater/Riverwatch/Data/

Timberline Aquatics, Inc.

Memo

To: Michaela Taylor, RW Program Manager

From: David E. Rees

Date: 4/12/2010

Subject: Summary of QA/QC and notes for 2009 samples

QA/QC

Macroinvertebrate Sorting and Identification

The sorting process involved separating macroinvertebrates from debris in each sample. Macroinvertebrates were picked from a random number of grids until 300 individual macroinvertebrates were obtained or exceeded. Any large and rare taxa in the macroinvertebrate sample were picked to 100%. As part of the QA/QC protocols at Timberline Aquatics, Inc., all sorted samples were checked by an additional taxonomist.

Benthic macroinvertebrates were identified to a taxonomic level based on examples of previous work from this study. As part of the QA/QC for identifications, portions of samples 326, 433, 529, 570, 751 and 2800 were checked for accuracy by Dr. Boris Kondratieff at Colorado State University.

Samples that were received by Timberline Aquatics, Inc. exhibited some variability in their condition. Some samples were not preserved well, which resulted in the partial decomposition of some taxa. Please emphasize to the volunteers the importance of proper preservation. Additional care in macroinvertebrate collection and preservation usually results in an increase in consistency and reliability in the data.

New Specimens

Several new taxa were added to the River Watch collection during 2009. Most of these came from high elevation sites in the upper Clear Creek drainage. These taxa were known from Colorado, but had not yet been collected as part of the River Watch program. One taxon (*Maccaffertium terminatum*) that had not been officially recorded in the State of Colorado was collected at site 529 (White River). Specimens were saved at the Colorado State University Entomology Museum. Rangely High School and River Watch received credit for this collection. Representatives of all new taxa were added to the River Watch reference collection.

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Macroinvertebrate Collection and Physical Habitat Assessment Instructions

Overview

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 should be collected at **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 species level.

2. RW staff will identify the station you will sample and send you supplies. Ten percent of participating groups will be chosen to provide a quality control sample.

3. A physical habitat and analysis **must** be completed with each macroinvertebrate sample. The habitat analysis describes the bug's residence and will record changes in aquatic environment over time. If you are asked to collect a macroinvertebrate sample, you need to submit a physical habitat assessment along with your collection. If you are not set up to collect a macroinvertebrate collection, a physical habitat analysis could be completed without a macroinvertebrate sample.

4. Full instructions and datasheets for both macroinvertebrate collection and physical habitat assessment are in this manual. A video/picture training is available via the RW staff that illustrates many of the steps and definitions.

5. Each bug collection or habitat assessment is a sampling event, given a unique combination of station number, date and time. If possible macroinvertebrates should be collected during one of your monthly water quality sample events. If water quality samples are collected at the same time, all these samples will have the same sample identifier.

6. Ship macroinvertebrates, data sheets and chain of custody within two weeks of 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 (**not** your River Watch bucket); a large whit bucket works best.
- 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
- A clipboard

Field Preparation Overview

1. Review macro collection video or instructions.

2. Retrieve blank data sheets and complete the information above Part 1. Check all appropriate boxes. If you have been chosen to collect a QA/QC sample, check that box also. Be sure to check box for either Rocky or Sandy Substrate. See step 2 in next section to determine if your site is Rocky or Sandy.

3. Using a permanent marker (sharpie), label each macroinvertebrate sample bottle with river name, station name, station number, organization, time and date. Fill out your labels for the inside of the jars using a pencil.

4. Gather gear from list above.

5. If you are collecting water quality samples, prepare to do so BEFORE any macroinvertebrate sampling. **This method involves disturbing the substrate and could contaminate a surface water sample.**

Choosing and Recording Your Sampling Site

1. The first step in your collection is to determine the general area where you are going to collect a sample. 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, not the slow deep pool areas (see the Rocky Substrate Collection section below for further discussion). For SANDY substrate sampling, look for a segment you can sample in multiple habitats (i.e. submerged vegetation, large woody debris, vegetated banks, water column and substrate) for 4 full minutes.

    - 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.
    - Choose reaches with habitats that are representative of the entire stream (i.e. riffles that looks like all the other riffles in the area).
    - You may have to walk around the entire are of your 200 foot reach to find habitats to sample, they do not need to be right next to each other.

2. Next, determine if your segment is classified as rocky or sandy. Take a look at your sample site. Is the bottom of your stream (where you will be collecting your sample) more that 50% sand? Or is does your site consist mostly of rocks and boulders? If your site is mostly sandy, then you are sampling a sandy substrate. If your site is mostly rocks and boulders, then you are sampling a rocky substrate.

3. On part 1 of the Macroinvertebrate collection data sheet, **fill in the top portion with information about your collection. Be sure to include station number, date and time. This is very important.** Next, draw a map of the 200 foot section, scanning 100 feet above and 100 feet below the area sampled, including the riparian zone (the area immediately adjacent to the stream channel). 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 area and the number of the kick (1-4) inside the square of where you will be sampling. **SANDY substrate folks**: include riparian and instream vegetation, sandbars, etc.

4. On part 2 of the data sheets, **Average Depth Profile of representative sample transect**, there is a location for recording the longitudinal profile of the stream reach. Record this information (see data sheet section below).

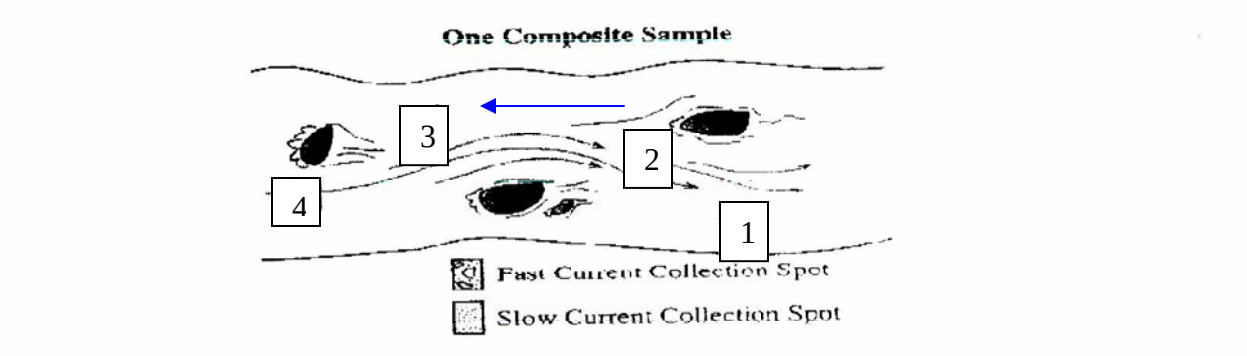
5. Once these two sections of the Macroinvertebrate collection datasheet are complete, it is time to collect your sample.

Rocky Substrate Collection

Remember, it's best to collect your water quality samples and field parameter at the same time as your macro collection. 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 and field parameter 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 4conduct longitudinal profile.

A rocky substrate collection is made up of four "kicks" in four different locations. You will need to collect in two different riffles and two different pools. A riffle is a shallow, fast flowing, usually bubbly area (flowing over rocks/pebbles) and a pool is a deeper area where the water moves much slower. This is a composite collection, so your sample will be contained in the net until the final kick is finished. Be VERY careful not to lose any part of your sample in between changing locations.

1. Review the four specific sites where you will collect a kick net sample. (These should be the four boxes on part 1 of your data sheet.) You want to work your way from downstream to upstream to minimize disturbing the stream substrate. Look at the diagram below. If the water is flowing right to left, you should begin your collection at the furthest point downstream (box 1) and end collecting the final sample upstream (box 4).



2. Approach the most downstream sample area for the first kick (box 1 above). Visualize an area on the stream bottom that is equivalent to about a 3 x 5 foot rectangle (1 x 1 .7 meter) area. Measure this kick area by laying the net down 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 kick area.

3. Complete the **Part 3 Substrate Composition** data sheet for kick #1 for Rocky Substrate, columns 1 and 2. All of these questions focus on the kick area being sampled only. You will need to circle "**Rocky"** on the top part of the data sheet and in column 1 circle whether this was a riffle or a pool collection.

4. Columns 1 and 2 address inorganic substrate composition and organic composition of the habitat sampled. For column 1, inorganic component, use a ruler to measure various substrate sizes and the size guides on part 3 of the data sheet. KICKER

estimates the percent of each substrate size and the RECORDER records the estimate in the appropriate shaded box. 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. For column 2 organic substrate components, estimate the percent of the total rectangle that is covered by various types of organic matter. This may not add up to 100%; it is dependant upon how much organic material is covering the substrate (i.e. the kick area described above containing 10% leaf litter).

5. With the net open to oncoming water and at the most downstream position, place the net in the stream, 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 where 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 the net is set and the water is flowing through the net, you are ready to conduct your first kick.

6. Conduct kick #1:

    a. One person will hold the net open downstream.
    b. Second person will kick and disturb from upstream to downstream. Make sure this person is holding the brush.
    c. A third person will time for 60 seconds.
    d. A fourth person 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 toes 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. Recorder records total time “kicked” in this sample area.
    i. Record the average depth of the sample area in column 2 of the Substrate composition data sheet.
    j. Once the kick is complete, carefully pick up the net and move to sample collection area #2 as shown on your drawn map.

7. Repeat steps 1 through 6 for kicks 2, 3 and 4. Be careful when you raise the net out of the water between each kick so that no organisms are lost. Carry the net to each sample location; do not remove the bugs in-between kicks. After all sites are sampled, process the sample as described below.

8. Once all kicks are complete. Fill out the physical habitat data sheet. Habitat terms and descriptions are provided in the data sheet instructions.

Sandy Substrate Collection

Remember, it's best to collect your water quality samples and field parameter at the same time as your macro collection. 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 and field parameter 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 longitudinal profile.

1. Review the specific sites where you will collect a kick net sample. (These should be the boxes on part 1 of your data sheet.) You want to work your way from downstream to upstream to minimize disturbing the stream substrate. Sandy substrate tends to shift and doesn't have the large interstitial space many bugs prefer. In this habitat, the bugs will be in the water just above the substrate, or in aquatic vegetation in the stream amongst debris or along the banks. Identify **ALL** potential habitat types (vegetated banks, submerged vegetation, snags/debris, water column or sandy substrate) in the segment and plan to sample each habitat minimizing 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. Identify the variety of habitats along the 200 foot section and divide 4 minutes (i.e. 5 habitats/4min = 48 seconds each) into those habitats. This is a composite collection, so your sample will be contained in the net until the final collection is finished. Be VERY careful not to lose any part of your sample in between changing locations. Move in a downstream to upstream direction with minimal or no wading if possible.

    - 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 sampled first 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/or 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 when can.

- 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.

2. Complete the **Part 3 Substrate Composition** data sheet for kick 1 for Sandy Substrate, columns 2 &3. All of these questions focus on the kick area being sampled only. You will need to circle "**Sandy"** on the top part of the data sheet and input the number of seconds you will sample at this location.

3. Columns 2 address organic composition of the habitat sampled. For column 2 organic substrate components, estimate the percent of the total rectangle that is covered by various types of organic matter. This may not add up to 100%; it is dependant upon how much organic material is covering the substrate (i.e. the sampled area described above containing 10% leaf litter).

4. Column 3 is for the habitat sampled. Determine which habitat is being sampled and input the number of seconds the sample is collected and the habitat type.

5. Approach the most downstream sample area for the first collection. Visualize an area on the stream bottom that is equivalent to about a 3 x 5 foot rectangle (1 x 1 .7 meter) area. Measure this sampling area by laying the net down 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 kick area. This will be the area that you will probe, dip and collect your sample in. Depending on the number of sample collection sites, you will have an allotted time to kick, sweep and disturb each area. Note mental markers as to the beginning and end of this rectangle.

6. Conduct sampling #1.

   a) One person uses the net to collect the sample, starting downstream. Make sure to sample only in your 3 x 5 collection area.

   b) Second person assists with larger debris and substrate. Make sure this person is holding the brush.

   c) A third person is the time keeper. Make sure to sample for allotted time only.

   d) A fourth person fourth is needed to record amount of time spent sampling the habitat in part 3, column 3 of the data sheet.

   e) Sample the area using the techniques described above. Do not move 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 included in the net and examined later for clinging bugs.

f) Once the collection is complete at this location, carefully pick up the net and move to the next sample location as shown on your drawn map.

g) Repeat steps 1 through 3 for remaining habitats. Be careful when you raise the net out of the water between each collection so that no organisms are lost. Carry the net to each sample location; do not remove the bugs in-between sampling. After all sites are sampled, process the sample as described below.

7. Once all sampling areas are complete, fill out the physical habitat data sheet. Habitat terms and descriptions are provided in the data sheet instructions.

8. Repeat steps 1 through 6 for all remaining sampling areas. Raise the net all together out of the water between each kick so that no organisms are lost from the net. Carry the net to the next sample location. Be careful not to lose any bugs when placing net in the stream. After all sites are sampled, process the sample as described below.

Sample Processing

The goal of sample processing is to get all the macroinvertebrates into the sample jar from the kick net 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. 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 collection sites, carry the net to the shore. Fill the bucket a 1/2 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. Once you have all sample and debris in the bucket, separate the organisms from the debris by "swirling" the sample in the bucket. Add more water if you need to and really swirl! Swirling needs to be aggressive enough to dislodge clinging organisms. This will take approximately 10-20 swirls to make sure all organisms are dislodged. The lighter organisms and debris will rise to the top of the water and the heavier sediment will sink to the bottom. Look for bugs to float to the top. Pour off the top water and floating material into the sieve, leaving the sand, rocks, gravel, large debris and rocks in the bucket.
4. The sieve should contain bugs and small debris. Carefully examine the large debris (too big to fit in the sample container) to see if there are macroinvertebrates attached. If there are no bugs on the debris, discard the debris. Let the water drain from the sieve as much as possible and GENTLY put all the remaining debris and bugs from the sieve into the sample collection jar filled with alcohol. You may have to pick some lingering bugs from the sieve using the forceps. BE VERY CAREFUL NOT TO SMASH THE BUGS WHEN TRANSFERING THEM TO THE ALCOHOL FILLED CONTAINER.
5. Repeat swirling until lighter material and bugs no longer rise to the top. 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.
6. Repeat swirling/picking bugs in small manageable batches until all debris has been processed.

7. 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.

8. 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.

9. If you have been selected to collect a QA/QC sample, you will have received 4 jars, some 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, large sticks, rocks, sand and twigs) is placed in the other QA/QC 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 whereas your "normal" bug sample will have a similar label without the QA line checked.

10. 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.

11. Make sure your samples are labeled correctly. The collection containers should include sample #, station #, organization, date and time on the outside of the bottle. Place the other label inside the macroinvertebrate samples (these labels are located on the last page of this sampling protocol). The inside label is smaller and requests collector's name. **These labels need to be filled out in pencil.**

12. **The evening of sampling, take your collection bottle and carefully decant the first third the alcohol and then pour in fresh alcohol from the second bottle. 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. **Ensure that you have placed a label** located on the last page of this sampling protocol **in the sample**.

13. Ship or deliver the sample 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:

- School, 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 use.
- Circle the number of "kicks" performed. This should total four.

Part 1 of the Data Sheet

Part 1a

Draw a picture of the reach in which you sampled. You want to diagram from a birds eye view of the 200 foot collection area 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 represent which kick it is.

1e Describe where the station is relative to your water quality station **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 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 macroinvertebrate sample. As recording this data may disturb the very habitat you are sampling, this measurement should be taken after the macro invertebrate 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 bankfull and wet water measurements can be done as part of this step and the bankfull measurement can be recorded in **Part 4, Section F**. Measure bankfull 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, column 2 addresses and 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 and odors 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, 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 for get 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 sample collection site. For each bank check the predominant (top 1 or 2 most prevalent types) land uses 300 feet 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 or 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 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, stock or people access, roads and crossings, clearing 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

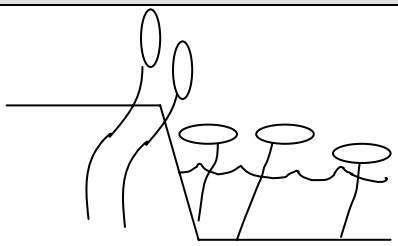
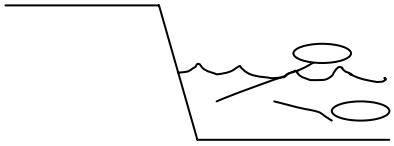
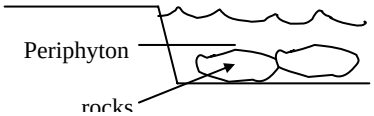
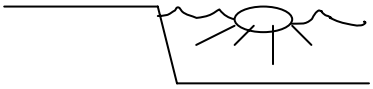
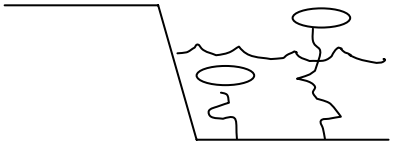
This section describes the vegetation type on each bank. Riparian vegetation along the bank and transitioning into the upland ecosystem provide food and habitat for a variety of animals in some aspect of their life cycles. It can also provide a migration corridor, soil stability, water quality filter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

In this section, please describe the predominant vegetation type for each bank. You don't need to know the species, just the type (tree, grass, shrub/bush or forbs) 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, 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 "came" loose and is 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, the most common in Rocky Mountains Stream.
- 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 foot 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, or 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

(page 1)

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

River_____ Station number:_____

Group (School)_____ RW Net / Other_____ Kit # _____

# of kicks: 1 2 3 4

Substrate type **Rocky** ☐ **Sandy** ☐

Part 1 **QA Sample Collected** ☐

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.

e. If not at water quality station, describe (distance from water quality station, etc.):

_____

_____

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: (UNIT=_____) Place transect on diagram above.

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_____

Part 3 Substrate Composition kick/sample area #1:
Sampling method: Circle one: **Rocky (Columns 1 and 2)** **Sandy (Column 3)**

Kick/sample area #1 time sampled _____ seconds

1			2			3			
Fast riffle / Slow Riffle (circle one) 1.5-2.5 ft/sec 0.5-1.5 ft/sec			Average Depth of sampled area =_____						
Inorganic Substrate Components (Rocky=should add to 100%)			**Organic Substrate Components (Rocky and MAYBE Sandy Substrates)**			**Habitat sampled (Sandy =should add to 100%)**			
Substrate Type	Diameter	% Composition in sample	Substrate Type	Describe Characteristics	% Composition in sample	Habitat Type	Time sampled ___seconds	Describe Characteristics	% Composition of sample
Bedrock			Detritus	Sticks, wood, coarse plant material, CPOM		Vegetated Banks			
Boulder	>256mm, 10inches					Submerged Vegetation			
Cobble	64-256mm, 2.5-10”		Muck-Mud	Black, very fine organic material, FPOM		Snags/Debris			
Gravel	2-64 mm, 0.1-2.5“					Water Column			
Sand	0.06-2 mm, Gritty					Sand/Subs			
Silt	0.004-0.06mm		Marl	Grey, shell fragments					
Clay	<0.004, slick/slimy								
	TOTAL %					**TOTAL TIME**		**TOTAL %**	
Rocky Only			**Rocky and MAYBE Sandy (if substrate part of sample habitat)**			**Sandy Only**			

Part 3 Substrate Composition kick/sample area #2:

Sampling method: Circle one: **Rocky (Columns 1 and 2)** **Sandy (Column 3)**

Kick/sample area #2 time sampled _____ seconds

1			2			3			
Fast riffle / Slow Riffle (circle one) 1.5-2.5 ft/sec 0.5-1.5 ft/sec			Average Depth of sampled area =_____						
Inorganic Substrate Components (Rocky=should add to 100%)			**Organic Substrate Components (Rocky and MAYBE Sandy Substrates)**			**Habitat sampled (Sandy =should add to 100%)**			
Substrate Type	Size	% Composition in sample	Substrate Type	Describe Characteristics	% Composition in sample	Habitat Type	Time sampled ___seconds	Describe Characteristics	% Composition of sample
Bedrock			Detritus	Sticks, wood, coarse plant material, CPOM		Vegetated Banks			
Boulder	basketball and larger					Submerged Vegetation			
Cobble	tennis ball to basketball		Muck-Mud	Black, very fine organic material, FPOM		Snags/Debris			
Gravel	ladybug to tennis ball					Water Column			
Sand	Gritty to ladybug					Sand/Subs			
Silt	Not gritty but silica based		Marl	Grey, shell fragments					
Clay	slick/slimy								
	TOTAL %					**TOTAL TIME**		**TOTAL %**	
Rocky Only			**Rocky and MAYBE Sandy (if substrate part of sample habitat)**			**Sandy Only**			

Substrate (page 4)

Part 3 Substrate Composition kick/sample area #3:

Sampling method: Circle one: **Rocky (Columns 1 and 2)** **Sandy (Column 3)**

Kick/sample area #3 time sampled _____ seconds

1			2			3			
Fast riffle / Slow Riffle (circle one) 1.5-2.5 ft/sec 0.5-1.5 ft/sec			Average Depth of sampled area =_____						
Inorganic Substrate Components (Rocky=should add to 100%)			**Organic Substrate Components (Rocky and MAYBE Sandy Substrates)**			**Habitat sampled (Sandy =should add to 100%)**			
Substrate Type	Size	% Composition in sample	Substrate Type	Describe Characteristics	% Composition in sample	Habitat Type	Time sampled ___seconds	Describe Characteristics	% Composition of sample
Bedrock			Detritus	Sticks, wood, coarse plant material, CPOM		Vegetated Banks			
Boulder	basketball and larger					Submerged Vegetation			
Cobble	tennis ball to basketball		Muck-Mud	Black, very fine organic material, FPOM		Snags/Debris			
Gravel	ladybug to tennis ball					Water Column			
Sand	Gritty to ladybug					Sand/Subs			
Silt	Not gritty but silica based		Marl	Grey, shell fragments					
Clay	slick/slimy								
	TOTAL %					**TOTAL TIME**		**TOTAL %**	
Rocky Only			**Rocky and MAYBE Sandy (if substrate part of sample habitat)**			**Sandy Only**			

Part 3 Substrate Composition kick/sample area#4:
Sampling method: Circle one: **Rocky (Columns 1 and 2)** **Sandy (Column 3)**

Kick/sample area #4 time sampled _____ seconds

1			2			3			
Fast riffle / Slow Riffle (circle one) 1.5-2.5 ft/sec 0.5-1.5 ft/sec			Average Depth of sampled area =_____						
Inorganic Substrate Components (Rocky=should add to 100%)			**Organic Substrate Components (Rocky and MAYBE Sandy Substrates)**			**Habitat sampled (Sandy =should add to 100%)**			
Substrate Type	Size	% Composition in sample	Substrate Type	Describe Characteristics	% Composition in sample	Habitat Type	Time sampled ___seconds	Describe Characteristics	% Composition of sample
Bedrock			Detritus	Sticks, wood, coarse plant material, CPOM		Vegetated Banks			
Boulder	basketball and larger					Submerged Vegetation			
Cobble	tennis ball to basketball		Muck-Mud	Black, very fine organic material, FPOM		Snags/Debris			
Gravel	ladybug to tennis ball					Water Column			
Sand	Gritty to ladybug					Sand/Subs			
Silt	Not gritty but silica based		Marl	Grey, shell fragments					
Clay	slick/slimy								
	TOTAL %					**TOTAL TIME**		**TOTAL %**	
Rocky Only			**Rocky and MAYBE Sandy (if substrate part of sample habitat)**			**Sandy Only**			

Physical Habitat

Part 4 Overall area physical habitat

Habitat Features	A	**Indicate % of each habitat type in reach (50 m above/below sample):** ☐ Cobble____% ☐ Sand_____% ☐ Snags_____% ☐ Vegetated Banks_____%			
Watershed Features	B	**Predominant Surrounding Land use Right Bank:** **Left Bank** ☐ Forest ☐ Dense housing ☐ Forest ☐ Dense housing ☐ Field/pasture ☐ Sparse housing ☐ Field/pasture ☐ Sparse housing ☐ Irrigated ☐ Commercial ☐ Irrigated ☐ Commercial ☐ RR/hwy ☐ Industrial ☐ RR/hwy ☐ Industrial ☐ Other_____ ☐ Other_____			
Localized Erosion	C	**% Bare Bank Soil** ☐ 80-100% ☐ 10-39% ☐ 40-79% ☐ 0-9%	**Erosion Amount** ☐ **extensive** ☐ **localized/occ** ☐ **some evidence** ☐ **no evidence**	**Bank movement** ☐ bank failures ☐ slight ☐ mod collapses ☐ none	
Riparian Vegetation Right bank ____ ft Left bank ____ ft	D	**Indicate the dominant vegetation type:** **Right bank:** **Left bank** ☐ Trees ☐ shrubs ☐ Trees ☐ shrubs ☐ grasses ☐ herbaceous ☐ grasses ☐ herbaceous ☐ other_____ ☐ other_____ ☐ dominant species_____ ☐ dominant species_____			
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 Sample

River Name ____________________________
Station Name ___________________________
Station Number _________________________
Date _____________ Time _____________
Organization _______________________
QA/QC (circle one) Yes No
Preserved with 95% ethanol / method 1

River Name ____________________________
Station Name ___________________________
Station Number _________________________
Date _____________ Time _____________
Organization _______________________
QA/QC (circle one) Yes No
Preserved with 95% ethanol / method 1

River Name ____________________________
Station Name ___________________________
Station Number _________________________
Date _____________ Time _____________
Organization _______________________
QA/QC (circle one) Yes No
Preserved with 95% ethanol / method 1

River Name ____________________________
Station Name ___________________________
Station Number _________________________
Date _____________ Time _____________
Organization _______________________
QA/QC (circle one) Yes No
Preserved with 95% ethanol / method 1

River Name ____________________________
Station Name ___________________________
Station Number _________________________
Date _____________ Time _____________
Organization _______________________
QA/QC (circle one) Yes No
Preserved with 95% ethanol / method 1

River Name ____________________________
Station Name ___________________________
Station Number _________________________
Date _____________ Time _____________
Organization _______________________
QA/QC (circle one) Yes No
Preserved with 95% ethanol / method 1

River Name ____________________________
Station Name ___________________________
Station Number _________________________
Date _____________ Time _____________
Organization _______________________
QA/QC (circle one) Yes No
Preserved with 95% ethanol / method 1

River Name ____________________________
Station Name ___________________________
Station Number _________________________
Date _____________ Time _____________
Organization _______________________
QA/QC (circle one) Yes No
Preserved with 95% ethanol / method 1

River Name ____________________________
Station Name ___________________________
Station Number _________________________
Date _____________ Time _____________
Organization _______________________
QA/QC (circle one) Yes No
Preserved with 95% ethanol / method 1

River Name ____________________________
Station Name ___________________________
Station Number _________________________
Date _____________ Time _____________
Organization _______________________
QA/QC (circle one) Yes No
Preserved with 95% ethanol / method 1