WATER QUALITY PROGRAMS DIVISION

Standard Operating Procedure for the Collection of Macroinvertebrates in Streams

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STANDARD OPERATING PROCEDURE FOR THE COLLECTION OF MACROINVERTEBRATES IN STREAMS[1]
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1.0 General Information

Most free flowing water bodies, with acceptable water quality and habitat conditions, support diverse macroinvertebrate communities in which there are 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, the flowing water community is more suitable for assessing the condition of a stream, than by 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 root masses, and woody debris. Where possible, a rocky riffle should be sampled, but if it is not present, or is of dubious quality, if rocky riffles cannot be found at all streams of a given ecoregion, both of the other two alternate habitats should be sampled. The sampling methodology for the three habitat types is included in this SOP.

[1] Much of this SOP is adopted from two documents: Oklahoma Water Resources Board (1999) Technical Report 99-3: Standard Operating Procedures for Stream Assessments and Biological Collections Related to Biological Criteria and Development, Oklahoma City, OK; Oklahoma Conservation Commission (2001), Standard Operating Procedure for Macroinvertebrate Collection, Subsampling, and Picking. Oklahoma City, OK.

Invertebrate 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 means that collections made during different times of the summer tend to be the same in lieu of water quality differences. This period of the summer when collections from different streams can be compared to each other is termed the Summer Index Period.

2.0 Definitions/Terms

- Team Leader—crew member of fish collection team who provides support, expertise, and opinions; gives instruction and has final say on how work will be done; must score a 95% on critical fish identification
- Team Member—crew member of fish collection team who provides support, expertise, and opinions; follows the instructions of the team leader
- 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.
- Streamside Vegetation—any streamside vegetation that offers fine structure for invertebrates to dwell within or upon that receives suitable flow. Most habitats are 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—July 1 to September 15.
- Ethanol—preservative used in macroinvertebrate collections. Proper precautions should be taken when handling 100% ethanol. It is flammable, an intoxicant, and an eye irritant.

3.0 Safety

Upon reaching the sampling location, site safety determinations should be made before proceeding. Please refer to the OWRB safety manual for instructions. During most fish collections a backpack electrofisher or 2.5 GPP pram/boat electrofisher will be used. Because electrofishers send an electrical current through the water, not following safety procedures may result in serious injury or death. General safety guidelines include:

- Proper precautions should be taken when handling 100% ethanol. It is flammable, an intoxicant, and an eye irritant.
    - Protective gloves and eyewear should be worn
    - Avoid inhalation of vapors

4.0 Quality of the Measurement

4.1 Training

Principle investigators for the OWRB are required to have degrees and/or experience with biological or other applicable sciences. Principle investigators are defined as crew leaders, and this designation may be made upon the leader of a multi- or a one person crew. Training is required for all SOPs dealing with water quality and quantity collections and measurements as well as habitat assessments and biological collections. In-house training will be conducted for the use of all meters and digital titrators used for water quality or quantity measurements. Investigators must be familiar with OWRB SOP document and all training will follow the methods outlined in that document. Extra training will be provided when new SOPs are developed. Training of field crews will be done through dry run exercises in the laboratory to familiarize field crews with sample collection, sample preservation, instrument operation, calibration, and maintenance. In addition, when new personnel are hired or new methods developed, qualified staff will train on sample collection, measurement, and field analysis methods through side-by-side field trips. These trips will familiarize staff with SOP requirements. When training is considered adequate, a qualified staff member will check field staff for adherence to SOPs. Prior to collecting macroinvertebrates, subsampling, and picking, all staff should familiarize themselves with this SOP and OWRB Technical Report 99-3 Standard Operating Procedures for Stream Assessments and Biological Collections Related to Biological Criteria and Development.

4.2 Kinds of Quality Assurance Samples

4.2.1 Replicate Collections

Replicate collection samples and duplicate subsampling and pick samples will be collected during each biological season. The scope and number of replicates will be determined by the project Quality Assurance Project Plan. They may include replicates for various habitat or stream order.

4.2.2 Vouchers and Photodocumentation

All unique reaches and special or unusual circumstances should be photodocumented.

4.2.3 Certification of Personnel for Laboratory Subsampling

Benthic Macro-Invertebrate (BMI) samples are collected in the field, in accordance with Standard Operating Procedures. BMI samples returned to the laboratory consist of large amounts of detritus, algae, and other organic matter. Processing BMI data requires sorting through this Organic matter, in order to remove BMI for taxonomic identification. Many BMI are not easily recognized in and amongst the organic matter present in field-collected samples. In order to minimize variance in data created when sorting, Quality Assurance/ Quality Control measures will be implemented.

Quality Control of laboratory sorting will consist of a certification process for anyone who will sort field collected BMI samples. If Field samples are sorted by anyone not certified,

a certified person must search the sorted organic matter for BMI before the sample is logged. No person, not certified, will discard any organic matter coming from a field sample that has not been inspected by a certified individual.

To minimize variability in BMI metrics there is a standard of quality, which will be required for certification. In order to be certified an individual must sort 4 out of 5 samples with out overlooking more than 10 percent of all BMI present in the sorted portion of a field collected sample. The individual seeking certification should not discard any organic matter he/she has sorted. This organic matter will be saved and reviewed by a certified individual.

All organic matter sorted by an individual, seeking certification, will be kept in a zip lock bag and labeled with appropriate site information. The certifier will then examine the remaining organic matter for any invertebrates. A check will be run to determine if the sample will receive a passing mark. A minimum of 90% of the total number of individuals should be found by the individual seeking certification, in order to receive a passing mark. Additionally, one annual random QA check will be performed in each of the two years following certification. The same checks will be made to determine a passing mark. An index card (5" x 7") will be filled out to track the progress of each person seeking certification. Table 4.1 details the information required on the index card.

Table 4.1: A template for Index card to Track progress of employee seeking certification for sub-sampling macroinvertebrates.

Picker's Name:_____ Site Name:_____
Section:_____ Project Code:_____

Date	Habitat type	# individuals	Certifier's Initials	# Individuals	% missed	Taxonomic groups missed	Pass/Fail

Annual QA check: Pass or Fail Certifier's Initials:_____
Annual QA check: Pass or Fail Certifier's Initials:_____

5.0 Personnel and Equipment
5.1 Personnel

Macroinvertebrate collection crews may consist of one to two people. The team will consist of a team leader and possibly one team members. The team leader is someone with one or more seasons of collection experience. Collection experience in other programs may be substituted for that with the OWRB. The team leader will have the final say on all crew activities. A team member is someone trained on macroinvertebrate collection, subsampling and picking protocols. Team members will be expected to participate in the decision-making and follow the team leaders direction.

5.2 Equipment and Supplies
5.2.1 Kick Net

OWRB uses a 1 m^2 kick net with 500 micron mesh. The kick net is composed of the net, brailles inserted and tied to the net, and a bottom leadline. The team leader will provide a detailed explanation of how to use the net. The kick net is used in riffles and only in flowing water. Bugs are collected by disturbing a 1 m^2 area upstream of the net by overturning, scrubbing, and agitating all material within the area. The small mesh selects for all bugs within the habitat.

The net should be checked regularly for holes and should be stored dry and free of snags and debris.

5.2.2 Dip Net

OWRB uses a modified dip net (a.k.a. D-net) with 500 micron mesh. The net is composed of a net attached to a D-ring and a one to three foot handle. The net is shrouded to protect against snags and increase longevity. The team leader will provide a detailed explanation of how to use the net. The dip net is used in streamside vegetation and woody debris and is only used in flowing water. Bugs are collected by disturbing the underside of root wads, undercut banks, wood, etc. The small mesh selects for all bugs within the habitat.

The net should be checked regularly for holes and should be stored dry and free of snags and debris.

5.2.3 General Supplies

Chemicals
Ethanol

Nets
Fishing line or dental floss to repair nets
Spare net

Containers

Wide mouth 1-quart polyethylene bottles and lids
Large bucket for instream subsampling

6.0 Collection of Macroinvertebrates

Before beginning collection, 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 too 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.

The collection of macroinvertebrates involves collection in three possible lotic habitats—riffles, streamside vegetation, and woody debris. The combination of methods was selected in order to produce a representative macroinvertebrate collection. Because Oklahoma streams vary widely in substrate, riffle habitats may not always be available.

In general, each stream is sampled for a distance of 400 meters. A representative stream reach is selected and measured such that all available habitats are sampled. The reach should also include representative microhabitats for streamside vegetation. Very small streams may be sampled at 200 meters if all representative habitats are in that reach. Larger rivers with long pools or runs and braided rivers may be sampled for up to 800 meters. Again, ensure that all representative habitats are in that reach. The reach should be located away from the influences of major tributaries and bridge/road crossings. Record reach length on the Field Notes.

Because streamside and woody habitats are timed collections and riffle kicks involve the use of three riffles, collections may be quite large. After exclusion of as much organic matter as possible, infield subsampling should be done to decrease the amount of each collection to no greater than 75% of a 1 quart sample bottle. This method is described is subsequent subsections. This sample is then subsampled and picked in the laboratory.

Sequence for collection is determined by site characteristics. In general, an appropriate riffle should be identified and sampled first. Subsequently, streamside vegetation can be sampled by going up the reach and woody debris sampled on the return trip.

6.1 Collection from Rocky Riffles

6.1.1 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.

6.1.2 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.

6.1.3 Method of Collecting the Sample in both Wadeable and Non-wadeable Waterbodies

Support a 1-m2 kick net composed of a double layer of fiberglass window screen or a net of number 30 mesh in such a way that the current will carry any organisms dislodged from the substrate into it. 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 jar three fourths (3/4) full or less, remove all of the material from the net and place it in the jar. In no case should the 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. **Record the number of sample divisions that are made. This number will be used to calculate back to the number of individuals in the sample.**

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

6.2 Collection from Streamside Vegetation

6.2.1 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.

6.2.2 Where to 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.

6.2.3 Method of Collecting the Sample in Wadeable Streams

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 jar three fourths (3/4) full or less, remove all of the material from the net and place it in the jar. In no case should the 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. **Record the number of sample divisions that are made. This number will be used to calculate back to the number of individuals in the sample.**

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

6.2.4 Method of Collecting the Sample in Non-wadeable Rivers

Sampling should continue for **6 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 jar three fourths (3/4) full or less, remove all of the material from the net and place it in the jar. In no case should the 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. **Record the number of sample divisions that are made. This number will be used to calculate back to the number of individuals in the sample.**

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

6.3 Collection from Woody Debris

6.3.1 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.

6.3.2 Where to 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.

6.3.3 Method of Collecting the Sample in Wadeable Streams

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 jar three fourths (3/4) full or less, remove all of the material from the net and place it in the jar. In no case should the 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. **Record the number of sample**

divisions that are made. This number will be used to calculate back to the number of individuals in the sample.

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

6.3.4 Method of Collecting the Sample in Non-wadeable Rivers

Sample for total of **10 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. Be sure to sample all wood sizes including large dead trees and logjams.

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 jar three fourths (3/4) full or less, remove all of the material from the net and place it in the jar. In no case should the 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. **Record the number of sample divisions that are made. This number will be used to calculate back to the number of individuals in the sample.**

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

6.4 Sample Handling & Preservation

- **CAUTION: Proper precautions should be taken when handling 100% ethanol. It is flammable, an intoxicant, and an eye irritant. Protective gloves and eyewear should be worn. Avoid inhalation of vapors.**

    1. **Pack the Jar Properly.** In no case should the 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 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
Stream Name
Habitat Type
Date of Collection
Time of Collection
Name of Collector(s)
County
Project Code

3. **Complete the Chain of Custody.** A Chain of Custody logbook is kept in the OWRB laboratory. Each jar should be entered into the COC as an individual sample. A sample number is assigned to each jar as it is registered on the COC. Sample numbers are added to labels and lids. Once samples are assigned a sample number and registered in the COC, they are stored in a refrigerator in the OWRB laboratory. Anytime a sample is removed from the refrigerator the COC will be updated to reflect this movement and track the sample.

4. **Transfer samples to the Macroinvertebrate Sample Custodian.** Correctly labeled macroinvertebrate samples, along with a COC form, should be transferred to the Project Manager 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.

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.

6.5 Subsampling and Picking of Macroinvertebrates from field Collected Samples

The waterbody assessment procedure utilized by OWRB requires that a sample of macroinvertebrates be collected, identified and enumerated, from a 400-meter 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 process used to subsample a field-collected sample, which may contain 200-10,000 organisms.

1. **Remove a Field Sample from Storage.** The field collected macroinvertebrate samples will be transferred to the individual(s) designated to complete the

macroinvertebrate sub-sampling. The individual who performs sub-sampling will initial and date the COC to indicate that a sample has been removed from the refrigerator. This individual is responsible for this sample until it is returned to the refrigerator and the COC is updated to annotate the samples return from sorting.

Samples will be sub-sampled in the order assigned on the COC form. If necessary to meet a reporting deadline or a data request, adjustments to this order may be made by a supervisor or project lead.

2. **Rinse Sample.** Without shaking or disturbing the contents, pour the liquid from the sample through a sieve made of #35 or finer screen. 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 removed from the sample.

3. **Remove Large Pieces of Detritus and Sediment.** Large leaves and big pieces of wood and bark should be removed. Visually inspect all large detritus and pick all macroinvertebrates before removing detritus. At this point, the material remaining in the sieve should consist of a mixture of sand, fine gravel, small organic detritus, pieces of leaves < 1-2 cm wide, fine roots, algae and macroinvertebrates. Make sure to rinse away all silt and small sediments. Drain as much water as possible out of the sieve before transferring material to gridded tray for homogenization.

4. **Homogenize the remaining substrata.** Spread the sample out in a rectangular tray that is divided into 28 equally sized squares. A pan with a white background will help contrast between the organisms and the pan. A red permanent marker is used with a straight edge to delineate square boundaries. Each square is assigned a number 1-28. Numbers do not need to be permanently marked on the pan. A diagram assigning each square a specific number will suffice. All detritus and sediment should be as uniformly distributed across the pan as possible. No water should be added to the sample at this point. Keeping the pan as dry as possible will prevent macroinvertebrates from drifting from square to square.

   At this point the remaining sample material should be spread out across the sampling grid. The composition of substrata in each square should be equal to one another, as well equal to the entire tray. For example if the whole sample is determined to contain 25% rotted leaves and 75% whole leaves, then 25% of each square should be rotted leaves and 75% should be whole leaves.

5. **Fill Out Sub-sampling/Picking Form.**
    - **Sample Number**. Write the sample number corresponding to the entry line in the Chain of Custody.
    - **Site Name.** Write the name of the site as it is written on the sample jar.
    - **Habitat Type.** Riffle, Streamside Vegetation, or Woody Debris.
    - **Project Code.** Write the appropriate project code here.
    - **County.** Write the Oklahoma County in which the sample was collected.
    - **Legal Land Description.** Write the section number, township, and range associated with the stream reach where the sample was collected.
    - **Collector.** Write the name of the person who collected the sample.
    - **Collection Date.** Write the military date when the sample was collected.
    - **Collection Time.** Write the 24-hour military time the sample was collected.
    - **Name of Sorter.** Write the name of the person who performed the Sub-sample.
    - **Date Sub-sampled.** Write the military date that sub-sampling occurred.
    - **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, rotted pieces of leaves, filamentous algae, and unidentifiable organic material. Record the percentage of each fraction.
    - **Square number.** Record the number of each square that was randomly selected for sorting.
    - **List the Number of Animals Picked from each square.** Record the number of individuals removed from each square.
    - **Simuliidae picked.** Record the number of black fly larva removed from each square.
    - **Decapods picked.** Record the number of crayfish removed from each square
    - **Large and Rare scan.** Record the number of individuals removed from the sample during the Large and Rare scan.

6. **100 count sub-sample.** Using the randomly generated numbers list provided in the macroinvertebrate data folder, select the next number from the list. Cross this number off. The contents of the selected square may be moved to a white sorting pan. In some instances organisms will be found with part of their body in one square and the rest in another. When this occurs move the organisms to the square in which, its head is found. Visually inspect the square in the gridded tray with a 10x lens to make sure no organisms were left in the selected square. Water may be added to the sorting pan to “float” organisms and assist in locating them. Locate and collect all the organisms in the sorting pan.

    Keep track of the number of organisms picked. Place the organisms picked in a scintillation vial that is filled up to the neck with 80% ethanol and labeled “100

Count Sub-sample". If any large organisms (too big to fit in the vial with the other organisms) are picked such as *Corydalidae* (hellgrammites), place them in a separate vial filled to the neck with 80% ethanol and labeled "100 count sub-sample (LG)". If the number of organisms, placed in the scintillation vial does not exceed 100, then select the next number from the provided randomly generated numbers list. If the next number on the list corresponds to a square already removed from the current sample, then cross the number off and move to the next number on the list. Continue to remove the contents of each selected square, placing the contents in an empty white sorting pan. Remove all organisms from each selected square. When the number of organisms removed exceeds 100, continue to remove all organisms from the currently selected square. When all organisms have been removed from all randomly selected squares and the number of organisms removed exceeds 100, the 100-count sub-sample is complete. Label the "100 Count Sub-sample" vial appropriately and seal the vial. If any large organisms (too big to fit in the vial with the other organisms) are picked such as *Corydalidae* (hellgrammites), then label the vial appropriately and seal the vial.

Special Considerations for 100 count sub-sample:

Black fly (Simuliidae) larvae:

Occasionally, field samples are dominated by Simuliidae (Black fly) larvae. It is argued that due to patchy distributions in nature, the entire stream reach may not truly be dominated by black flies. However, this patchiness may cause sub-samples to be inaccurately over populated by black fly larvae. This will in turn create a sub-sample not representative of the true population. Some protocols have been designed to account for this by sub-sampling 100 non black fly organisms (Oklahoma Conservation Commission 2001). It is also argued that exclusion of black flies from the 100 count will result in a sub-sample not representative of the true population. The Oklahoma Water Resources Board is currently investigating the effects that excluding black fly larvae will have on metrics used to describe macroinvertebrate community structure. To help facilitate this investigation an extra step is currently being used when performing the 100-count sub-sample. This extra step will allow metric to be calculated with and without black fly larvae.

Currently, black flies WILL be counted towards the 100 count. The number of black fly larva included in the 100 count will also be recorded. Once all organisms have been removed from all randomly selected squares and the number of organisms removed exceeds 100, the vial these organisms have been placed in is to be sealed and labeled "100 count sub-sample". At this point DO NOT move to the Large and Rare Scan.

Before moving to the large and rare scan, count the number of black fly larva included in the 100-count sub-sample. If the number of non black fly organisms

removed, during the 100 count sub sample, exceeds 100, then there is no need for a further step. You may now move to the Large and Rare scan.

If the number of non black fly organisms removed, during the 100 count sub sample is less than one hundred, then continue sampling. Move to the next square, on the randomly generated numbers list, and remove all NON black fly larvae, counting them as you go. These organisms should be placed in a new vial filled to the neck with 80% ethanol and labeled "Non Black Fly Organisms". When all non black fly organisms have been removed from a square, add the number of non black fly larvae in both vials. If the number of non black fly organisms does not exceed 100, then continue to sub-sample all non black fly organisms by moving to the next square. Continue this process until the number of non black fly organisms exceeds 100. When the number of non black fly organisms exceeds 100, continue to remove all non black flies from the current square. Now label the "Non Black Fly Organism" vial appropriately and seal the vial.

Decapoda (Crayfish)

Dichotomous keys used to determine taxonomic designation for crayfish are written describing sexually mature males. To further complicate crayfish taxonomy, mature males change forms seasonally. Reliable keys used to identify 288 recognized species of crayfish, describe only breeding males (Form I). Additionally, 130 species have been described and recognized since the last revision of the most widely accepted key (**Horton H. Hobbs, Jr. 1972. Biota of Freshwater Ecosystems: Identification Manual No. 9. Crayfishes (Astacidea) of North and Middle America. For the Environmental Protection Agency, Project # 18050 ELD.)** The vast majority of crayfish collected are not Form I males and can not reliably be identified.

Many small macroinvertebrates (e.g. Chironomidae) are often found attached to crayfish bodies. In order to avoid organisms attached to undersides of crayfish being overlooked, all crayfish are removed from selected squares and visually inspected for attached invertebrates. Crayfish ARE counted as part of the 100-count sub-sample. Invertebrates removed from crayfish bodies during this inspection are counted towards the 100-count sub-sample and tallied towards the square from which the crayfish was removed. The crayfish are placed into a separate vial filled to the neck with 80% ethanol and labeled with the appropriate sample number and the designation "Decapoda". Crayfish not found in selected squares are NOT removed from the gridded tray.

When all organisms have been removed from all randomly selected squares and the number of organisms removed exceeds 100, label the "Decapoda" vial appropriately and seal it. Crayfish are stored in a refrigerator in the OWRB laboratory. They are not sent to contracted taxonomists. Unless further reliable taxonomic resolution can be determined, crayfish are designated Decapoda for metric calculations.

7. **Large and Rare Scan.** Studies have shown that the number of organisms used in a fixed-count sub-sample has an effect on richness metrics used to describe community structure (Growns et al. 1997). Countermach (1996) suggests that the chance of removing rare species, during fixed-count sub-sampling, increases as the number of total organisms removed increases. Debate over the optimal number of organisms to remove in a fixed-count sub-sample is ongoing in current literature and professional societies. Due to the increased cost of removing more organisms in a fixed-count sub-sample, many bio-assessment protocols use a lower number and a "Large and Rare Scan". The purpose of this scan is to include large and rare species in sub-samples, without dramatically increasing resources needed to complete the sub-sample. The OWRB has adopted this practice and the protocol is described in this section.

   The entirety of contents remaining in the gridded tray will now be scanned for large and rare organisms. Water may be added to the gridded tray to "float" organisms and assist in their location. Large organisms, for purposes of this scan, are defined as organisms large enough to be located without sorting through sample material. Rare organisms, for purposes of this scan, are defined as species not included in the 100-count sub-sample. The designation of "rare" species is more easily determined as experience sub-sampling increases. If you are not 100% certain if a species is rare or not, then consider rare.

   Spend three minutes inspecting the remaining sample material for large and rare organisms. Place all organisms removed during the large and rare scan into a new scintillation vial, filled to the neck with 80% ethanol and labeled "Large and Rare Scan". After three minutes have elapsed label the "Large and Rare Scan" vial appropriately and seal the vial.

8. **Label the Vial(s).** Each vial should be labeled on the lid, the side, and the interior. Use a fine point permanent ink marker to label each lid. Use a white sticky label on the side of each vial. Use an indelible ink pen and "write on rain" paper to create the interior label.

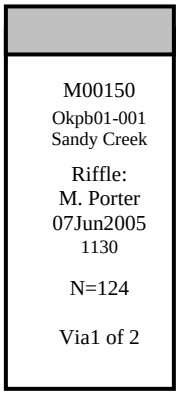
   The lid of each vial should be labeled with the following information:
   - Sample number
   - "100 ct. sub-sample", "100 ct. sub-sample (LG)", "Non Black Fly Organisms", "Decapoda", or "Large and Rare Scan"
   - Vial number (e.g. 1 of x, where x = total number vials for the sample number)

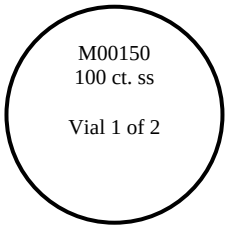
   The side of each vial should be labeled with the following information:
   - Sample number
   - Project Code
   - Water body name
   - Habitat type (riffle, woody, vegetation)

- Name of collector, Date of collection, and Time of collection
- Number of individual organisms placed in the vial
- Number of vials for this sample (e.g. 1 of x, where x = total number vials for the sample number)

The interior label should contain the following information:

- Sample number
- Project Code
- Water body name
- Habitat type (riffle, woody, vegetation)
- Name of collector, Date of collection, and Time of collection
- Name of Sub-sampler
- Date of Sub-sample
- Number of individual organisms placed in the vial
- Number of vials for this sample (e.g. 1 of x, where x = total number vials for the sample number)





Scintillation Vial Scintillation Vial Cap

Figure 1 Example labels for vial lids and exteriors.

9. **Place Clear Tape Over exterior Labels.** To protect the label from the ethanol, place clear tape (scotch tape) over the lid and side labels. This also ensures that the sub-sample has not been tampered with after it was completed.

10. **Return Sub-sample vials to Storage**. Once macroinvertebrate samples have been picked and placed in properly labeled scintillation vials, the vials will be transferred to the laboratory refrigerator for storage. The individual who performed the sub-sample should update the COC, indicating the samples return to storage and the number of vials associated with the sample number. The

responsibility for tracking of sub-sample vials is now returned to the Macroinvertebrate sample custodian.

11. **Remaining Sample.** The remainder of the field collected macroinvertebrate samples can now be discarded. No sample material will ever be discarded until an individual who is QA/QC certified for sub-sampling has investigated and signed off on the sub-sampling procedure.

7.0 Forms

7.1 Field Notes

Field notes are documents used to annotate and record information that is gathered at the project site. They are a data sheet and should be treated as such. Therefore, they should be written, legible, and complete. To avoid confusion and loss of data, a new sheet should be used at each new project site. Field notes should be initialed and dated by the collecting personnel and data entry personnel. For guidance on proper procedure to complete the field notes, refer to your supervisor and or FTE. Field notes can be found at S:\Monitoring\STREAMS\forms\. All field notes should be relinquished to the macroinvertebrate sample custodian, for appropriate filing and storage.

7.2 Laboratory Log-in Sheets

Log-in sheets are documents turned into the analytical laboratory for each sample collected. These forms are used to denote the parameters that should be analyzed. They are a data sheet and should be treated as such. Therefore, they should include the date and time of sample collection and be legible and complete. To avoid confusion and loss of data, a new sheet should be used at each new project site. For guidance on proper procedure to complete the log-in sheets, refer to your supervisor and or FTE. Log-in sheets can be found at S:\Monitoring\STREAMS\forms\.

7.3 Chains of Custody

Chains of custody are documents turned into the analytical laboratory for each group of samples collected. These forms are used for several purposes. They act as a legal document to show proper delivery of samples occurred and they make a general list of the parameters that should be analyzed. They are a data sheet and should be treated as such. Therefore, they should include the date and time for each sample collected and be legible and complete. They should also be signed and dated by field and laboratory receiving personnel at the time of delivery. To avoid confusion and loss of data, a new chain of custody should be used for each group of samples. For guidance on proper procedure to complete the chains of custody, refer to your supervisor and or FTE. Chains of custody can be found at S:\Monitoring\STREAMS\forms\.

8.0 Data Storage

All completed paper copies of forms and data sheets should be maintained with the appropriate station notebook. The data from the field notes and laboratory data sheets should be either entered into or uploaded to the Water Quality Biological Database.

Each sample should be maintained electronically in the database under a unique sample number.

9.0 References

Countermach, D.L. 1996. Commentary on the subsampling procedures used for rapid bioassessmens. Journal of the North American Benthological Society 15:381-385.

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Growns, J.E., B.C. Chessman, J.E. Jackson, AND G. Ross. 1997. Rapid assessment of Australian rivers using macroinvertebrates: cost and efficiency of 6 methods of sample processing. Journal of the North American Benthological Society 16: 682-693.

Oklahoma Water Resources Board (1999) Technical Report 99-3: Standard Operating Procedures for Stream Assessments and Biological Collections Related to Biological Criteria and Development, Oklahoma City, OK.

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