OKLAHOMA CONSERVATION COMMISSION
WATER QUALITY DIVISION

STANDARD OPERATING PROCEDURE

MACROINVERTEBRATE COLLECTION, SUBSAMPLING, AND PICKING

1.0 PROCEDURAL SECTION

1.1 Scope and Application[19,20]

Most free flowing water bodies with acceptable water quality and habitat conditions support diverse macroinvertebrate communities in which there is a reasonably balanced distribution of species among the total number of individuals present. Macroinvertebrate community responses to environmental perturbations are useful in assessing water quality and habitat impacts. The composition and density of macroinvertebrate communities in flowing water are reasonably stable from year to year. However, seasonal fluctuation associated with life-cycle dynamics of individual species may result in extreme variation at specific sites within any calendar year. Assessing the impact of pollution generally involves comparison of macroinvertebrate communities and their habitats at sites influenced by pollution with those collected from adjacent unaffected sites.

Macroinvertebrate collections, for purposes of stream assessment, are made from the community that requires or prefers flowing (lotic) water. Reasons why this community type is sampled rather than various lentic communities include:

1. The flowing water community is routinely exposed to the average water quality of the stream;
2. The metrics used to analyze the macroinvertebrate community of streams were designed for the flowing water community;
3. The database of pollution tolerance of macroinvertebrates found in Oklahoma is much larger for lotic communities; and
4. The organisms most sensitive to water quality degradation tend to live in flowing water.

Due to these factors, looking at the flowing water community is more suitable for assessing the condition of a stream than looking at the pool community where more tolerant organisms are found, regardless of the stream's water quality.

Lotic communities require a substrate of some type to attach to. The most common substrates of this type include rocky riffles, streamside vegetation/root masses, and woody debris. Where possible, a rocky riffle should be sampled. If a rocky riffle is not present, if the riffle is of dubious quality, or if rocky riffles cannot be found at all streams of a given ecoregion, both of the other two alternate habitats (root masses and woody debris) should be sampled. At present, it appears that the streamside vegetation is superior to woody debris for macroinvertebrates, but until that is definitely established, both should be sampled. The sampling methodology for the three habitat types is included in this SOP.

Macroinvertebrate communities are constantly changing throughout the year as species emerge and new species hatch. Consequently, it is not possible to infer water quality from the invertebrate community of a stream by comparing it to a reference stream community that was collected at a different time of year. The springtime communities are especially unstable, as many of the insects that over-winter as larvae begin to emerge. By summertime, however, the insects that only have one generation per year have mostly emerged, and the insects left are ones that hatch repeatedly throughout the summer. This period of the summer when collections from different streams can be compared to each other is termed the Summer Index Period.

Fall is also a poor time to collect to be used for comparing the water quality of different streams. Many insects lay eggs in the summer, and these do not hatch until the water temperature cools down. As these insects hatch and grow large enough to see, they start appearing in collections. Since they hatch at different times and grow at different rates, collections can be very different if they are sampled at different times in the fall. Wintertime communities, on the other hand, tend to be stable. Very few insects emerge in the wintertime, and Oklahoma streams stay warm enough that the invertebrates in them remain actively growing. The wintertime period in which macroinvertebrate collections from different streams can be compared to each other is called the Winter Index Period.

1.2 Summary of Method

A modified version of EPA Rapid Bioassessment Protocol (RBPs) was adopted for macroinvertebrate collections. As stated above, the collection methods are geared toward assessing communities that require or prefer flowing water. Lotic communities require a substrate of some type to attach to. The most common substrates encountered are rocky riffles, streamside vegetation, and woody debris. All three substrates can be sampled (when available) to provide an accurate representation of the various communities in the stream. A combination of collection techniques is used for each habitat. Organisms collected from these habitats are subsampled and sent to a professional macroinvertebrate taxonomist and enumerated to genus level, when possible.

[19] Text taken directly or in part from Standard Methods (APHA, AWWA, WPCF, 1995).

[20] Text taken directly or in part from Dan Butler, Senior Biologist, Oklahoma Conservation Commission (2000)

1.2.1 Definitions

- Riffle: Any sudden downward change in the level of the streambed such that the surface of the water becomes disrupted by small waves. A riffle substrate must be composed of gravel, or cobble from 1" to 12" in the longest dimension; substrates of bedrock or tight clay are not considered suitable. If composed of gravel and sand, it must be >50% gravel.
- Streamside Vegetation: Any streamside vegetation which offers fine structure for invertebrates to dwell within or upon that receives suitable flow. Most habitat is located along undercut banks where fine roots of riparian vegetation are hanging in the water.
- Woody Debris: Any dead wood with or without bark located in the stream with suitable current flowing over it.
- Summer Index Period: **June 1** to **September 15.**
- Winter Index Period: **January 1** to **March 15**

1.3 Health and Safety Warnings

- Proper precautions should be taken when handling 100% ethanol.
  - Flammable
  - Intoxicant
  - Eye irritant

1.4 Cautions

- Stream stage must not be greater than 3 cm (~1 inch) above base flow during the collection.
- Collections must be done in flowing water.
- In no case should the Mason jar be filled more than 3/4 full of loose sample.
- There should always be enough room in the jar to have at least 5 cm (~2 inch) of free ethanol over the sample.

1.5 Interference

None

1.6 Personnel Qualification

Field personal must be trained and evaluated on sample collection technique. Sample collection is subject to approval by the QA Officer and/or the Environmental Monitoring Coordinator. Training will be done through dry run exercises in the laboratory and field to familiarize field personnel with procedures and techniques.

1.7 Apparatus & Materials

- Absolute ethanol (200 proof; ~100%)
- Clean quart size Mason jars
- New mason jar lids
- Pencil & indelible marker
- 1 m^2 kick net composed of # 30 nylon mesh
- Handheld dip net composed of #30 size nylon mesh

1.8 Instrument/Method Calibration

Not applicable

1.9 Preparation

Determine if flow conditions are suitable for collection. Samples must be collected in flowing water no greater than 3 cm (~1 inch) above the seasonal base flow. After a high flow event, 5 – 7 days should lapse before a collection is made to allow the benthic organisms to return to the preferred substrate. Furthermore, collection should be delayed for two weeks after a stream has gone from no flow (interrupted, or dry conditions) to base flow conditions.

1.10 Sample Collection

There are three possible habitat types for collection. The methods for each are described below.

1.10.1 Collection of Benthic Macroinvertebrates from Rocky Riffles

- **Suitable Substrate** - A riffle is defined as any sudden downward change in the level of the streambed such that the surface of the water becomes disrupted by small waves. For this collection method the substrate of the riffle must be

composed of gravel, or cobble from 1" to 12" in the longest dimension. Riffles with substrates of bedrock or tight clay are not suitable. If the riffle substrate is composed of only gravel and sand it must contain at least 50% gravel.

- **Where to Sample the Riffle** - Three 1 m^2 areas of the riffle must be sampled. They can be square, rectangular or trapezoidal so long as each area equals 1 m^2 in area. One should be in the fastest part of the riffle where the largest rocks and the smallest amount of interstitial sediment will generally be found. The second should be in the slowest part of the riffle, often near the edge of the stream where the smallest rocks and the greatest amount of interstitial sediment will be found. The third sample should be in an area intermediate between the first two

- **Method of Collecting the Sample** - Support a 1 m^2 kick net composed of a double layer of fiberglass window screen or a net of number 30 mesh in such a way that any organisms dislodged from the substrate will be carried into it by the current. The bottom of the net should be tight against the bottom of the stream and the current must be sufficient to insure that dense organisms such as small mollusks will be carried into the net from the sampling area. There is no definite cutoff for stream velocity in the sampling area, but if possible, riffles with average velocities of 1 foot/second or greater are preferred and should be chosen if possible.

  By kicking the substrate, vigorously agitate the substrate of a 1 m^2 area of the bed of the riffle immediately upstream of the net until all rocks and sediment to a depth of at least five inches have been thoroughly disturbed. Organisms living between and upon the rocks will have been dislodged and carried into the net by the current. Any rocks too large to kick should be brushed by hand on all surfaces. This can be done using your hands or with the aid of a brush. If a brush is used, you must be very careful to clean it after each site to prevent contamination of the next sample with invertebrates from the previous site. Continue agitation and brushing until it can be seen that the area being sampled is producing no new detritus, organisms, or fine sediment.

  At this point, rinse leaves, sticks and other large debris caught in the net in the current in a manner such that organisms on them are carried into the net. When the volume of the sample is reduced so that three 1 m^2 samples will loosely fill a 1-quart mason jar three fourths (3/4) full or less, remove all of the material from the net and place it in the Mason jar. In no case should the Mason jar be filled more than 3/4 full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. There should always be enough room in the jar to have at least 5 cm (2 inches) of free ethanol over the sample.

  Label the sample appropriately following the instructions presented in section 1.11 Sample Handling & Preservation.

1.10.2 Collection of Macroinvertebrates from Streamside Vegetation

- **Suitable Substrate** - Any streamside vegetation in current that offers fine structure for invertebrates to dwell within or upon is suitable. The vegetation being sampled must be in the current so that it offers suitable habitat for organisms which collect drifting particles or which need flowing water for other reasons. This habitat will often be found along the undercut banks of runs and bends where the fine roots of grasses, sedges, and trees, such as willow and sycamore, hang in the water.

- **Method of Collecting the Sample** - This type of sample should be collected with a dip net made of #30 size mesh material. The net should be placed around or immediately downstream of the vegetation being sampled. The organisms can be dislodged from the roots either by vigorously shaking the net around the roots or by shaking the roots by hand while the roots are inside the net.

- **Where and How Long to Sample** - Sampling should continue for **3 minutes** of actual root shaking. Do not count the time that elapses between sampling areas. Be careful to only sample roots in current. Usually, only one or two sides of a given root mass are in current. Be careful not to sample the backside of a root mass that is in still water.

At this point, rinse leaves, sticks and other large debris caught in the net so that organisms are not lost. When the volume of the sample is reduced so that it will loosely fill a 1-quart mason jar three fourths (3/4) full or less, remove all of the material from the net and place it in the Mason jar. In no case should the Mason jar be filled more than 3/4 full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. There should always be enough room in the jar to have at least 5 cm (2 inches) of free ethanol over the sample. Label the sample appropriately following the instructions presented in section 1.11 Sample Handling & Preservation.

1.10.3 Collection of Macroinvertebrates from Woody Debris

- **Suitable Substrate** - Any dead wood with or without bark in the stream is suitable as long as it is in current fast enough to offer suitable habitat for organisms which collect drifting particles or which need flowing water for other reasons. The final sample should consist of organisms collected from an even mixture of wood of all sizes and in all stages of decay.

- **Method of Collecting the Sample** - This type of sample should be collected with a dip net made of #30 size mesh material. The net should be placed around or immediately downstream of the debris being sampled. The organisms can be dislodged from the debris either by vigorously shaking the net around the woody debris or by shaking the debris by hand while the debris is inside the net. Large logs that are too big to shake should be brushed or rubbed vigorously by hand while the net is held immediately downstream.

- **Where and How Long to Sample** - Sample for total of **5 minutes** counting only the time that debris is actually being agitated. Include as many types of debris in the sample as possible. These types often include wood that is very rotten and spongy with or without bark, wood that is fairly solid which has loose and rotten bark, wood that is solid with firmly attached bark and any combination of these states. They should range in size from 1/4" to about 8" in diameter.

After sampling, rinse leaves, sticks and other large debris caught in the net so that organisms are not lost. When the volume of the sample is reduced so that it will loosely fill a 1-quart mason jar three fourths (3/4) full or less, remove all of the material from the net and place it in the Mason jar. In no case should the Mason jar be filled more than 3/4 full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. There should always be enough room in the jar to have at least 5 cm (2 inches) of free ethanol over the sample.

Label the sample appropriately following the instructions presented in section 1.11 Sample Handling & Preservation.

1.11 Sample Handling & Preservation

1. **Pack the Mason Jar Properly.** In no case should the Mason jar be filled more than 3/4 full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. There should always be enough room in the jar to have at least 5 cm (2 inches) of free ethanol over the sample.
2. **Label the Sample.** The Mason jar should be labeled on the lid using a fine tip permanent ink marker (Sharpie) as described below. In addition, a small sheet of paper (approximately 2" x 2") should be filled out with the same information written in pencil and placed in the jar.

Jar Lid & Sample Insert

Site Date
Stream Name
Waterbody ID #
Site Time
Legal Description
County
Type of sample (riffle, woody, vegetation)
Sampler's Initials

3. **Complete the Chain of Custody Form (COC).** Follow the instructions in the **Chain of Custody and Sample Labeling SOP**. A new COC should be completed for each collection episode. Each substrate collection (riffle, woody, vegetation) should occupy a separate line on the COC. There should be only one box of samples per COC, i.e., one box, one COC.
4. **Transfer samples to the Macroinvertebrate Sample Custodian.** Correctly labeled macroinvertebrate samples, along with a Chain of Custody form (COC), should be transferred to the Macroinvertebrate Sample Custodian for subsampling. The box should be conspicuously labeled with COC number. Once the samples have been received and the COC signed, the field sampler should make a photocopy of the COC for their records.

1.12 Sample Preparation and Analysis

In some instances it may be necessary to drain the liquid from the sample and add fresh 100% ethanol. This is necessary when the sample contains a large amount of algae or other material with high water content or material that will rapidly become rancid. This will help preserve the morphological integrity of the invertebrates and greatly aid in taxonomic identification.

1.12.1 Subsampling and Picking of Macroinvertebrates from field Collected Samples

The waterbody assessment procedure utilized by OCC requires that a random sample of macroinvertebrates be collected, identified and enumerated, from the portion of the waterbody being assessed. In order to make this test cost effective it is not possible to identify more than about 150 organisms from each site. This procedure describes the procedure used to subsample a field-collected sample, which may contain 200-10,000 organisms.

1. **Obtain the Field Sample to be Subsampled.** The field collected macroinvertebrate samples and the original COC will be transferred to the individual(s) designated to complete the macroinvertebrate subsampling and picking. At this time a copy of the COC (signed by the subsampling designee) will be sent to the Data Manager.

   The sample will come from the field in 1-quart mason jars preserved with 100% ethanol. Mason jar lids have a sealing compound that is not particularly resilient. Care must be taken so that the lids are not damaged when they are opened or resealed. If a lid is damaged it must be replaced with a new one. Keep a fresh supply of lids handy in case this happens. If you use a new lid, label it exactly the same as the one that was originally used.

   Samples will be subsampled/picked in the order assigned on the Chain of Custody form.

2. **Decant Ethanol.** Without shaking or disturbing the contents, pour the liquid from the sample through a sieve made of #30 or finer screen. Save the ethanol to preserve the unused portion of the sample.

3. **Rinse Sample.** At this point, any silt, clay or fine sand in the sample should be GENTLY rinsed out of the sample. Be careful not to break off any of the delicate appendages that are used for identification of the animals. The sample will be easier to process if any large pieces of leaf, bark, stones, etc., are discarded. **Any material to be discarded must first be carefully rinsed within the sieve.**

4. **Prepare Sample for Picking.** Spread the sample out in a rectangular tray/pan that is divided into 28 sections of equal area. The size and shape of the divisions are not important so long as they are all equal in size. A pan with a white background may facilitate the collection since there will be a contrast between the organisms and the pan.

   A clear glass pan or baking dish can be effectively used by creating a grid system on the bottom of the pan using a permanent marker. Each square should be numbered (1-28), and a sheet of white paper can be glued or taped over the outside bottom of the pan. If very many samples will be subsampled it will be worth your time to construct a divider for the tray similar in construction to an ice cube tray divider. This will not only demarcate the subsampling squares, but it will also prevent animals from drifting from one square to another during subsampling.

5. **Remove Large Pieces of Detritus and Sediment.** Large leaves and big pieces of wood and bark should be removed. Be VERY CAREFUL to pick all macroinvertebrates off of them before discarding. At this stage, any debris removed must be viewed through a magnifying lens to ensure the removal of all small invertebrates. At this point, the material remaining in the dish should consist of a mixture of sand, fine gravel, small organic detritus, pieces of leaves < 1-2 cm wide, fine roots, algae and macroinvertebrates.

6. **Spread the Sample Out.** All detritus and sediment should be as uniformly distributed over the bottom of the dish as possible.

7. **Visually Estimate Invertebrate Density of the Sample.** Determine if the sample must be subdivided. The decision will be based on three requirements: (1) individual squares MUST have AT LEAST 3 animals in them (providing the entire sample has at least 100 animals total), (2) you MUST pick AT LEAST 5 squares, and (3) each square can have absolutely NO MORE THAN 25 animals. Simulid (blackfly) larvae are not to be counted as individuals for this purpose. That is, the density estimate should be independent of any blackfly larvae present. The goal is to have roughly 10 TO 20 ANIMALS PER SQUARE. This is a compromise between the statistical ideal of very few organisms per square and ease of subsampling where the entire sample is picked from one square. If you estimate that there are less than 200 animals in the entire sample, you should process the entire sample.

The purpose of this estimate is to make the sample statistically valid. Fewer than 80 animals does not provide a good representation of the population to draw conclusions from, and more than 130 animals biases the sample making it appear that that stream has more taxa than it really does. A total of 100 invertebrates is the absolute minimum number of individual invertebrates to pick from a sample, except when a sample contains fewer than 100. Although 80 is the minimum number of individuals for statistical analysis, it is imperative that a cushion is incorporated to allow for the potential difference in the sub-samplers count and the final count by the taxonomist. Often invertebrates are tossed out by the taxonomist due to an inability to identify individuals caused by missing or damaged body parts or other various reasons.

8. **Subdivide the Sample.** If each square is estimated to have more than 50 animals, it is important to grossly subdivide the sample prior to picking. For instance, divide the sample in half or quarters depending on the animal density. Ideally, 10 to 20 animals per square is desirable.

   For dividing in fourths, cut the sample into top and bottom halves and then into right and left halves. After dividing the sample, the four portions should appear as equal as possible in terms of the amount of detritus and sediment present. Choose one quarter by random means. For instance, flip a coin to select either the top half or the bottom half, and then flipping the coin again to select either the right or left side of the first half selected. If the sample is not too dense, that is the selected quarter has a density of 10 to 20 animals per square, then no additional subdivision is necessary. If the number of invertebrates is still too dense, divide the remaining portion in half and select one half by flipping the coin again. Continue dividing the sample until the density appears to fall in the correct range.

9. **Return the Unselected Portion(s) to the Mason Jar.** Return the unselected portion(s) of the sample to the original Mason jar and add the reserved alcohol. Be very careful to remove the entire portion of unselected subsample. A proportionately high density of small macroinvertebrates can remain hidden in the sediment and detritus.

10. **Fill the Tray About 1 to 2 cm Deep With Water.** Add enough tap water to fill the tray to a depth of 1–2 cm (~0.5 to 0.75 inches) or the depth necessary to cover all sample material (debris and invertebrates). The water aids in the subsampling process. The organisms and individual pieces of detritus do not clump together as when they are dry. If the water is run into the tray very slowly, the remaining leaves and large pieces of detritus can be rinsed and discarded.

11. **Distribute the Sample Evenly.** Make sure that all materials in the tray are evenly distributed, especially the gravel and leaves. This is most easily accomplished by gently homogenizing the sample mixture by hand in the tray and distributing the mixture evenly over the entire pan. If a divider is to be used, place it in the tray now. Once the sample has been distributed, do not move the pan. Jostling can cause the organisms to move outside of their designated square. This could lead to a sampling bias.

12. **Fill Out Macroinvertebrate Picking Data Sheet.**
    Complete the Macroinvertebrate Picking Data Sheet (see SOP Appendix: Data Sheets) as described below:

    SITE / SUBSAMPLING INFORMATION

    - **Picking Date.** The date of subsampling and picking should be recorded in MM/DD/YY format.
    - **Site Name.** The name of the site as it is written on the sample jar.
    - **WBID #.** The waterbody identification number on the sample jar
    - **Picker.** The name of the person subsampling / picking.
    - **Site Date.** The date the sample was collected.
    - **COC #.**
    - **Lab Log #.**
    - **Site Time.** Record the site time in military format. The "site time" is when initial activities began at the site.
    - **Sample Type.** Type of sample "Woody", "Vegetation", or "Riffle"
    - **Sample Description.** Exclusive of invertebrates, estimate the composition of the sample according to the following list: silt and clay, sand, fine gravel (<2mm), coarse gravel (>2mm), woody debris (twigs, bark, roots, etc.), whole leaves, rotted pieces of leaves, filamentous algae, and unidentifiable organic material. Record the percentage of each type.
    - **Proportion Picked.** Record the fraction of the sample that was placed into the tray for sampling –e.g. 1/2, 1/4, or 1/8 of the original Mason jar sample. This is important because the density calculations depend on this number.

- **Square # / # Organisms.** List the number of the square that was picked on the lab notebook along with the number of organisms that were picked from that square.
- **Total Number of Organisms.** Record the sum total of organisms from all squares picked. This number is not used in the calculation of the IBI scores, but provides an estimate of the total number of organisms.

INFORMATION TO INCLUDE	SAMPLE NOTEBOOK PAGE						
Subsampling Date	11/18/99						
Site Date	07/16/99						
Stream Name	Griever Creek						
Site Time	13:30						
Legal Description & County	E 9 T22N R15W, Major County						
WBID #	OK620920-01-0130g						
COC #	COC# 1772						
Sampling Type	Riffle kick						
Sample Description	40%fine gravel	10% film. algae	30% well-rotted leaves				
	10% whole leaves	5% woody debris	5% coarse gravel				
Amount of Sample Picked	¼ of the original sample was prepared for picking						
Squares Picked	Square #	1	12	3	23	28	10
# of animals found in each square	# picked	15	27	10	18	8	24
Subsampler's name	John Hassell						

13. **Randomly Select Squares.** Using some method to generate a series of random numbers (random number generator or number table), select at least 6 squares. The random number generators found on most pocket calculators or the Excel spreadsheet function will give a series of three digit numbers—usually >0 but <1. For OCC purpose, use only the last two numbers. Starting with the first random number generated, record all numbers between 01 and 28 until there are 6. These numbers represent the numbered squares to pick. Picking must follow the order in which the number were generated.

14. **Confine the Organisms to the Selected Square.** Place rectangles or squares constructed of clear plastic or other material that are the same or greater height as the water in each square. This will keep the organisms from drifting out of the squares during the picking process. If a large piece of detritus (leaves, roots, algae masses) crosses the boundary of two or more squares, it may be sliced along the edge of the square so it is contained with the boundary of the selected square.

    If there are any organisms that cross square boundaries, do not cut them. Place them in the square in which their head is already lying.

15. **Pick <u>All</u> the Invertebrates Out of the First Square Selected.** Locate and collect all the organisms in the selected square. Keep track of the number of non-blackfly organisms picked. Place the organisms picked in a scintillation vial that is filled up to the neck with 70-100% ethanol. If any large organisms (that are too big to fit in the vial with the other organisms) are picked such as crayfish or hellgrammites, place them in a separate vial. If there is some question if something is an organism, place it in the vial but <u>DO NOT COUNT</u> it as part of the total. Place five (5) blackflies and five (5) scuds in the vial but <u>DO NOT COUNT</u> them as part of the total. Pickers should be trained to identify blackfly larval forms—if the picker cannot identify blackflies, they should not be subsampling without further training. Note the general abundance of blackflies and scuds in the comments.

    When all of the organisms are picked out of the square, record the number of non-blackflies/scuds that were picked from that square under the number of that square.

16. **Continue to Pick Squares Until 100 Organisms Have Been Collected.** Using the random number list, continue to select squares until 100 (non-blackfly) animals have been collected. Once a square has been started, all of the animals must be collected from that square. A subsample will typically have 100-130 organisms / vial. If there are more than

130, the sample was not properly subdivided. If the sample has more than 150 organisms in it, it must be mixed back in with the rest of the "unpicked sample" and re-picked. If the finished invertebrate sample has less than 100 organisms in it and there is unpicked sample available, add all of the picked sample (debris and invertebrates) back to the unpicked sample portion and begin the process again with the full sample. The ONLY time that it is acceptable to have a sample with less than 100 organisms is when the entire mason jar of material has been picked. All other samples containing less than 100 invertebrates will be rejected as data with bad QA/QC.

17. **Label the Vial(s).** Using a pencil and a fine point permanent ink marker, label the vial. The top and side of the vial should both be labeled.

    The vial should be labeled IN PENCIL on waterproof paper taped to the vial after labeling with the following information:
    - Stream name
    - WBID #
    - Site date
    - Site time
    - Legal location and County
    - Type of sample (riffle, woody, vegetation)
    - Number of vials for this sample (e.g. 1 of x, where x = total number vials for one site (Mason jar))

    The cap should be labeled IN PERMANENT INK with the following information:
    - Stream name
    - WBID #
    - Site date
    - Time
    - Type of collection
    - Number of vials for this sample

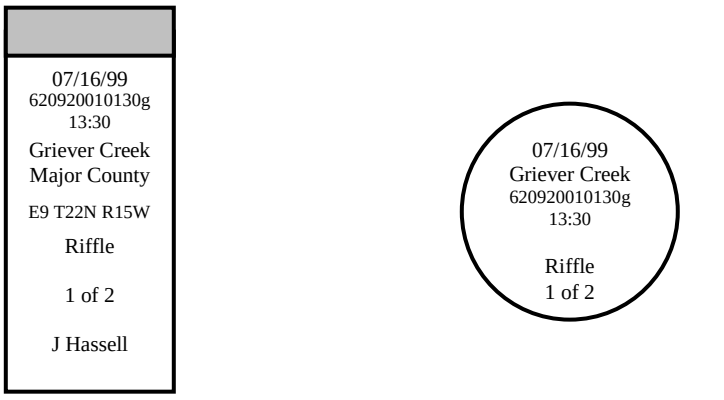


Figure 1: Example label for bug picking sample.

18. **Place Clear Tape Over the Label.** To protect the pencil-written label from wear, place clear tape (scotch tape) over the writing.

19. **Transfer Picked Samples to the Taxonomist**. Once macroinvertebrate samples have been picked and placed in properly labeled scintillation vials, the vials and the original COC will be transferred to the laboratory for taxonomic identification. At this point, a copy of the COC should be forwarded to the Data Manager with the signature of the taxonomist. The original COC will be returned to the Data Manager by the taxonomist or laboratory custodian.

20. **Archive Remaining Sample.** The remainder of the field collected macroinvertebrate samples (un-picked) should be delivered to the OCC designee for archive purposes. The COC number should be conspicuously labeled on the end of the box.

1.13 Troubleshooting

Consult with the Environmental Monitoring Coordinator

1.14 Data Acquisition, Calculation & Data Reduction

Not applicable

1.15 Computer Hardware & Software

Not applicable

1.16 Data Management & Records Management

1.16.1 Field Notation

A duplicate sample should be collected for every 10 sampling sites and noted on the **Sampling Episode Sheet** (see **SOP Appendix: Data Sheets**). All measurements and observations made at each site should be recorded on the **Site Collection Sheet** (see **SOP Appendix: Data Sheets**). Data should be recorded following procedures outlined in the **Procedure for Completing Field Data Sheets SOP**.

1.16.2 Habitat Form

Regardless of the habitat sampled, a **Macroinvertebrate Habitat Assessment Sheet** (see **SOP Appendix: Data Sheets**) must be filled out at each collection site.

The following bullets describe how to fill out the Macroinvertebrate Habitat Sheet:

DATA SHEET HEADER INFORMATION:

- SITE NAME: Record the stream name from the USGS 7-1/2' map name. If a county map, soil map, or other map has a different name, the USGS 7-1/2' map takes precedence. If a stream is unnamed on the USGS map, but named on another map, use that name, but write the name of the map in parentheses beside the stream name.
- WBID #: Record the Water Body Identification number.
- LEAD INVESTIGATOR: Record the name of the person responsible for data custody and reporting
- DATE: Record the site date in MM/DD/YR format
- TIME: Record the site time in military format. The "site time" is when initial activities began at the site. The site time should be the same on all forms associated with this site.

The form is broken into three columns, one for each habitat type (riffle, streamside vegetation and woody debris). Fill out the appropriate information for each habitat type collected. If one or two of the three sample types are not collected, write "not collected" above the habitat type.

RIFFLE

- % of SAMPLE COLLECTED Usually all of the sample collected will fit in a one quart mason jar (3/4 full). If the sample will not fit, even after removing leaves, rocks, and sticks, without overfilling the jar, mix the sample until all the components (algae, leaves, twigs, rocks, sand, etc.) appear to be uniformly mixed and discard enough of it so that the remainder will fit without over packing the jar. Write down the % of sample you estimate that you have placed in the jar

- UNIT of EFFORT: Refers to the area of riffle sampled. Three 1 m^2 samples should be collected. Record the area sampled.

- EMBEDDEDNESS: This quantifies the amount of silt, clay and sand that has been **DEPOSITED IN RIFFLES.** If there is no fine material surrounding the cobble and gravel of riffles, and there is at least some free space under the rocks, that is 0 percent embedded. If the free space under the rocks is filled but the sides are untouched, count that as 5 percent embedded. As the level of fines rises up the cobble sides, estimate the percentage of the total height of the cobbles that is covered. This is the embeddedness estimate. You can often see this line quite distinctly if you lift the rocks out of the water.

- CPOM in SAMPLE: "Coarse Particulate Organic Matter" Refers to the % of sample composed of partially or well-rotted plant material not counting the substrate being sampled. This should mostly be composed of leaf material. Do not count freshly fallen leaves that have not started to rot. Circle the appropriate number.
  1. Absent 0%
  2. Sparse > 0% but < 5%
  3. Moderate 5% to 25%
  4. Abundant > 25%

- SUBSTRATE TYPE & %: This is an approximate classification of the riffle substrate <u>where the collection is being made</u>. Estimate the proportion each type comprises of the entire substrate. The total of all substrate components should add up to 100%.
  1. Silt & Clay Refers to loose particles < 0.05 mm.
  2. Sand Refers to particles 0.1 to 2 mm is size.
  3. Gravel Refers to particles 2 to 50 mm is size.
  4. Cobble Refers to particles 50 to 250 mm is size.
  5. Boulder Refers to particles >250 mm is size.
  6. Bedrock Refers to rock that is attached to the earth's crust. If a rock can be moved by any means, it is not bedrock.
  7. Hard Pan Clay Refers to a smooth (relatively) surface of clayey material, firm to hard that is moderately resistant to erosion, and provides stable habitat.

- SUBSTRATE ROUGHNESS: Refers to the roughness of the rocks in the riffle. If you can easily assign the riffle to one of these categories by a visual estimate of the roughness no scraping is necessary. If you are not sure, pick up a typical rock and scrape it with a pocketknife. Circle the appropriate number.
  1. Low >75% of the visible periphyton is removed when scraped with a pocketknife or spatula.
  2. Moderate 25 to 75% of the visible periphyton is removed when scraped with a pocketknife or spatula.
  3. High <25% of the visible periphyton is removed when scraped with a pocketknife or spatula.

- VELOCITY TYPICAL MAX: This is an estimate of the average velocity of the habitat sampled in the fastest part of the stream. In a riffle this would be the thalweg. This velocity can be estimated using a floating object and a watch. Circle the appropriate number.
  1. Low (0.2-0.5 FPS; 0.061-0.15 MPS) FTS = feet/second; MPS = meters/second
  2. Moderate (0.5-1 FPS; 0.152-0.305 MPS)
  3. High (>1 FPS; 0.305 MPS)

- NON-FILAMENTOUS ALGAE: Refers to the areal percent of the substrate sampled which is covered with algae that lacks a stringy appearance. Circle the appropriate number.
  1. Sparse When rocks, bedrock, limbs, trash, etc., are free of attached algae or have only a thin film of greenish or brownish algae that cannot be measured by holding a ruler perpendicular to the surface of the submerged object.
  2. Moderate When the submerged surfaces have a slight fuzzy or blanketed appearance. The thickness of the attached algae does not exceed 5mm.
  3. Abundant Submerged surfaces have a definite fuzzy or blanketed (covered with gelatinous mat) appearance. The thickness of attached growth exceeds 5mm.

- FILAMENTOUS ALGAE: Refers to the areal percent of the substrate sampled which is covered with algae that is hair-like or stringy in appearance. Circle the appropriate number.
  1. Absent 0%
  2. Sparse > 0% but < 5%
  3. Moderate 5% to 25%
  4. Abundant > 25%

- AQUATIC MOSS: Refers to the areal percent of the substrate sampled which is covered with aquatic moss. Circle the appropriate number.
  1. Absent 0%
  2. Sparse > 0% but < 5%
  3. Moderate 5% to 25%
  4. Abundant > 25%

STREAMSIDE VEGETATION

- % of SAMPLE COLLECTED Usually all of the sample collected will fit in a one quart mason jar. If the sample will not fit, even after removing leaves and sticks, without overfilling the jar, mix the sample until all the components (algae, leaves, twigs, rocks, sand, etc.) appear to be uniformly mixed and discard enough of it so that the remainder will fit without over packing the jar. Write down the % of the total sample that is placed in the jar

- UNIT of EFFORT: Refers to the amount of time the vegetation was agitated. The collection should proceed for **3** minutes. Record the actual time of collection in minutes.

- CPOM in SAMPLE: "Coarse Particulate Organic Matter" Refers to the % of sample composed of partially or well-rotted plant material not counting the substrate being sampled. This should mostly be composed of leaf material. Do not count freshly fallen leaves that have not started to rot. Circle the appropriate number.
  1. Absent 0%

2. Sparse > 0% but < 5%
3. Moderate 5% to 25%
4. Abundant > 25%

- PRESENCE: Refers to the amount of suitable streamside vegetation habitat present in the stream. Circle the appropriate number.
  1. Occasional Indicates that you must walk more than 50 meters to get a good 3-minute sample.
  2. Common Indicates that you must walk 10 to 50 meters to get your sample.
  3. Abundant Indicates that a good sample can be collected in less than 10 meters of stream.

- TYPE: Refers to the type of streamside vegetation sampled. Circle all that makes up at least ¼ of the total habitat sampled.
  1. Grass-like Leaves Leaves of aquatic or semi aquatic grasses & sedges which have been hanging in the water long enough to develop a periphyton and/or slime coat.
  2. Fine Roots Root masses where most of the roots are <2 mm in diameter.
  3. Coarse Roots Root masses where most of the roots are >2 mm but <6 mm in diameter.
  4. *Ludwigia* Stems Stream macrophyte—not suitable habitat.

- VELOCITY TYPICAL MAX: This is an estimate of the average velocity of the habitat sampled in the fastest part of the stream. For streamside vegetation it would be on the outside (streamside) edge of the root mass. This velocity can be estimated using a floating object and a watch.
  1. Low 0.2 to 0.5 ft/sec
  2. Medium 0.5 to 1.0 ft/sec
  3. High >1.0 ft/sec.

- NON-FILAMENTOUS ALGAE: Refers to the areal percent of the substrate sampled which is covered with algae that are not stringy in appearance. Circle the appropriate number.
  1. Sparse When rocks, bedrock, limbs, trash, etc., are free of attached algae or have only a thin film of greenish or brownish algae that cannot be measured by holding a ruler perpendicular to the surface of the submerged object.
  2. Moderate When the submerged surfaces have a slight fuzzy or blanketed appearance. The thickness of the attached algae does not exceed 5mm.
  3. Abundant Submerged surfaces have a definite fuzzy or blanketed (covered with gelatinous mat) appearance. The thickness of attached growth exceeds 5mm.

- FILAMENTOUS ALGAE: Refers to the areal, percent of the substrate sampled which is covered with algae that is hair-like or stringy in appearance. Circle the appropriate number.
  1. Absent 0%
  2. Sparse > 0% but < 5%
  3. Moderate 5% to 25%
  4. Abundant > 25%

WOODY DEBRIS

- % of SAMPLE COLLECTED Usually all of the sample collected will fit in a one quart mason jar. If the sample will not fit, even after removing leaves and sticks, without overfilling the jar, mix the sample until all the components (algae, leaves, twigs, rocks, sand, etc.) appear to be uniformly mixed and discard enough of it so that the remainder will fit without over packing the jar. Write down the % of sample you estimate that you have placed in the jar

- UNIT of EFFORT: Refers to the amount of time the vegetation was agitated. The collection should proceed for **5** minutes. Record the actual time of collection in minutes.

- CPOM in SAMPLE: "Coarse Particulate Organic Matter" Refers to the % of sample composed of partially or well-rotted plant material not counting the substrate being sampled.

This should mostly be composed of leaf material. Do not count freshly fallen leaves that have not started to rot. Circle the appropriate number.

1. Absent 0%
2. Sparse > 0% but < 5%
3. Moderate 5% to 25%
4. Abundant > 25%

- PRESENCE: Refers to the amount of suitable woody debris habitat present in the stream. Circle the appropriate number.
  1. Occasional Indicates that you must walk more than 50 meters to get a good 3-minute sample.
  2. Common Indicates that you must walk 10 to 50 meters to get your sample.
  3. Abundant Indicates that a good sample can be collected in less than 10 meters of stream.

- SIZE: Refers to the average diameter of the woody debris sampled. Check all lines where that size class makes up at least 1/4 of the habitat sampled.
  1. Small 0.6 to 2.0 cm
  2. Medium 2.0 to 7.5 cm
  3. Large >7.5 cm

- STATE OF DECAY: Refers to the state of decay of the woody debris sampled. Circle all that apply where debris of this type makes up at least ¼ of the habitat sampled. All of these categories may or may not have bark on them. These categories are determined by firmly pressing your thumbnail into the wood (not bark) of the debris sampled perpendicular to the grain. The depth of the indentation, if any that remains when your thumbnail is removed is measured to determine the state of decay.
  1. Low Indentation is 0 to 0.5 mm deep
  2. Moderate Indentation is 0.5 to 2 mm deep
  3. High Indentation is > 2 mm deep

- VELOCITY TYPICAL MAX: This is an estimate of the average velocity of the habitat sampled in the fastest part of the stream. For woody debris, it would be the average velocity of the water passing over the sides of the wood. This velocity can be estimated using a floating object and a watch.
  1. Low 0.2 to 0.5 ft/sec
  2. Medium 0.5 to 1.0 ft/sec
  3. High >1.0 ft/sec.

- NON-FILAMENTOUS ALGAE: Refers to the areal percent of the substrate sampled which is covered with algae that are not stringy in appearance. Circle the appropriate number.
  1. Sparse When rocks, bedrock, limbs, trash, etc., are free of attached algae or have only a thin film of greenish or brownish algae that cannot be measured by holding a ruler perpendicular to the surface of the submerged object.
  2. Moderate When the submerged surfaces have a slight fuzzy or blanketed appearance. The thickness of the attached algae does not exceed 5mm.
  3. Abundant Submerged surfaces have a definite fuzzy or blanketed (covered with gelatinous mat) appearance. The thickness of attached growth exceeds 5mm.

- FILAMENTOUS ALGAE: Refers to the areal percent of the substrate sampled which is covered with algae that is hair-like or stringy in appearance. Circle the appropriate number.
  1. Absent 0%
  2. Sparse > 0% but < 5%
  3. Moderate 5% to 25%
  4. Abundant > 25%

COMMENTS Record any useful information that provides insight to the sample collection process, conditions, or miscellaneous information.

1.16.3 Chain of Custody Procedure

Collection of inorganic sample requires the use of a Chain of Custody form (COC). The handling of COC should follow the procedures described in the **Chain of Custody and Sample Labeling SOP.** The manifest is routed as follows:

1. Macroinvertebrate samples are collected in the field and the COC is completed and signed by the field personnel involved with collection.
2. Samples are submitted to the Macroinvertebrate Sample Custodian. That person signs the COC and forwards a copy to Data Manager or logs the information on the web page.
3. Samples are assigned to subsampling/picking personnel for processing. They must sign the COC.
4. Processed samples are sent to the taxonomist for identification. The taxonomist must sign the COC. The person who sends the samples to the taxonomist, forwards a copy of the COC to the Data Manager.
5. After identification, the taxonomic identification sheets will be forwarded with the signed COC to the Data Manager. The laboratory will include the laboratory tracking or log numbers used to reference the identification sheet.

2.0 QA/QC SECTION

2.1 Training

Training of field personnel will be done through dry run exercises in the laboratory to familiarize them with instrument operation, calibration and maintenance. All operators are required to become familiar with the SOP documents. Prior to solo sample collection, subsampling or picking, personnel are evaluated in for proper use of equipment and sample collection protocol. Annual audits are performed on sample collectors following procedures outlined in the **Quality Management Plan**.

2.2 Maintenance

Not applicable

2.3 QC Procedures

A set of field QA samples will be collected for every sampling episode or one set per 10 sampling sites (10%). The QA samples will include at a minimum a Field Replicate. Spatial replicates should be obtained by implementing the aforementioned sampling procedures upstream of the sampling site being careful to sample with equal effort a similar composition of habitat to the original sampling site. If required by the QAPP, Field Splits will be collected. Subsampling and picking QA/QC is the responsibility of the contracted facility. The OCC will evaluate QA/QC procedures through blind checks and spot inspections.

3.0 REFERENCES

APHA, AWWA, and WPCF (1995) Standard Methods for the Examination of Water and Wastewater, 19th edition, eds. L.S. Clesceri, A.E. Greenberg, and R.R. Trussell, American Public Health Association, Washington, D.C.

Butler, D., (1999) Personal Communication, Senior Biologist, Oklahoma Conservation Commission, Oklahoma City, OK.

4.0 APPENDIX A

STANDARD OPERATING PROCEDURE
Field Summary

Summary of Method
A modified version of EPA Rapid Bioassessment Protocol (RBPs) was adopted for macroinvertebrate collections. As stated above, the collection methods are geared toward assessing communities that require or prefer flowing water. Lotic communities require a substrate of some type to attach to. The most common substrates encountered are rocky riffles, streamside vegetation, and woody debris. All three substrates can be sampled (when available) to provide an accurate representation of the various communities in the stream. A combination of collection techniques is used for each habitat. Organisms collected from these habitats are subsampled and sent to a professional macroinvertebrate taxonomist and enumerated to genus level, when possible.

Definitions
- Riffle: Any sudden downward change in the level of the streambed such that the surface of the water becomes disrupted by small waves. A riffle substrate must be composed of gravel, or cobble from 1" to 12" in the longest dimension; substrates of bedrock or tight clay are not considered suitable. In riffle with sand & gravel, it must contain >50% gravel.
- Streamside Vegetation: Any streamside vegetation which offers fine structure for invertebrates to dwell within or upon that receives suitable flow. Most habitat is located along undercut banks where fine roots of riparian vegetation are hanging in the water.
- Woody Debris: Any dead wood with or without bark located in the stream with suitable current flowing over it.
- Summer Index Period: **June 1** to **September 15.**
- Winter Index Period: **January 1** to **March 15**

Health and Safety Warnings
- Proper precautions should be taken when handling 100% ethanol.

Cautions
- In no case should the Mason jar be filled more than 3/4 full of loose sample.
- There should always be enough room in the jar to have at least 5 cm (~2 inch) of free ethanol over the sample.
- Collections must be done in flowing water
- Stream stage must not be greater than 3 cm (~1 inch) above base flow during collection.

Collection of Benthic Macroinvertebrates from Rocky Riffles

- **Suitable Substrate** - A riffle is defined as any sudden downward change in the level of the streambed such that the surface of the water becomes disrupted by small waves. For this collection method the substrate of the riffle must be composed of gravel, or cobble from 1" to 12" in the longest dimension. Riffles with substrates of bedrock or tight clay are not suitable. If the riffle substrate is composed of only sand and gravel it must contain at least 50% gravel.

- **Where to Sample the Riffle** - Three 1 m^2 areas of the riffle must be sampled. They can be square, rectangular or trapezoidal so long as each area equals 1 m^2 in area. One should be in the fastest part of the riffle where the largest rocks and the smallest amount of interstitial sediment will generally be found. The second should be in the slowest part of the riffle, often near the edge of the stream where the smallest rocks and the greatest amount of interstitial sediment will be found. The third sample should be in an area intermediate between the first two

- **Method of Collecting the Sample** - Support a 1 m^2 kick net composed of a double layer of fiberglass window screen or a net of number 30 mesh in such a way that any organisms dislodged from the substrate will be carried into it by the current. The bottom of the net should be tight against the bottom of the stream and the current must be sufficient to insure that dense organisms such as small mollusks will be carried into the net from the sampling area. There is no definite cutoff for stream velocity in the sampling area, but if possible, riffles with average velocities of 1 foot/second or greater are preferred and should be chosen if possible.
  By kicking the substrate, vigorously agitate the substrate of a 1 m^2 area of the bed of the riffle immediately upstream of the riffle until all rocks and sediment to a depth of at least five inches have been thoroughly scraped against each other.

Organisms living between and upon the rocks will have been dislodged and carried into the net by the current. Any rocks too large to kick should be brushed by hand on all surfaces. This can be done using your hands or with the aid of a brush. If a brush is used, you must be very careful to clean it after each site to prevent contamination of the next sample with invertebrates from the previous site. Continue agitation and brushing until it can be seen that the area being sampled is producing no new detritus, organisms, or fine sediment.

At this point, rinse leaves, sticks and other large debris caught in the net in the current in a manner such that organisms on them are carried into the net. When the volume of the sample is reduced so that three 1 m^2 samples will loosely fill a 1 quart mason jar three fourths (3/4) full or less, remove all of the material from the net and place it in the Mason jar. In no case should the Mason jar be filled more than 3/4 full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. There should always be enough room in the jar to have at least 2 inches (5 cm) of free ethanol over the sample.

Label the sample appropriately following the instructions presented in section 1.11 Sample Handling & Preservation.

Collection of Macroinvertebrates from Streamside Vegetation

- **Suitable Substrate** - Any streamside vegetation in current that offers fine structure for invertebrates to dwell within or upon is suitable. The vegetation being sampled must be in the current so that it offers suitable habitat for organisms which collect drifting particles or which need flowing water for other reasons. This habitat will often be found along the undercut banks of runs and bends where the fine roots of grasses, sedges, and trees, such as willow and sycamore, hang in the water.

- **Method of Collecting the Sample** - This type of sample should be collected with a dip net made of #30 size mesh material. The net should be placed around or immediately downstream of the vegetation being sampled. The organisms can be dislodged from the roots either by vigorously shaking the net around the roots or by shaking the roots by hand while the roots are inside the net.

- **Where and How Long to Sample** - Sampling should continue for **3 minutes** of actual root shaking. Do not count the time that elapses between sampling areas. Be careful to only sample roots in current. Usually, only one or two sides of a given root mass are in current. Be careful not to sample the backside of a root mass that is in still water.

  At this point, rinse leaves, sticks and other large debris caught in the net so that organisms are not lost. When the volume of the sample is reduced so that it will loosely fill a 1-quart mason jar three fourths (3/4) full or less, remove all of the material from the net and place it in the Mason jar. In no case should the Mason jar be filled more than 3/4 full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. There should always be enough room in the jar to have at least 2 inches (5 cm) of free ethanol over the sample.

  Label the sample appropriately following the instructions presented in section 1.11 Sample Handling & Preservation.

Collection of Macroinvertebrates from Woody Debris

- **Suitable Substrate** - Any dead wood with or without bark in the stream is suitable as long as it is in current fast enough to offer suitable habitat for organisms which collect drifting particles or which need flowing water for other reasons. The final sample should consist of organisms collected from an even mixture of wood of all sizes and in all stages of decay.

- **Method of Collecting the Sample** - This type of sample should be collected with a dip net made of #30 size mesh material. The net should be placed around or immediately downstream of the debris being sampled. The organisms can be dislodged from the debris either by vigorously shaking the net around the woody debris or by shaking the debris by hand while the debris is inside the net. Large logs that are too big to shake should be brushed or rubbed vigorously by hand while the net is held immediately downstream.

- **Where and How Long to Sample** - Sample for total of **5 minutes** counting only the time that debris is actually being agitated. Include as many types of debris in the sample as possible. These types often include wood that is very rotten and spongy with or without bark, wood that is fairly solid which has loose and rotten bark, wood that is solid with firmly attached bark and any combination of these states. They should range in size from 1/4" to about 8" in diameter.

After sampling, rinse leaves, sticks and other large debris caught in the net so that organisms are not lost. When the volume of the sample is reduced so that it will loosely fill a 1-quart mason jar three fourths (3/4) full or less, remove all of the material from the net and place it in the Mason jar. In no case should the Mason jar be filled more than 3/4 full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. There should always be enough room in the jar to have at least 2 inches (5 cm) of free ethanol over the sample.

Label the sample appropriately following the instructions presented in section 1.11 Sample Handling & Preservation.

Sample Handling & Preservation

1. **Pack the Mason Jar Properly.** In no case should the Mason jar be filled more than 3/4 full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. There should always be enough room in the jar to have at least 2 inches (5 cm) of free ethanol over the sample.

2. **Label the Sample.** The Mason jar should be labeled on the lid using a fine tip permanent ink marker (Sharpie) as described below. In addition, a small sheet of paper (approx. 2" x 2") should be filled out with the same information written in pencil and placed in the jar.

Jar Lid & Sample Insert

Site Date
Stream Name
Waterbody ID #
Site Time
Legal Description
County
Type of sample (riffle, woody, vegetation)
Sampler's Initials

3. **Complete the Chain of Custody.** Follow the instructions in the Chain of Custody and Sample Labeling SOP. A new COC should be completed for each collection episode. Each substrate collection (riffle, woody, vegetation) should occupy a separate line on the COC. There should be only one box of samples per COC, i.e., one box, one COC.

4. **Transfer samples to the Macroinvertebrate Sample Custodian.** Correctly labeled macroinvertebrate samples, along with a Chain of Custody form, should be transferred to the Macroinvertebrate Sample Custodian (Nathan Carter) for subsampling. The box should be conspicuously labeled with Chain of Custody number. Once the samples have been received and the Chain of Custody signed, the field sampler should make a photocopy of the Chain of Custody form for their records.

5. A duplicate sample should be collected and noted on the **Sampling Episode Sheet** (see SOP Appendix: Data Sheets). All measurements and observations made at each site should be recorded on the **Site Collection Sheet** and on the **Macroinvertebrate Habitat Sheet.**

Instructions for filling out the Macroinvertebrate Habitat Sheet

DATA SHEET HEADER INFORMATION:

- SITE NAME: Record the stream named from the USGS 7-1/2' map name. If a county map, soil map, or other map has a different name, the USGS 7-1/2' map takes precedence. If a stream is unnamed on the USGS map, but named on another map, use that name, but write the name of the map in parentheses beside the stream name.
- WBID #: Record the Water Body Identification number.
- LEAD INVESTIGATOR: Record the name of the person responsible for data custody and reporting
- DATE: Record the site data in MM/DD/YR format

- TIME: Record the site time in military format. The "site time" is when initial activities began at the site. The site time should be the same on all forms associated with this site.

The form is broken into three columns, one for each habitat type (riffle, streamside vegetation and woody debris). Fill out the appropriate information for each habitat type collected. If one or two of the three sample types are not collected, write "not collected" above the habitat type.

RIFFLE

- % of SAMPLE COLLECTED Usually all of the sample collected will fit in a one quart mason jar (3/4 full). If the sample will not fit, even after removing leaves, rocks, and sticks, without overfilling the jar, mix the sample until all the components (algae, leaves, twigs, rocks, sand, etc.) appear to be uniformly mixed and discard enough of it so that the remainder will fit without over packing the jar. Write down the % of sample you estimate that you have placed in the jar

- UNIT of EFFORT: Refers to the area of riffle sampled. Three 1 m^2 samples should be collected. Record the area sampled.

- CPOM in SAMPLE: "Coarse Particulate Organic Matter" Refers to the % of sample composed of partially or well-rotted plant material not counting the substrate being sampled. This should mostly be composed of leaf material. Do not count freshly fallen leaves that have not started to rot. Circle the appropriate number.
  1. Absent 0%
  2. Sparse > 0% but < 5%
  3. Moderate 5% to 25%
  4. Abundant > 25%

- EMBEDDEDNESS: This quantifies the amount of silt, clay and sand that has been **DEPOSITED IN RIFFLES.** If there is no fine material surrounding the cobble and gravel of riffles, and there is at least some free space under the rocks, that is 0 percent embedded. If the free space under the rocks is filled but the sides are untouched, count that as 5 percent embedded. As the level of fines rises up the cobble sides, estimate the percentage of the total height of the cobbles that is covered. This is the embeddedness estimate. You can often see this line quite distinctly if you lift the rocks out of the water.

- SUBSTRATE TYPE & %: This is an approximate classification of the riffle substrate <u>where the collection is being made</u>. Estimate the proportion each type comprises of the entire substrate. The total of all substrate components should add up to 100%.
  1. Silt & Clay Refers to loose particles < 0.05 mm.
  2. Sand Refers to particles 0.1 to 2 mm is size.
  3. Gravel Refers to particles 2 to 50 mm is size.
  4. Cobble Refers to particles 50 to 250 mm is size.
  5. Boulder Refers to particles >250 mm is size.
  6. Bedrock Refers to rock that is attached to the earth's crust. If a rock can be moved by any means, it is not bedrock.
  7. Hard Pan Clay Refers to a smooth (relatively) surface of clayey material, firm to hard that is moderately resistant to erosion, and provides stable habitat.

- SUBSTRATE ROUGHNESS: Refers to the roughness of the rocks in the riffle. If you can easily assign the riffle to one of these categories by a visual estimate of the roughness no scraping is necessary. If you are not sure, pick up a typical rock and scrape it with a pocketknife. Circle the appropriate number.
  1. Low >75% of the visible periphyton is removed when scraped with a pocketknife or spatula.

2. Moderate — 25 to 75% of the visible periphyton is removed when scraped with a pocketknife or spatula.
3. High — <25% of the visible periphyton is removed when scraped with a pocketknife or spatula.

- VELOCITY TYPICAL MAX: This is an estimate of the average velocity of the habitat sampled in the fastest part of the stream. In a riffle this would be the thalweg. This velocity can be estimated using a floating object and a watch. Circle the appropriate number.
  1. Low (0.2-0.5 FPS; 0.061-0.15 MPS) FTS = feet/second; MPS = meters/second
  2. Moderate (0.5-1 FPS; 0.152-0.305 MPS)
  3. High (>1 FPS; 0.305 MPS)

- NON-FILAMENTOUS ALGAE: Refers to the areal percent of the substrate sampled which is covered with algae that are not stringy in appearance. Circle the appropriate number.
  1. Sparse — When rocks, bedrock, limbs, trash, etc., are free of attached algae or have only a thin film of greenish or brownish algae that cannot be measured by holding a ruler perpendicular to the surface of the submerged object.
  2. Moderate — When the submerged surfaces have a slight fuzzy or blanketed appearance. The thickness of the attached algae does not exceed 5mm.
  3. Abundant — Submerged surfaces have a definite fuzzy or blanketed (covered with gelatinous mat) appearance. The thickness of attached growth exceeds 5mm.

- FILAMENTOUS ALGAE: Refers to the areal percent of the substrate sampled which is covered with algae that is hair-like or stringy in appearance. Circle the appropriate number.
  1. Absent — 0%
  2. Sparse — > 0% but < 5%
  3. Moderate — 5% to 25%
  4. Abundant — > 25%

- AQUATIC MOSS: Refers to the areal percent of the substrate sampled which is covered with aquatic moss. Circle the appropriate number.
  1. Absent — 0%
  2. Sparse — > 0% but < 5%
  3. Moderate — 5% to 25%
  4. Abundant — > 25%

STREAMSIDE VEGETATION

- % of SAMPLE COLLECTED Usually all of the sample collected will fit in a one quart mason jar. If the sample will not fit, even after removing leaves and sticks, without overfilling the jar, mix the sample until all the components (algae, leaves, twigs, rocks, sand, etc.) appear to be uniformly mixed and discard enough of it so that the remainder will fit without over packing the jar. Write down the % of the total sample that is placed in the jar
- UNIT of EFFORT: Refers to the amount of time the vegetation was agitated. The collection should proceed for **3** minutes. Record the actual time of collection in minutes.
- CPOM in SAMPLE: "Coarse Particulate Organic Matter" Refers to the % of sample composed of partially or well-rotted plant material not counting the substrate being sampled. This should mostly be composed of leaf material. Do not count freshly fallen leaves that have not started to rot. Circle the appropriate number.
  1. Absent — 0%
  2. Sparse — > 0% but < 5%
  3. Moderate — 5% to 25%
  4. Abundant — > 25%

- PRESENCE: Refers to the amount of suitable streamside vegetation or woody debris habitat present in the stream. Circle the appropriate number.
  1. Occasional — Indicates that you must walk more than 50 meters to get a good 3-minute sample.
  2. Common — Indicates that you must walk 10 to 50 meters to get your sample.

3. Abundant — Indicates that a good sample can be collected in less than 10 meters of stream.

- TYPE: Refers to the type of streamside vegetation sampled. Circle <u>all</u> that makes up at least ¼ of the total habitat sampled.
  1. Grass-like Leaves — Leaves of aquatic or semi aquatic grasses & sedges which have been hanging in the water long enough to develop a periphyton and/or slime coat.
  2. Fine Roots — Root masses where most of the roots are <2 mm in diameter.
  3. Coarse Roots — Root masses where most of the roots are >2 mm but <6 mm in diameter.
  4. *Ludwigia* Stems — Stream macrophyte—not suitable habitat.

- VELOCITY TYPICAL MAX: This is an estimate of the average velocity of the habitat sampled in the fastest part of the stream. For streamside vegetation it would be on the outside (streamside) edge of the root mass. This velocity can be estimated using a floating object and a watch.
  1. Low — 0.2 to 0.5 ft/sec
  2. Medium — 0.5 to 1.0 ft/sec
  3. High — >1.0 ft/sec.

- NON-FILAMENTOUS ALGAE: Refers to the areal percent of the substrate sampled which is covered with algae that are not stringy in appearance. Circle the appropriate number.
  1. Sparse — When rocks, bedrock, limbs, trash, etc., are free of attached algae or have only a thin film of greenish or brownish algae that cannot be measured by holding a ruler perpendicular to the surface of the submerged object.
  2. Moderate — When the submerged surfaces have a slight fuzzy or blanketed appearance. The thickness of the attached algae does not exceed 5mm.
  3. Abundant — Submerged surfaces have a definite fuzzy or blanketed (covered with gelatinous mat) appearance. The thickness of attached growth exceeds 5mm.

- FILAMENTOUS ALGAE: Refers to the areal percent of the substrate sampled which is covered with algae that is hair-like or stringy in appearance. Circle the appropriate number.
  1. Absent — 0%
  2. Sparse — > 0% but < 5%
  3. Moderate — 5% to 25%
  4. Abundant — > 25%

WOODY DEBRIS

- % of SAMPLE COLLECTED: Usually all of the sample collected will fit in a one quart mason jar. If the sample will not fit, even after removing leaves and sticks, without overfilling the jar, mix the sample until all the components (algae, leaves, twigs, rocks, sand, etc.) appear to be uniformly mixed and discard enough of it so that the remainder will fit without over packing the jar. Write down the % of sample you estimate that you have placed in the jar

- UNIT of EFFORT: Refers to the amount of time the vegetation was agitated. The collection should proceed for **5** minutes. Record the actual time of collection in minutes.

- CPOM in SAMPLE: "Coarse Particulate Organic Matter" Refers to the % of sample composed of partially or well-rotted plant material not counting the substrate being sampled. This should mostly be composed of leaf material. Do not count freshly fallen leaves that have not started to rot. Circle the appropriate number.
  1. Absent — 0%
  2. Sparse — > 0% but < 5%
  3. Moderate — 5% to 25%
  4. Abundant — > 25%

- PRESENCE: Refers to the amount of suitable streamside vegetation or woody debris habitat present in the stream. Circle the appropriate number.

1. Occasional — Indicates that you must walk more than 50 meters to get a good 5-minute sample.
2. Common — Indicates that you must walk 10 to 50 meters to get your sample.
3. Abundant — Indicates that a good sample can be collected in less than 10 meters of stream.

- SIZE: Refers to the average diameter of the woody debris sampled. Check all lines where that size class makes up at least 1/4 of the habitat sampled.
  1. Small — 0.6 to 2.0 cm
  2. Medium — 2.0 to 7.5 cm
  3. Large — >7.5 cm

- STATE OF DECAY: Refers to the state of decay of the woody debris sampled. Circle all that apply where debris of this type makes up at least ¼ of the habitat sampled. All of these categories may or may not have bark on them. These categories are determined by firmly pressing your thumbnail into the wood (not bark) of the debris sampled perpendicular to the grain. The depth of the indentation, if any, which remains when your thumbnail is removed is measured to determine the state of decay.
  1. Low — Indentation is 0 to 0.5 mm deep
  2. Moderate — Indentation is 0.5 to 2 mm deep
  3. High — Indentation is > 2 mm deep

- VELOCITY TYPICAL MAX: This is an estimate of the average velocity of the habitat sampled in the fastest part of the stream. For woody debris, it would be the average velocity of the water passing over the sides of the wood. This velocity can be estimated using a floating object and a watch.
  1. Low — 0.2 to 0.5 ft/sec
  2. Medium — 0.5 to 1.0 ft/sec
  3. High — >1.0 ft/sec.

- NON-FILAMENTOUS ALGAE: Refers to the areal percent of the substrate sampled which is covered with algae that are not stringy in appearance. Circle the appropriate number.
  1. Sparse — When rocks, bedrock, limbs, trash, etc., are free of attached algae or have only a thin film of greenish or brownish algae that cannot be measured by holding a ruler perpendicular to the surface of the submerged object.
  2. Moderate — When the submerged surfaces have a slight fuzzy or blanketed appearance. The thickness of the attached algae does not exceed 5mm.
  3. Abundant — Submerged surfaces have a definite fuzzy or blanketed (covered with gelatinous mat) appearance. The thickness of attached growth exceeds 5mm.

- FILAMENTOUS ALGAE: Refers to the areal percent of the substrate sampled which is covered with algae that is hair-like or stringy in appearance. Circle the appropriate number.
  1. Absent — 0%
  2. Sparse — > 0% but < 5%
  3. Moderate — 5% to 25%
  4. Abundant — > 25%

COMMENTS Record any useful information that provides insight to the sample collection process, conditions, or miscellaneous information.

4.0 APPENDIX B

STANDARD OPERATING PROCEDURE
Subsampling and Picking Summary

Subsampling and Picking of Macroinvertebrates from field Collected Samples
The waterbody assessment procedure utilized by OCC requires that a random sample of macroinvertebrates be collected, identified and enumerated, from the portion of the waterbody being assessed. In order to make this test cost effective it is not possible to identify more than about 150 organisms from each site. This procedure describes the procedure used to subsample a field-collected sample, which may contain 200-10,000 organisms.

1. **Obtain the Field Sample to be Subsampled.** The field collected macroinvertebrate samples and the original Chain of Custody will be transferred to the individual(s) designated to complete the macroinvertebrate subsampling and picking. At this time a copy of the Chain of Custody (signed by the subsampling designee) will be sent to the Data Manager.

   The sample will come from the field in 1-quart Mason jars preserved with 100% ethanol. Mason jar lids have a sealing compound that is not particularly resilient. Care must be taken so that the lids are not damaged when they are opened or resealed. If a lid is damaged it must be replaced with a new one. Keep a fresh supply of lids handy in case this happens. If you use a new lid, label it exactly the same as the one that was originally used.

   Samples will be subsampled/picked in the order assigned on the Chain of Custody form.

2. **Decant Ethanol.** Without shaking or disturbing the contents, pour the liquid from the sample through a sieve made of #30 or finer screen. Save the ethanol to preserve the unused portion of the sample.

3. **Rinse Sample.** At this point, any silt, clay or fine sand in the sample should be GENTLY rinsed out of the sample. Be careful not to break off any of the delicate appendages that are used for identification of the animals. The sample will be easier to process if any large pieces of leaf, bark, stones, etc., are washed carefully and discarded.

4. **Prepare Sample for Picking.** Spread the selected portion out in a rectangular tray/pan that is divided into 28 sections of equal area. The size and shape of the divisions are not important so long as they are all equal in size. A pan with a white background may facilitate the picking since there will be a contrast between organism and the pan.

   A clear glass pan or baking dish can be effectively used by creating a grid system on the bottom of the pan using a permanent marker. Each square should be numbered (1-28), and a sheet of white paper can be glued or taped over the outside bottom of the pan. If very many samples will be subsampled it will be worth your time to construct a divider for the tray similar in construction to an ice cube tray divider. This will not only demarcate the subsampling squares, but it will also prevent animals from drifting from one square to another during subsampling.

5. **Remove Large Pieces of Detritus and Sediment.** Large leaves and big pieces of wood and bark should be removed. Be VERY CAREFUL to pick all macroinvertebrates off of them before discarding. At this point, the material remaining in the dish should consist of a mixture of sand, fine gravel, small organic detritus, pieces of leaves < 1-2 cm wide, fine roots, algae and macroinvertebrates.

6. **Spread Sample Out.** All detritus and sediment should be as uniformly distributed over the bottom of the dish as possible.

7. **Visually Estimate Invertebrate Density of the Sample.** Determine if the sample must be subdivided. The decision will be based on three requirements: (1) individual squares MUST have AT LEAST 3 animals in them (providing the entire sample has at least 100 animals total), (2) you must pick at least five (5) squares, and (3) each square can have absolutely NO MORE THAN 25 animals. Simulid (blackfly) larvae are not to be counted as individuals for this purpose. That is, the density estimate should be independent of any blackfly larvae present. The goal is to have roughly 10 TO 20 ANIMALS PER SQUARE. This is a compromise between the statistical ideal of very few organisms per square and ease of subsampling where the entire sample is picked from one square. If you estimate that there are less than 200 animals in the entire sample, you should process the entire sample.

The purpose of this estimate is to make the sample statistically valid. Fewer than 80 animals does not provide a good representation of the population to draw conclusions from, and more than 130 animals biases the sample making it appear that that stream has more taxa, that it really does. Correctly estimating densities is something that can only be done with experience, do not become discouraged.

8. **Subdivide the Sample.** If each square is estimated to have more than 25 animals, it is important to grossly subdivide the sample prior to picking. For instance, divide the sample in half or quarters depending on the animal density. Ideally 10 to 20 animals per square is desirable.

   For dividing in fourths, cut the sample into top and bottom halves and then into right and left halves. After dividing the sample, the four portions should appear as equal as possible in terms of the amount of detritus and sediment present. Choose one quarter by random means. For instance, flip a coin to select either the top half or the bottom half, and then flipping the coin again to select either the right or left side of the first half selected. If the sample is not too dense, that is the selected quarter has a density of 10 to 20 animals per square, then no additional subdivision is necessary. If the number of invertebrates is still too dense, divide the remaining portion in half and select one half by flipping the coin again. Continue dividing the sample until the density appears to fall in the correct range.

9. **Return the Unselected Portion(s) to the Mason Jar.** Return the unselected portion(s) of the sample to the original Mason jar and add the reserved alcohol. Be very careful to remove the entire portion of unselected subsample. A proportionately high density of small macroinvertebrates can remain hidden in the sediment and detritus. .

10. **Fill the Tray About 1 to 2 cm Deep With Water.** Add enough tap water to fill the tray to a depth of 1–2 cm (~0.5 to 0.75 inches). The water aids in the subsampling process. The organisms and individual pieces of detritus do not clump together as when they are dry. If the water is run into the tray very slowly, the remaining leaves and large pieces of detritus can be rinsed and discarded.

11. **Distribute the Sample Evenly.** Make sure that all materials in the tray are evenly distributed, especially the gravel and leaves. If a divider is to be used, place it in the tray now. Once the sample has been distributed, do not move the pan. Jostling can cause the organisms to move outside of their designated square. This could lead to a sampling bias.

12. **Fill Out the Macroinvertebrate Picking Data sheet.**
    - **Stream Information.** Record the date of subsampling, the site date and time, stream name, waterbody identification number (WBID #), legal description, Chain of Custody form number (COC#), and sample type (woody, vegetation, riffle).
    - **Estimate the Composition of the Sample.** Exclusive of invertebrates, estimate the composition of the sample according to the following list: silt and clay, sand, fine gravel (<2mm), course gravel (>2mm), woody debris (twigs, bark, roots, etc.), whole leaves, well-rotted pieces of leaves, filamentous algae, and unidentifiable organic material. Record the percentage of each fraction.
    - **Record the Fraction of the Sample That Was Picked.** Record the fraction of the sample that was placed into the tray for sampling –e.g. 1/2, 1/4, or 1/8 of the original Mason jar sample. This is important because the density calculations depend on this number.
    - **List Each of the Squares That Was Picked.** List the number of the square that was picked on the **Macroinvertebrate Picking Data** sheet along with the number of organisms that were picked from that square.

INFORMATION TO INCLUDE	SAMPLE NOTEBOOK PAGE						
Subsampling Date	11/18/99						
Site Date	07/16/99						
Stream Name	Griever Creek						
Site Time	13:10						
Legal Description & County	E 9 T22N R15W, Major County						
WBID #	OK620920-01-0130g						
COC #	COC# 1772						
Sampling Type	Riffle kick						
Sample Description	40%fine gravel	10% film. algae	30% well-rotted leaves				
	10% whole leaves	5% woody debris	5% coarse gravel				
Amount of Sample Picked	¼ of the original sample was prepared for picking						
Squares Picked	Square #	1	12	3	23	28	10
# of animals found in each square	# picked	15	27	10	18	8	24
Subsampler's name	John Hassell						

13. **Randomly Select 6 Squares.** Using some method to generate a series of random numbers (random number generator or number table), select at least 6. The random number generators found on most pocket calculators or the Excel spreadsheet function will give a series of three digit numbers—usually >0 but <1. For OCC purpose, use only the last two numbers. Starting with the first random number generated, record all numbers between 01 and 28 until there are 6. These numbers represent the numbered squares to pick. Picking must follow the order in which the number were generated.

14. **Confine the Organisms to the Selected Square.** Place rectangles or squares constructed of clear plastic or other material that are the same or greater height as the water in each square. This will keep the organisms from drifting out of the squares during the picking process. If a large piece of detritus (leaves, roots, algae masses) crosses the boundary of two or more squares, it may be sliced along the edge of the square so it is contained with the boundary of the selected square.

    If there are any organisms that cross square boundaries, do not cut them. Place them in the square in which their head is already lying.

15. **Pick <u>All</u> the Invertebrates Out of the First Square Selected.** Locate and collect all the organisms in the selected square. Keep track of the number of non-blackfly organisms picked. Place the organisms picked in a scintillation vial that is filled up to the neck with 70-100% ethanol. If any large organisms (that are too big to fit in the vial with the other organisms) are picked such as crayfish or hellgrammites, place them in a separate vial. If there is some question if something is an organism, place it in the vial but <u>DO NOT COUNT</u> it as part of the total. Place five (5) blackflies and five (5) scuds in the vial but <u>DO NOT COUNT</u> them as part of the total. Pickers should be trained to identify blackfly larval forms—if the picker cannot identify blackflies, they should not be subsampling without further training. Note the general abundance of blackflies and scuds in the comments.

    When all of the organisms are picked out of the square, record the number of non-blackflies that were picked from that square under the number of that square on the data sheet. See example page listed above.

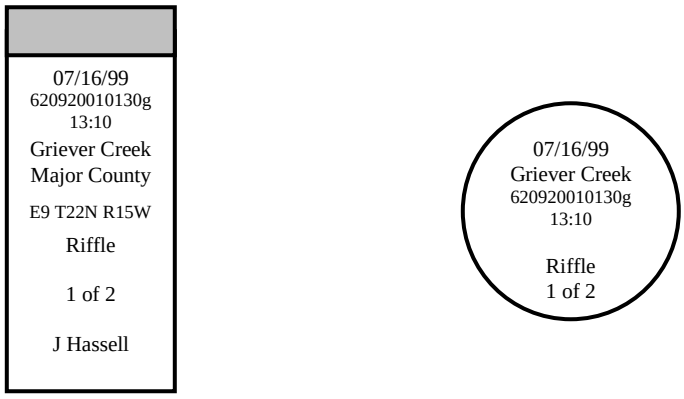
16. **Continue to Pick Squares Until 100 Organisms Have Been Collected.** Using the random number list, continue to select squares until 100 (non-blackfly) animals have been collected. Once a square has been started, all of the animals must be collected from that square. A subsample will typically have 100-130 organisms / vial. If there are more than this, the sample was not properly subdivided. If the sample has more than 150 organisms in it, it must be mixed back in with the rest of the "unpicked sample" and re-picked. If the finished invertebrate sample has less than 80 organisms

in it and there is unpicked sample available, another tray full of sample must be picked. The ONLY time that it is acceptable to have a sample with less than 100 organisms is when the entire Mason jar of material has been picked. All other samples containing less than 100 invertebrates will be rejected as data with bad QA/QC.

17. **Label the Vial(s).** Using a pencil and a fine point permanent ink marker label the vial. The top and side of the vial should both be labeled.

    The vial should be labeled in pencil on waterproof paper taped to the vial after labeling with the following information:
    - Stream name
    - WBID #
    - Legal location and County
    - Site Time
    - Site Date
    - Type of sample
    - Number of vials for this sample (e.g. 1 of x, where x = total number vials for one site (Mason jar))

    The cap should be labeled in permanent ink with the following information:
    - Stream name
    - Site time
    - WBID #
    - Site Date
    - Type of collection
    - Number of vials for this sample



Scintillation Vial Scintillation Vial Cap

Figure 1: Example label for bug picking sample.

18. **Place Clear Tape Over the Label.** To protect the label from the ethanol, place clear tape (scotch tape) over the writing (pencil).

19. **Transfer Picked Samples to the Taxonomist**. Once macroinvertebrate samples have been picked and placed in a properly labeled scintillation vials, the vials and the original Chain of Custody form will be transferred to the laboratory for taxonomic identification. The original Chain of Custody form should be returned to the Data Manager with the signature of the taxonomist or laboratory custodian.

20. **Archive Remaining Sample.** The remainder of the field collected macroinvertebrate samples (un-picked) should be delivered to Nathan Carter for archive purposes. The Chain of Custody form number should be conspicuously labeled on the outside of the box.