CHAPTER 5
FRESHWATER BENTHIC MACROINVERTEBRATES

Objective

The objective of this chapter is to describe the methods used by the TCEQ for the collection and assessment of benthic macroinvertebrate samples from freshwater systems. In general, the TCEQ uses benthic macroinvertebrate samples collected according to these methods in conjunction with fish community surveys and physical habitat assessments in an attempt to provide a holistic evaluation of the health of instream biological assemblages. Benthic macroinvertebrate samples collected from freshwater rivers and streams using the rapid bioassessment protocols (RBPs) are currently used by TCEQ programs in establishing the appropriate ALU for unclassified water bodies as well as for evaluating the appropriateness and/or attainment of the existing ALU for classified water bodies.

Scientific Collection Permit

Aquatic insects are not protected under state law; however, a SCP is required for the collection of certain benthic macroinvertebrates. This requirement applies to certain protected native mussels and amphipods as well as to oysters, shrimp, clams, mussels, and crabs that are subject to license requirements, possession limits, means and methods of take, and size restrictions. If native mussels are included in a benthic macroinvertebrate sample, the collector is encouraged to report this information along with annual fish lists to the TPWD. Contact the TPWD for information on protected benthic organisms.

Overview of Sample Collection Methods

The TCEQ currently uses the following primary techniques to collect benthic macroinvertebrate samples from freshwater systems:

- Wadeable streams and rivers
  - Riffles and runs/glides
    - RBPs
      - *5-minute kicknet*
      - *Snag sampling*
    - "Quantitative" protocols
      - *Surber sampler*
      - *Snag sampling*
- Lakes, reservoirs, and depositional zones of streams and rivers (pools)
  - Grabs: Ekman, Ponar, or Van Veen

RBPs were originally developed as cost-effective screening tools for evaluating the biotic integrity of benthic macroinvertebrate assemblages. Benthic macroinvertebrate samples are usually collected with a D-frame kicknet, preferably from riffle habitat, or secondarily, from run or glide habitats by kicking and disturbing the streambed, hence the name "kicknet." Dislodged material and associated benthic macroinvertebrates are collected in the net. In deeper streams, or in shallow wadeable streams with relatively unstable sand or silt bottoms, RBP samples can be collected from snag habitats. Snags consist of woody debris, stems of emergent vegetation, and roots of riparian vegetation exposed to flow. Snag samples are collected by gathering loose woody debris and, if necessary, by using lopping shears to remove sections of exposed roots along the stream banks.

Alternatively, benthic macroinvertebrate samples may be collected using a Surber sampler. The Surber sampler is listed as a "quantitative" technique since the use of a Surber sampler allows results to be expressed per unit area; for example, numbers of individuals per square meter.

Similarly, "quantitative" snag samples may be collected which will provide a density estimate. However, because benthic macroinvertebrates exhibit a clumped distribution, resulting in high variability in the number of individuals per unit area, density results for both methods are difficult to interpret. Also, since the methodology requires all benthic macroinvertebrates to be picked from the sample, the method is highly labor intensive. Surber samplers are used mainly for special studies where an objective is a density estimate.

Detailed methods for each of these collection techniques are described in other sections of this chapter.

Equipment

The field equipment and materials necessary to conduct freshwater benthic macroinvertebrate sampling are listed in Appendix A. Forms needed for biological assessments are included in Appendix C. Electronic copies of all the tables and forms in the appendices are available on the TCEQ's SWQM web site at: <www.tceq.state.tx.us/goto/swqm/resources>. Technical terms are defined in Appendix F.

Records

In addition to sample labeling requirements as specified in this chapter, maintain the following records:

Field logbook. For each freshwater benthic macroinvertebrate sample event, record in a field logbook all relevant information, including the date and time of sample collection, the location of the sample site (Station ID), collector,s name(s), method of collection, number and type of samples collected, number of sample containers, and preservative used.

Sample tracking logbook. Maintain a sample tracking logbook that contains the information described in the QA chapter of this manual. This logbook documents when samples arrive at the laboratory or headquarters facility, when each sample enters each sample processing step, and who has custody or responsibility for the sample.

Laboratory bench sheets. Laboratory bench sheets, as described in the QA chapter of this manual, are maintained at the location where specimen identification and enumeration occur. These bench sheets document the raw numbers of individuals for each taxon and notes relevant to the identification and enumeration process.

Wadeable Streams and Rivers

Note: These procedures apply to benthic macroinvertebrate samples collected with the intent of using the data in conjunction with either the RBP *benthic macroinvertebrate IBI* or the *benthic index of biotic integrity* (BIBI) for Surber samples to make an ALU determination or evaluate an existing ALU. They may not apply for special studies with other objectives that do not involve making an ALU determination, such as assessing the differences between benthic macroinvertebrate assemblages on bedrock versus sand substrates, or comparing pool benthic macroinvertebrate assemblages to riffle assemblages.

Where to Collect Samples

While the assessment of the biotic integrity of fish assemblages is based on a sample collected from multiple habitat types in a reach of a stream or river, benthic macroinvertebrate biotic integrity assessments are usually based on a sample collected from a single habitat type within a stream reach. An exception would be a benthic snag sample that might be collected across more than one habitat type. Since the benthic macroinvertebrate assemblage can vary considerably in response to changes in the character and quality of the physical habitat, careful consideration

must be given to where to collect a benthic macroinvertebrate sample within a stream reach with multiple habitat types. The overall objective must be to collect the sample from the optimal benthic macroinvertebrate habitat within the reach, in other words, the best physicochemical conditions in reference to the needs of the benthic macroinvertebrate assemblage. Once the habitat, fish, and benthic macroinvertebrate sampling crew leaders have agreed where sampling will be conducted, the habitat crew marks the ends of the reach with bright survey flagging. Sampling from areas outside those boundaries is discouraged.

There are three general habitat types in streams: riffles, runs and glides, and pools. These are *listed in order of preference for collecting benthic macroinvertebrate samples in streams*.

Riffles

Riffles are characterized by relatively fast-moving water, relatively shallow depth, and a water surface usually "broken" by flow over rocks, logs, or other similar obstructions (Platts et al. 1983). In most streams, the riffle habitat provides the optimal habitat for benthic macroinvertebrates. The rapid, turbulent flow facilitates reaeration of the water and optimal respiratory function, especially for those benthic macroinvertebrates that rely on water movement for respiration needs. The rapid water movement also provides a constantly renewed source of food for filter-feeding macroinvertebrates, as well as nutrients for primary producers. The shallow depth typically allows the development of attached algae, which provides an important food and microhabitat resource. Often, because of the many microhabitats found in riffles, habitat heterogeneity is greater than that found in runs or pools. A riffle microhabitat includes small eddy pools that are created behind obstructions, such as large rocks or logs. Microhabitats are also found across the riffle and longitudinally along the length of the riffle, where water velocity and depth vary. This microhabitat heterogeneity contributes to the diversity of benthic macroinvertebrates found within the riffle, as different taxa are best adapted to use each microhabitat type.

If there are multiple riffles within a reach, each must be inspected and evaluated for substrate characteristics and microhabitat heterogeneity. Substrate characteristics must be evaluated relative to the following prioritized list:

1. Cobble/gravel
2. Debris jams
3. Emergent vegetation
4. Rootwads
5. Sand
6. Bedrock

For example, if among several riffles in a reach, one contains primarily cobble and gravel substrate and all the rest contain primarily bedrock, collect the sample in the riffle that contains primarily cobble and gravel substrate. If all of the riffles contain primarily bedrock or sand, each must be inspected for the availability of microhabitats, such as pockets of gravel or debris jams.

If these types of microhabitats are present, collect the sample from the riffle(s) making sure to spend most of the "kick" time in these microhabitats. *If the substrate in all of the riffles in a reach are essentially homogeneously bedrock or sand, then the runs and glides in the reach must be evaluated as potential alternative sample collection habitats.*

Runs and Glides

Run- and glide-type habitats are areas of the stream that are characterized by relatively rapid, nonturbulent flow. These habitat types are similar to an inclined plane—all of the water flows at the same fast pace, but not rapidly enough or with sufficient depth to cause significant surface rippling. Runs and glides cannot be classified as either riffles or pools (Platts et al. 1983). Evaluate the substrate within a run or glide habitat according to the priorities listed above for riffles, with cobble and gravel habitats being given the highest priority.

If no riffle, run, or glide habitats can be found that are appropriate, as described above, for collecting a kicknet sample, it may be necessary to collect a snag sample, as described in the "RBP Snag Sample Collection Procedures" section.

Pools

Pool habitats are areas of the stream characterized by relatively slow water velocity, and are usually deeper than a riffle or a run (Platts et al. 1983). For most purposes, *pools are the least preferable habitat type for benthic macroinvertebrate samples collection.*

Benthic macroinvertebrate samples are not collected from pools routinely, but only for specific objectives, such as evaluating the effects of excessive sedimentation or of toxicants associated with particulates that tend to settle out most readily in pools because of the reduced current velocity.

If a suitable site for collecting benthic macroinvertebrates cannot be found in the sample reach, do not collect benthic macroinvertebrate samples. Consider an alternate sample reach.

Rapid Bioassessment Protocols

Note: The standard D-frame kicknet sample as described below is the primary or sole method of collection in riffles, runs, and glides when the predominant substrate type is gravel and cobble. The kicknet is used as a supplement to snag samples in both riffles and runs when the predominant substrate type is sand or silt.

RBP Kicknet Sample Collection Procedures

The goal of collecting a benthic macroinvertebrate RBP kicknet sample is to collect, properly preserve, identify and enumerate 175 ($\pm$ 20 percent) individual benthic macroinvertebrates according to the methods outlined below.

If the count of individuals is low (< 100), the sample is considered inadequate for ALU assessments. Thus, it is important to visually inspect the RBP sample prior to leaving the site. If it appears that the sample contains less than 140 individual benthic macroinvertebrates (175 - [0.2 x 175]), repeat the sample collection process.

If it is determined that it is most appropriate to collect an RBP kicknet sample, based on the guidance given in the section on "Where to Collect," proceed according to the following guidelines.

Equipment

Use a standard D-frame kicknet with mesh size $\leq$590 µm to collect the RBP kicknet sample. The kicknet is the primary or sole method of collection in riffles and runs when the predominant substrate type is gravel and cobble. Before collecting the sample, carefully inspect the net and replace or repair it if there are any holes in the net.

Collecting a Sample

Collect the kicknet sample by placing the straight edge of the kicknet on the stream bottom, close to the stream bank at the downstream end of the riffle or run, with the opening facing upstream.

The toe or heel of the boot is used to disturb the substrate in an area covering approximately 0.3 m^2 immediately upstream of the net. Allow the dislodged material to be carried into the net by the current. It may be necessary to pick up and rub or brush larger substrate particles to remove attached organisms. After all of the dislodged material has been collected in the net, move a short distance upstream, toward the opposite bank, and repeat procedure. Continue this technique for 5 minutes of actual "kick time" in a "zig-zag" pattern beginning at the downstream end of the riffle or run, and proceeding upstream making sure to cover as much of the length and width of the riffle as is possible.

Processing a Sample in the Field

If the intent is to process the RBP kicknet sample in the field, place the contents of the net into a sorting and subsampling tray. Carefully inspect the net and use forceps to remove any remaining benthic macroinvertebrates and place in the sorting (subsampling) pan with the remainder of the sample.

If the sample includes snags or other debris, use a squirt bottle to thoroughly wash any benthic macroinvertebrates from the surface of the snag or debris into the pan with the rest of the sample.

Carefully inspect the snag, including cracks, crevices, and under loose bark for any remaining macroinvertebrates. Place any organisms found in the sorting pan along with the rest of the sample. After all organisms have been removed from large pieces of snag or leaves, remove those pieces of detritus from the sorting pan. After it has been determined that all organisms present in the sample have been successfully transferred from the collecting net to the sample pan, inspect the sample and visually estimate the abundance of individuals. If it appears that there are at least 140 individuals in the sample pan, proceed with sample processing by following procedures given in the "Benthic Macroinvertebrate RBP Sample Processing Procedures" section of this chapter. If it appears that there are less than 140 individuals in the sample pan, collect another 5-minute kicknet sample and combine with the first sample prior to processing. Record in field notes that it was necessary to collect an additional kicknet sample.

Processing a Sample in the Lab

If the intent is to process the RBP kicknet sample in the laboratory, transfer the entire sample from the net to a sample pan. Carefully inspect the sample and visually estimate the number of individuals in the sample. If it appears that there are at least 140 individuals in the sample, transfer the entire sample to the sample container(s). If it appears that there are less than 140 individuals in the initial sample, collect one more 5-minute kicknet sample, combine it with the first kicknet sample, and transfer to the sample container(s). Carefully follow the guidelines given above to ensure that the collecting net and all large pieces of debris are carefully inspected, and preserve according to guidelines in the "Sample Preservation Procedures for RBP Samples" section of this chapter.

RBP Snag Sample Collection Procedures

Note: The snag sample collection method as described below is the primary collection method in riffles or runs when the predominant substrate type is sand or silt. The standard D-frame kicknet sample as described in the "RBP Kicknet Sample Collection Procedures" must be used as a supplemental method for collection in riffles and runs when the predominant substrate type is sand or silt. A triangular-frame kicknet may be substituted for the D-frame kicknet for snag and undercut bank sampling.

When to Use the Snag Sample Collection Method

Collect a 5-minute kicknet sample as a supplement to the snag sample in order to provide an adequate representation of the benthic community. Base the decision to collect a snag sample supplemented with a kicknet sample on guidance given in the "Where to Collect Samples" section of this chapter.

Selecting Snags

Snags are submerged woody debris (for example: sticks, logs, or roots) that are exposed to the current. Optimally, snags are 0.5 - 2.5 cm in diameter and submerged in the stream for a minimum of two weeks. Moss, algae, or fungal growth on the snags can be taken as evidence that the snag has been in the stream for an adequate time period to allow colonization by benthic macroinvertebrates.

Collecting a Sample

For RBP snag samples, collect woody debris accumulated in debris piles or jams *in areas exposed to good flow*. Use lopping shears to cut off sections of submerged woody debris. Avoid depositional zones (for example: pools) and backwater areas. Place a D-frame net immediately downstream of the snag while cutting to minimize loss of macroinvertebrates. Once cut, place the snag immediately in sorting tray, sieve bucket, or net with No. 30 or smaller mesh size ($\leq$590 μm).

Emergent vegetation and rootwads in undercut banks that are exposed to good flow may be sampled by sweeping the kicknet under the roots and agitating them by hand or by a jabbing motion with the net. Place the dislodged macroinvertebrates and associated debris in the sorting tray or sieve bucket along with any woody debris or other kicknet sample.

Using a squirt bottle, wash the surface of the snags and collect the dislodged benthic macroinvertebrates and associated debris in a sorting tray. Carefully inspect the snag, including cracks, crevices, and under loose bark for any remaining macroinvertebrates. Place any organisms found in the sorting pan along with the rest of the sample.

Processing a Sample in the Field

Before completing the sample event, and prior to preserving the sample, visually inspect the RBP sample. *If it appears that the sample contains less than 140 individual benthic macroinvertebrates (175 -[0.2 x 175]), repeat the sample collection process.* Record in field notes that it was necessary to collect a second RBP sample to obtain enough organisms.

If the intent is to process the RBP snag sample in the field, combine all individuals from supplemental kicknet sample(s) with all individuals from the snag sample in the sample pan, inspect the sample and estimate the abundance of individuals. If it appears that there are at least 140 individuals in the sample, follow procedures in the “RBP Sample Processing Procedures” section of this chapter. Preserve according to guidelines in the “Sample Preservation Procedures for RBP Samples” section of this chapter.

Processing a Sample in the Lab

If the intent is to process the RBP snag sample in the laboratory, visually inspect the sample and estimate the abundance of individuals. If it appears that there are at least 140 individuals in the sample, combine the entire sample, including the kicknet sample, in the sorting tray. Transfer the combined sample to sample container(s). Preserve according to guidelines in the “Sample Preservation Procedures for RBP Samples” section of this chapter. Process the sample by following guidelines in the “RBP Sample Processing Section” of this chapter.

For either field or laboratory processing, if the visual inspection indicates that there are less than 140 individuals in the sample, repeat the collection process for both supplemental 5-minute kicknet and snags and combine them with the first sample in either the sorting tray or container(s).

Benthic Macroinvertebrate Sample Preservation Procedures for RBP Samples

Preservation for Processing a Sample in the Field

If individual benthic macroinvertebrates are separated from other debris in the sample in the field (picked), place the organisms (with no organic detritus) directly in 70 percent ethanol or 40 percent isopropyl alcohol. Take care to use adequate preservative to cover the sample.

Labeling the Sample

In each sample container, place a label that includes, at minimum, the following information. Use pencil or waterproof ink on paper with a high rag content for each label.

- Station number and location description
- Date and time of collection
- Collection method (for example: 5-minute kicknet or snag)
- Preservative used
- An estimate of the number of individuals in the subsample
- Name of collector(s)
- Container replicate number (for example: 1 of 2 or 2 of 2), if needed

Preservation for Processing a Sample in the Lab

If the intent is to sort and subsample in the laboratory, transfer the entire sample from the net or sorting tray to sample container(s). Preserve the sample in 10 percent formalin (one part full-

strength formalin and nine parts water). Alternatively, if the sample is to be sorted soon after reaching the laboratory, preserve the sample in 95 percent ethanol.

> **Safety Note:** Avoid breathing **formalin** fumes! Formalin is corrosive to the eyes, skin, and respiratory tract. Wear safety glasses and latex gloves when working with this suspected carcinogen. Always work in a well ventilated area or under a hood when preparing formalin solutions.
>
> **Alcohol** is highly flammable. Take care in storage and handling.
>
> Check the alcohol and formalin solution Material Safety Data Sheets for proper handling requirements.

Use adequate preservative to cover the sample. To ensure adequate preservation of benthic macroinvertebrate collections, fill sample containers no more than one-half full with the sample,so the amount of preservative is at least equal to the volume of organic material, including detritus. Avoid placing too much sample in one jar. If there is too much organic matter in the jar, the sample may begin to decompose before processing.

Labeling the Field Sample for Laboratory Processing

Place a label in each sample container that includes, at minimum, the following information. Use pencil or waterproof ink on paper with a high rag content for each label.

- Station number and location description
- Date and time of collection
- Collection method (for example: 5-minute kicknet, or snag)
- Preservative used
- Name of collector(s)
- Container replicate number (for example: 1 of 2 or 2 of 2), if needed

Sample Processing Procedures for RBP Kicknet or Snag Samples

RBP kicknet or snag samples may be processed either in the field or in the lab. Field processing is often easier—the movement of living organisms makes them more easily detected and any organisms not picked can be returned to the stream, decreasing the impact of sample collection on the benthic community. Also, it is not unusual, even at minimally impacted streams, to fail to obtain a minimum of 140 organisms with a single 5-minute kicknet sample. Thus, if samples are picked in the field it will be possible to determine whether the required number of individuals has been collected in-situ, and to collect another kicknet sample if necessary.

It is often difficult to clean samples adequately in the field; lighting is often inadequate, and time is often limited due to weather, terrestrial pests, or safety considerations. For these reasons, it may be more appropriate to process the RBP sample in the lab. Lab processing allows the allocation of more time in a well lighted, controlled environment as well as the use of magnification equipment when necessary. One limitation of working with preserved specimens is loss of movement to aid in detection and loss of natural coloration that assists in identification.

Field Processing RBP Kicknet or RBP Snag Samples

The goal of processing the RBP kicknet or RBP snag sample is to produce a properly preserved subsample of 175 ($\pm$ 20 percent) individuals derived from the entire kicknet or snag sample according to the following guidelines.

Cleaning a sample. Thoroughly wash the sample using the collecting net or No. 30 sieve or sieve bucket (mesh size $\leq$ 595 µm) to remove fine sediment. After rinsing large organic material (for example: whole leaves, twigs, algae, or macrophyte material), visually inspect for any attached organisms and then discard the large material. Place the rinsed sample in a shallow white sorting pan and add enough water to allow the organisms to move around (1 to 2 cm). Gently swirl the pan to disperse contents as evenly as possible.

Subsampling. Use either a mason jar lid or square from the USEPA sample processing trays (Caton 1991) to decrease bias. Place the square or mason jar lid in the tray containing the whole sample to isolate a small portion of the sample. Remove the portion isolated in the square and place in another shallow white sorting pan. Add a small amount of water to facilitate sorting.

In this manner, remove a total of four portions from the sample pan and place all four in the sorting pan. Inspect the contents of the sorting pan, pick and count all organisms, and transfer to a sample bottle or vial containing 70 percent ethanol. Organisms of varying species may be combined in the vials. Do not overcrowd the vials. Use a fine set of forceps to "pick" (remove) organisms. Continue this process until a minimum of 140 organisms have been collected. Pick and count the remaining macroinvertebrates from the last square even after a 140 organism count is exceeded.

High-density samples. If the density of the four subsamples appears to be greater than 175 organisms, it will be necessary to subsample again from the subsample tray. Using a mason jar lid or other device, isolate one portion at a time from the subsample in the sorting pan and place in a secondary sorting pan. Pick the macroinvertebrates from that single portion and return to the subsample tray for another isolated portion. Pick each portion placed in the secondary sorting pan one at a time until the range of 140 to 210 organisms is counted.

Low-density samples. If it is necessary to pick all macroinvertebrates from the sample in order to obtain the required number of organisms, then subsampling, as described above, is not required. Pick and count organisms as they are observed with an effort to pick all macroinvertebrates from the sample.

Labeling the subsampled vials. Label the sample bottle(s) or vial(s) containing the sorted and counted benthic macroinvertebrates with the following information. Use pencil or waterproof ink on paper with a high rag content for each label.

- Station number and location description
- Date and time of collection
- Collection method (for example: 5-minute kicknet, or snag)
- Preservative used
- Name of collector(s)
- An estimate of the number of individuals in the subsample
- Name of person conducting subsampling procedure, if different from collector
- An estimate of the number of individuals in the vial
- Container replicate number (for example: 1 of 2 or 2 of 2), if needed

Sample Tracking Requirements for RBP Kicknet or RBP Snag Samples

Upon returning to the laboratory, assign a unique sample tracking number to the vial(s) containing the macroinvertebrates according to the sequence in the benthic macroinvertebrate sample tracking logbook for both the field-processed samples and the whole samples brought back for laboratory processing. For example, a system of numbering may look like BM 040 04, where "BM" refers to "benthic macroinvertebrate", "040" refers to sample number 40, and "04" refers to the year 2004.

The sample log will contain the following information:

- Sample tracking number
- Collection date and time
- Station number and location description
- Name of collector(s)
- Collection method(s)
- Name of person conducting subsampling procedure, if different from collector, and if field processed
- Number of vials in the sample

Once the sample tracking number has been assigned, affix a label with the sample tracking number to the outside of the container—*but never on the lid*. Wrap the label with clear tape to ensure it will not come off.

Laboratory Processing RBP Kicknet or RBP Snag Samples

Whole samples returned for processing in the laboratory must first be washed. Thoroughly wash the sample in a sieve with mesh size ≤ 595 to remove preservative and fine sediment. After the preservative has been rinsed away, proceed with processing the sample using the protocols for cleaning, subsampling, and labeling outlined in the *Field Processing RBP Kicknet or RBP Snag Samples* section of this chapter.

> **Safety Note:** To reduce your exposure to formalin, rinse the sample with water in a sieve with mesh size ≤ 595 µm under a vent hood, or if a hood is not available, in an area with good ventilation. Transfer to alcohol before sorting.

Laboratory Procedures for Identification of Specimens Collected in RBP Kicknet or RBP Snag Samples

Use the appropriate references, a stereo dissecting microscope, and compound phase contrast microscope to identify the organisms to the taxonomic levels specified below. Chapter 11 provides a complete listing of required and recommended freshwater macroinvertebrates identification references.

- Insecta, identify to genus, except leave Chironomidae at family
- Oligochaeta, leave at Oligochaeta
- Hirudinea, leave at Hirudinea
- Hydracarina, leave at Hydracarina
- Isopoda, identify to genus
- Amphipoda, identify to genus
- Nematoda, leave at Nematoda
- Ostracoda, leave at Ostracoda
- Palaemonidae, identify to genus
- Cambaridae, leave at Cambaridae
- Gastropoda, identify to genus
- Turbellaria, identify to family
- Pelecypoda, identify to genus

Voucher Specimens

Retain at least one representative of each benthic macroinvertebrate taxon collected as a voucher specimen for a minimum of five years or until the conclusion of applicable regulatory decision (whichever is longer) to allow identification verification if necessary. Voucher specimens serve as long-term physical representations that substantiate the names applied to organisms collected as part of the TCEQ database. Voucher specimens ensure the credibility of TCEQ bioassessment data by documenting the identity of the organisms and making them available for review by the general scientific community.

Take the following into consideration when storing voucher specimens:

- Long-term maintenance of wet (alcohol-preserved) and mounted specimens
- Adequate quantity and quality of space to store specimens
- An effective mechanism for locating and retrieving specimens upon request
- Personnel experience in invertebrate taxonomy

The organization maintaining voucher specimens must have a history that indicates it will be able to preserve the specimens into the future (USGS 2000). This could include in-house provisions for maintenance of samples or archiving at a natural history collection at a university.

Data Evaluation Procedures for RBP Kicknet or RBP Snag Samples

For benthic macroinvertebrate data collected with a D-frame kicknet or RBP snag samples, evaluate data in accordance with the metric criteria as shown in Table B-11 in Appendix B.

This technique includes 12 metrics that integrate structural and functional attributes of macroinvertebrate assemblages to assess biotic integrity (Harrison 1996). Use this metric set to evaluate benthic macroinvertebrate RBP kicknet and snag samples collected in wadeable streams and rivers. These metrics help establish the appropriate ALU for unclassified freshwater bodies and help to evaluate the appropriateness or attainment of the existing ALU for classified water bodies. Report metric scoring on the form *Metrics and Scoring for Kick Samples, Rapid Bioassessment Protocol–Benthic Macroinvertebrates* in Appendix C or on a comparable form.

The criteria set includes 12 metrics.

1. **Taxa richness**. This metric is the total number of benthic macroinvertebrate taxa. Separate all macroinvertebrates into appropriate taxonomic categories and count the number of categories present. See the "Laboratory Procedures for Identification of Specimens Collected in RBP Kicknet or RBP Snag Sample" for the taxonomic categories. In general, relatively lower taxa richness values reflect lower biotic integrity. Decreases in taxa richness may result from disturbance of physicochemical factors.

2. **EPT richness**. This metric is the total number of distinct taxa (genera) within the orders of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). In general, this metric tends to decrease with increasing disturbance of physicochemical factors as the majority of taxa in these orders are considered pollution sensitive.

3. **Hilsenhoff Biotic Index (HBI**). This index is calculated as $\sum n_i t_i / N$ where n_i is the number of individuals of a particular taxa (for example: genus or family), t_i is the tolerance value of that taxon, and N is the total number of organisms in a sample. Tolerance values are assigned on a scale of 0 to10 (see Table B-13, Appendix B), with increasing tolerance values reflecting increasing tolerance to physicochemical degradation. N must include counts of organisms only from those taxa that have tolerance values. The index weights the relative abundance of each taxon in terms of its pollution tolerance in determining a community score. In general, the index increases as the relative abundance of tolerant taxa increases. The increase of these tolerant taxa is due to increasing degradation of physicochemical conditions.

4. **Percent Chironomidae**. This metric is the ratio of the number of individuals in the family Chironomidae to the total number of individuals in the sample multiplied by 100. Chironomidae are relatively ubiquitous in aquatic habitats. Although the Chironomidae are often considered pollution tolerant, the variability in tolerance at the species level is apparently quite large.

5. **Percent dominant taxon**. This metric is the ratio of the number of individuals in the numerically dominant taxon to the total number of individuals in the sample multiplied by 100. In general, a community dominated by relatively few taxa may indicate environmental stress, and a high percent contribution by one or two taxa represents an imbalance in community structure.

6. **Percent dominant functional group**. This metric is the ratio of the number of individuals in the numerically dominant functional group to the total number of individuals in the sample multiplied by 100. See Table B-4 in Appendix B. This metric is based on the well supported premise that physicochemical disturbance can result in modification of the resource base available to consumers in aquatic systems and subsequently cause an imbalanced trophic structure.

   Place aquatic macroinvertebrates in functional feeding groups (FFG) according to Merritt and Cummins (1996). See Table B-6 in Appendix B. Calculate the percentage represented by each group. The FFG classification places taxa in categories based on morpho-behavioral mechanisms of food acquisition (Merritt and Cummins 1996). Note that the functional

classification is independent of taxonomy, meaning that one functional group may contain several taxa. The five FFG categories used include:

- **Scrapers (grazers**). Benthic macroinvertebrates morpho-behaviorally adapted to use the fungal, bacterial, algal complex closely attached to the substrata as the primary food resource. The fungal, bacterial, algal complex is referred to as periphyton.

- **Collector-gatherers (deposit feeders**). Benthic macroinvertebrates morpho-behaviorally adapted to use fine particulate organic matter (FPOM) deposited both interstitially or on the surface of the substrata as the primary food resource.

- **Filtering collectors (suspension feeders**). Benthic macroinvertebrates morpho-behaviorally adapted to use particulate organic matter (POM) suspended in the water column as the primary food resource.

- **Predators (engulfers and piercers**). Benthic macroinvertebrates morpho-behaviorally adapted to use other living organisms (prey) as the primary food resource.

- **Shredders (living or dead plant material**). Benthic macroinvertebrates morpho-behaviorally adapted to use coarse particulate organic matter (CPOM), especially leaf litter and the associated algal, bacterial, and fungal complex, as the primary food resource.

Note: The groups are not mutually exclusive, that is, one taxon may be considered a scraper and collector-gatherer. In this situation, place half of the organisms from that taxon in the scraper category and half in the collector-gatherer category. For example, for four individuals from the genus *Baetis*, which is a scraper and collector-gatherer, place two in the scraper category and two in the collector-gatherer category.

Scoring for the metric is based on the premise that relatively low to moderate percentages for all functional groups reflects a balanced trophic structure, while extremely high or low percentages reflect an imbalance, possibly due to physicochemical perturbation.

7. **Percent predators**. This metric is the ratio of the number of individuals in the predator functional group (see Table B-6, Appendix B) to the total number of individuals in the sample multiplied by 100. Variability in the percentage of predators should be less correlated to changes in prey availability resulting from natural changes in habitat and more correlated to changes in prey availability resulting from other effects such as toxicity, nutrients, etc. Further, most predators have relatively long aquatic life stages, usually greater than six months. Thus, they integrate physicochemical conditions over longer periods of time than other groups, such as mayflies, which complete their aquatic existence in less than two weeks. Scoring for the metric is based on the premise that relatively low to moderate percentages of predators reflect a balanced trophic structure, while extremely high or low percentages reflect an imbalance, possibly due to physicochemical perturbation.

8. **Ratio of intolerant to tolerant taxa**. This metric is the ratio of the number of individuals in taxa with tolerance values < 6 to the number of individuals in taxa with tolerance values ≥ 6 (see Table B-6, Appendix B). This metric provides a measure of the relative contribution of tolerant and intolerant taxa to the composition of the community. The metric increases as the relative numbers of intolerant individuals increases and thus, higher values must reflect favorable physicochemical conditions.

9. **Percent of total Trichoptera as Hydropsychidae**. This metric is the ratio of the number of individuals in the family Hydropsychidae to the total number of individuals in the sample in the order Trichoptera multiplied by 100. Trichoptera are ubiquitous in Texas streams. Among the Trichoptera, the family Hydropsychidae is perhaps most commonly collected. Further, the Hydropsychidae tend to be among the most tolerant of Trichoptera. This metric is based on the observation that samples from reference streams in Texas typically contain representatives of Hydropsychidae as well as representatives from other families in the order Trichoptera. Thus, a high relative percent of total Trichoptera accounted for by the Hydropsychidae, or a complete lack of Trichoptera, likely reflect physicochemical degradation.

10. **Number of non-insect taxa**. This metric is based on the finding that kicknet samples from reference streams in Texas typically include representatives from several non-insect taxa and that the number of non-insect taxa typically is lower in impaired streams.

11. **Percent collector-gatherers**. This metric is the ratio of the number of individuals in the collector-gatherer functional group (see Table B-13, Appendix B) to the total number of individuals in the sample multiplied by 100. Collector-gatherers use FPOM as the primary food resource. Physicochemical disturbance, especially organic enrichment, can cause an increase in the availability of FPOM via several mechanisms including direct input of FPOM and/or increased microbial activity. A high percentage of collector-gatherers indicates degradation.

12. **Percent as Elmidae**. This metric is the ratio of the number of the individuals from the family Elmidae to the total number of individuals in the sample multiplied by 100. Riffle beetles are typically found in samples from reference streams in Texas. *Stenelmis* sp., perhaps the most commonly encountered genus, is relatively tolerant to pollution and thus apparently may become dominant in situations where a moderate tolerance to organic enrichment offers an advantage. Thus, low scores for this metric are associated with either an extremely high percentage of, or a complete absence of, Elmidae.

Surber Sampler Protocols

Sample Collection Procedures

The objective of the Surber sampling effort is to collect a minimum of three replicate Surber samples, and to remove and preserve all individual benthic macroinvertebrates from each replicate sample.

Where to Collect Samples

Collect samples only in riffle-type habitats with depths < 0.3 m. If there are multiple riffles within a reach, inspect and evaluate each for substrate characteristics and microhabitat heterogeneity. Evaluate substrate characteristics relative to the following prioritized list.

1. Cobble and gravel
2. Debris jams
3. Sand
4. Bedrock

For example, if one riffle among several riffles in a reach contains primarily cobble and gravel substrate and all the rest contain primarily bedrock, collect the sample in the riffle that contains primarily cobble and gravel substrate. If all of the riffles contain primarily bedrock or sand, inspect each one for available microhabitats, such as pockets of gravel or debris jams. If these types of microhabitats are present, collect the sample from the riffle(s), being sure to spend most of the sample time in these microhabitats.

Consider alternative sampling methods if the riffles are essentially homogeneously bedrock or sand. For example, the runs and glides in the reach must be evaluated as potential alternative candidate habitats for collecting either snag sample or RBP kicknet samples.

Collecting a Surber Sample

To collect the Surber sample, firmly push sampler down on the substrate with the net mouth facing upstream. Lift larger rocks individually and scrub them off at the mouth of the net. Thoroughly disturb the remaining sediment by repeatedly digging and stirring as deeply as possible, allowing the current to sweep organisms and detritus into the bag net. Repeat this process a minimum of three times to produce three individual replicate Surber samples.

Collect the three replicates in a manner that represents the longitudinal and cross-sectional heterogeneity of the riffle. For example, collect the first replicate at the lower end of the riffle a suitable distance away from the right bank; collect the second replicate mid-stream about

halfway up the riffle; and collect the third replicate a suitable distance from the left bank at the upper end of the riffle. If the riffle is large, it may be desirable to establish transects and use a random number generator to decide where to locate each replicate.

Quantitative Snag Samples

***Note*:** The snag sample collection method as described below is the primary collection method in riffles or runs when the predominant substrate type is sand or silt.

Selecting Snags

Snags are submerged pieces of woody debris (for example: sticks, logs, or roots) that are exposed to the current. Optimally, snags are 0.5 to 2.5 cm in diameter and have been submerged in the stream for a minimum of two weeks. Moss, algae, or fungal growth on the snags can be taken as evidence that the snag has been in the stream for an adequate time period to allow colonization by benthic macroinvertebrates.

Collecting a Sample

For quantitative snag samples, collect woody debris accumulated in debris piles or jams *in areas exposed to good flow*. Use lopping shears to cut off sections of the submerged woody debris. The section should be of a length appropriate to fit in a 1-qt mason jar. Avoid depositional zones (for example: pools) and backwater areas. Place a D-frame net immediately downstream of the snag, while cutting, to minimize loss of macroinvertebrates. Place snag samples directly into the mason jars containing 10 percent formalin. Collect enough snag sample to fill two 1-qt mason jars.

Field Processing and Preserving a Surber Sample

The objective of a Surber sample is to count and identify every individual benthic macroinvertebrate collected in a known area. Since sorting usually takes several hours to several days, sorting must be done in the laboratory. Inspect all of the sample under magnification to ensure that all individuals are counted and identified.

Transfer the entire sample from the net to sample container(s) and preserve in 10 percent formalin (one part full-strength formalin and nine parts water). Alternatively, if the sample is to be sorted soon after reaching the laboratory, the sample may be preserved in 95 percent ethanol.

Use adequate preservative to cover the sample. To ensure adequate preservation of benthic macroinvertebrate collections, fill sample containers no more than one-half full with the sample,so the amount of preservative is at least equal to the volume of organic material, including detritus. Avoid placing too much sample in one jar. If there is too much organic matter in the jar, the sample may begin to decompose before processing.

Labeling the Sample Container

Place a label in each sample container that includes, at minimum, the following information. Use pencil or waterproof ink on paper with a high rag content for each label.

- Station number and location description
- Date and time of collection
- Collection method (for example: Surber or quantitative snag)
- Sample container replicate number (for example: 1 of 3, 2 of 3, or 3 of 3), if needed
- Preservative used
- Name of collector(s)

Repeat this labeling process for each individual replicate sample. For example, if three replicate Surber samples or quantitative snag samples are collected at a site, there must be three separate jars or three separate sets of jars with a single jar or set of jars corresponding to an individual replicate sample.

Sample Tracking Requirements for Surber Samples

Upon return to the laboratory, assign a unique sample tracking number to the jars containing the Surber or snag samples, according to the sequence in the sample tracking logbook. For example, a system of numbering may look like BM 040 04, where “BM” refers to “benthic macroinvertebrate”, “040” refers to sample number 40, and “04” refers to the year 2004.

The sample log will contain the following information:

- Sample tracking number
- Collection date and time
- Station number and location description
- Name of collector(s)
- Collection method(s)
- Name of person conducting subsampling procedure in the lab, if different from collector
- Number of jars in the sample

Once the sample tracking number has been assigned, affix a label with the sample tracking number to the outside of the container—*but never on the lid*. Wrap the label with clear tape to ensure the label will not come off.

Laboratory Processing for Surber Samples

The objective of processing a benthic macroinvertebrate sample in the laboratory is to count and identify every individual benthic macroinvertebrate collected in the Surber sampler. Process each of the three replicate samples individually. Place all of the individual benthic macroinvertebrates from each replicate Surber sample in a separate vial. Once sorting is complete, there will be three separate vials, each containing all of the specimens from each individual replicate. This sorting is critical because it allows the variability between replicates to be evaluated.

Rinsing a sample. Thoroughly rinse the sample using a No. 30 or smaller ($\leq$ 595 μm) sieve to remove preservative and fine sediments. Rinse samples under a fume hood to minimize inhalation of fumes from the preservative. Place rinsed sample in a shallow white pan.

Sorting a sample. Put 1 to 2 cm of water in the bottom of the pan to disperse the contents as evenly as possible. Pick *all macroinvertebrates* visible to the unaided eye from the sample and place in a sample bottle or vial containing 70 percent ethanol or isopropyl alcohol.

After thoroughly inspecting the sample and removing all macroinvertebrates visible to the unaided eye, place small portions of the remaining sample in a petri dish and inspect using a dissecting scope. Repeat this process until the entire replicate sample has been inspected under magnification.

Labeling a sample. Label the sample bottle or vial containing the benthic macroinvertebrates obtained using this sorting and counting procedure with the following information. Use pencil or waterproof ink on paper with a high rag content for each label.

- Station number and location description
- Sample tracking number
- Date and time of collection
- Collection method (for example: Surber, snag, Ekman dredge)
- Container replicate number (for example: 1 of 3, 2 of 3, 3 of 3)
- Preservative used
- Name of collector(s)
- Name of person conducting subsampling procedure, if different from collector

Repeat this labeling process for each of the three replicate samples.

Affix a label with the sample tracking number and container replicate number to the outside of the container—*but never on the lid*. Make sure the container is dry, and wrap with clear tape to ensure the label will not come off.

Laboratory Processing for Quantitative Snag Samples

Using a squirt bottle, wash the surface of the snags and collect the dislodged benthic macroinvertebrates and associated debris in a sorting tray. Carefully inspect the snag, including cracks, crevices, and under loose bark for any remaining macroinvertebrates. Use a dissecting microscope, if necessary, to ensure all organisms are removed from the snags. It may be appropriate to use a soft bristled brush to remove the macroinvertebrates from the snag surface

taking care not to damage the organisms. Once all macroinvertebrates are removed from the snags, follow the procedures outlined in Laboratory Processing for Surber Samples in this chapter.

Before discarding snags, measure length and diameter of the snags in order to calculate the surface area of the snags. This allows the results to be expressed as numbers of individuals per unit area of snag surface.

Laboratory Procedures for Identification of Specimens Collected Using a Surber Sampler or Quantitative Snag Samples

Use the appropriate reference(s), a stereo dissecting microscope, and compound phase contrast microscope to identify organisms to the appropriate taxonomic level indicated on pages 5-9 of this chapter, genus in most cases. *Maintain a separate count of individuals and list of taxa for each replicate Surber sample to allow an evaluation of variability between replicates.* Chapter 11 provides a complete listing of required and recommended freshwater benthic macroinvertebrate identification references.

Voucher Specimens

Retain at least one representative of each benthic macroinvertebrate taxon collected as a voucher specimen for a minimum of five years or until the conclusion of applicable regulatory decision (whichever is longer) to allow identification verification if necessary. Voucher specimens serve as long-term physical representations that substantiate the names applied to organisms collected as part of the TCEQ SWQM Program. Voucher specimens ensure the credibility of TCEQ bioassessment data by documenting the identity of the organisms and making them available for review by the general scientific community.

Take the following into consideration when storing voucher specimens:

- Long-term maintenance of wet (alcohol-preserved) and mounted specimens
- Adequate quantity and quality of space to store specimens
- An effective mechanism for locating and retrieving specimens upon request
- Personnel experience in invertebrate taxonomy

The organization maintaining voucher specimens must have a history that indicates it will be able to preserve the specimens into the future (USGS 2000). This could include in-house provisions for maintenance of samples or archiving at a natural history collection at a university.

Data Evaluation Procedures for Surber Samples

Calculation of the BIBI is based upon the combined results, counts, and number of individuals from all three replicates. Evaluate data in accordance with the draft BIBI metric criteria as shown in Table B-12 in Appendix B (Davis 1997). The BIBI criteria were derived for three bioregions (central, east, and north) that overlap ecoregions as defined by Omernik and Gallant (1987). Figure B-10 in Appendix B illustrates the three bioregions. All bioregion boundaries coincide with ecoregion lines. The bioregions include:

Central bioregion. The region comprised of Ecoregions 23, 24, 27, 29, 30, 31, and 32, which includes a disjunct portion of Ecoregion 27 in the Texas panhandle and an isolated fragment of Ecoregion 32 in southeastern Texas.

East bioregion. The region encompassing Ecoregions 33, 34, and 35.

North bioregion. The region consisting of Ecoregions 25 and 26.

The BIBI was designed for definitive evaluation of quantitative benthic macroinvertebrate data and is applicable for lotic-erosional habitats under low-flow hydrological regimes. Regional criteria include 11 metrics that integrate structural and functional attributes of macroinvertebrate

assemblages to assess biotic integrity. The method was designed to determine ALUs using a Surber sampler. Report metric scoring on a form as shown in Appendix C or on a comparable form.

The draft criteria set includes the following 11 metrics:

1. **Taxa richness**. This metric is the total number of benthic macroinvertebrate taxa. Macroinvertebrates are identified to the lowest taxonomic level possible, generally genus or species, and the number of taxonomic categories are counted. In general, relatively lower taxa richness values reflect lower biotic integrity. Decreases in taxa richness may result from disturbance of physicochemical factors.

2. **Diptera taxa**. This metric is the total number of benthic macroinvertebrate taxa within the order Diptera. It reflects the condition of the most ecologically diverse insect order in aquatic ecosystems. This metric usually reflects the order with the highest number of species present. The Diptera taxa usually increase with increasing perturbation.

3. **Ephemeroptera taxa**. This metric is the total number of benthic macroinvertebrate taxa within the order Ephemeroptera. It reflects the status of one of the more environmentally sensitive aquatic insect orders, making it a valuable indicator of ambient conditions. A decrease in Ephemerpotera taxa usually indicates increasing stream perturbation.

4. **Intolerant taxa**. This metric is the total number of intolerant benthic macroinvertebrate taxa. Analysis of tolerance and intolerance conforms to the protocol of Fore et al. (1996), where the most tolerant and least tolerant taxa are used. The tolerant taxa metric is expressed as a percentage of total abundance, and the intolerant taxa metric as taxa richness, as has been shown to be the optimal approach (Karr et al. 1986; Fore et al. 1996). Designation of tolerant and intolerant taxa is based primarily on information in Lenat (1993), as outlined in Table B-6, Appendix B. Tolerant taxa are defined as those having tolerance values $\geq$ 8.5, and intolerant taxa, values $\leq$ 4.0. This metric embodies the axiom that sensitive organisms seldom are numerically abundant, yet their presence provides valuable insight into environmental suitability (Fore et al. 1996).

5. **Percent EPT taxa**. This metric is the ratio of the number of individuals within the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) to the total number of individuals in the sample multiplied by 100. In general, this metric tends to decrease with increasing disturbance of physicochemical factors as the majority of taxa in these orders are pollution sensitive.

6. **Percent Chironomidae**. This metric is the ratio of the number of individuals in the family Chironomidae to the total number of individuals in the sample multiplied by 100. Chironomidae are relatively ubiquitous in aquatic habitats and many of the species are facultative or pollution tolerant. Excessive representation within the community often reflects environmental perturbation.

7. **Percent tolerant taxa**. This metric is the ratio of the number of individuals classified as tolerant taxa to the total number of individuals in the sample multiplied by 100. See Table B-6, Appendix B. Refer to the intolerant taxa metric (metric no. 4) for further discussion.

8-10. **Percent grazers, percent gatherers, and percent filterers**. This metric is the ratio of the number of individuals in the grazer, gatherer, and filterer FFGs to the total number of individuals in the sample multiplied by 100. Community trophic structure is assessed following the convention of Minshall (1981), in which six FFGs are used:

- **Grazers**. Scrapers of periphyton, piercers of living macrophyte tissues or filamentous algal cells
- **Gatherers**. Gatherers of deposited FPOM
- **Filterers**. Filterers of suspended FPOM
- **Miners**. Burrowers in deposited FPOM

- **Shredders**. Chewers, miners, and borers of living macrophyte tissues or CPOM
- **Predators**. Piercers, engulfers, and parasites of living animal tissues

FFG assignments are mainly based on information in Merritt and Cummins (1996)—insects—and Pennak (1989)—non-insects. Some investigators employ only five FFGs, typically lumping gatherers and miners into a single group (collector-gatherers). For the present index, gatherers and miners are treated separately to maximize functional feeding resolution. Taxa categorized as collector-gatherers by Merritt and Cummins (1996) are differentiated on the basis of described habit. Taxa having habits other than burrowing (sprawling, climbing, clinging) are considered gatherers; burrowers are regarded as miners. For some taxa, the literature presents multiple indications for trophic relationships and habit. In these cases, the number of individuals in the taxon was apportioned among appropriate FFGs.

11. **Percent dominance**. The ratio of the number of individuals in the three most abundant taxa to the total number of individuals in the sample multiplied by 100. In general, a community dominated by relatively few taxa may indicate environmental stress, and a high percent contribution by a few taxa often represents an imbalance in community structure.

Pool, Reservoir, or Lake: Ekman, Ponar, Petersen, or Van Veen Dredge Sample Protocols

Methodologies for assessing ALUs have not been developed for Texas depositional habitats such as reservoirs and pools. Any private entity, such as a consulting firm, considering an assessment using reservoir or pool benthic macroinvertebrates must consult closely with TCEQ staff before planning the study.

Sample Collection Procedures

The Ekman dredge is the preferred sampler for collecting benthic macroinvertebrate samples from lentic or depositional habitats, such as pools or reservoirs whose bottom is primarily comprised of mud, silt, and/or fine sand. It is considered a quantitative sampling effort and should be collected and processed similar to Surber samples. In pools or reservoirs with substrates comprised of gravel, hard sand, or clay it may be necessary to use a Ponar or Van Veen dredge. Before using any of these devices, inspect them carefully to ensure that all parts are in good operational condition. The following collection methods refer to the Ekman dredge but, with only minor exceptions, apply to the Ponar and Van Veen as well.

Collecting a Sample

Collect a minimum of four Ekman dredge samples, each placed and preserved in a separate sample container, according to the following procedures.

Before collecting the sample, thoroughly rinse the Ekman dredge in ambient water. Once the Ekman has been cleaned, use the line (or pole in shallower pools) to lower the dredge to the bottom. Avoid lowering the sampler too rapidly as this could cause a pressure wave that can disturb the topmost sediment or give a directional signal to invertebrates capable of retreating from the sample area. Once the Ekman reaches the bottom, and you have determined that the line is vertical and taut, drop the messenger. After the dredge jaws are triggered, retrieve the closed dredge at a moderate speed (< 1 m/sec). At the water's surface, make sure the jaws are closed and the surface layer of fine silt is intact. Water must cover the sediment sample in the dredge. Do not drain the water off as this may cause the loss of organisms. Bring the dredge on board and empty it into a large container, such as a large plastic tub. Collect the remaining replicates in the same way, placing each into a separate tub.

Rinsing the sample. Insert the dredge into the mouth of a No. 30 or smaller sieve (mesh size $\leq$ 595 μm) bucket and open the jaws of the dredge to allow all of the material collected in the Ekman to fall into the sieve bucket. It may be necessary to rinse any remaining material into the sieve. After rinsing, thoroughly inspect the Ekman and place any remaining invertebrates or other material contained in the Ekman in the sieve bucket. Wash fine sediments from the sample by

submerging the mesh of the sieve bucket in the pool or reservoir and gently wash, taking care to minimize destruction of soft-bodied organisms.

Repeat this process a minimum of four times to produce four separate replicate benthic macroinvertebrate samples.

Preserving a Sample

Empty the washed contents of the sieve bucket into a clean, wide-mouthed bottle. Transfer the entire sample from the sieve bucket to sample container(s) and preserve in 10 percent formalin (one part full-strength formalin and nine parts water). Alternatively, if the sample is to be sorted shortly after reaching the laboratory, preserve the sample in 95 percent ethanol.

Use adequate preservative to cover the sample. To ensure adequate preservation of benthic macroinvertebrate collections, fill sample containers no more than one-half full with the sample,so the amount of preservative is at least equal to the volume of organic material, including detritus. Avoid placing too much sample in one jar. If there is too much organic matter in the jar, the sample may begin to decompose before processing.

Labeling the Sample Container

Place a label in each sample container that includes, at minimum, the following information.

- Station number and location description
- Date and time of collection
- Collection method (for example: Ekman or Van Veen dredge)
- Sample container replicate number (for example: 1 of 4 or 2 of 4)
- Preservative used
- Name of collector(s)

Repeat this labeling process for each individual replicate sample. If four replicate Ekman dredge samples are collected at a site, there must be four separate jars or four separate sets of jars. Each jar, or set of jars, corresponds to each individual replicate.

Upon returning to the laboratory, assign a unique sample tracking number to each individual replicate sample according to the sequence in the benthic macroinvertebrate sample tracking logbook. Follow the procedures outlined in the Sample Tracking Requirements for Surber and Snag Samples section in this chapter.

Laboratory Processing

The objective of laboratory processing is to count and identify every individual benthic macroinvertebrate collected. Begin by logging the sample(s) into the benthic macroinvertebrate sample tracking log with the pertinent information listed above in *Sample Tracking Requirements for Surber Samples*. Process each of the four replicate samples individually. Place all of the individual macroinvertebrates from each replicate grab sample in a separate vial so that, once sorting is complete, there will be four separate vials, each containing all of the specimens from each individual replicate. Processing each replicate sample separately is important since it will allow the variability between replicates to be evaluated.

Thoroughly rinse the sample using a No. 30 or smaller ($\leq$ 595 μm) sieve to remove preservative and fine sediments. Rinse samples under a fume hood to minimize inhalation of fumes from the preservative. Place rinsed sample in a shallow white pan.

Put 1 to 2 cm of water in the bottom of the pan to disperse the contents as evenly as possible. Pick *all macroinvertebrates* visible to the unaided eye from the sample and place in a sample bottle or vial containing 70 percent ethanol or isopropyl alcohol.

After thoroughly inspecting the sample and removing all macroinvertebrates visible to the unaided eye, place small portions of washed sample a in petri dish and inspect using a dissecting scope. Repeat this process until the entire replicate sample has been inspected under magnification and all organisms are removed.

Labeling the Sample Container

Label the sample bottle or vial containing the sorted and counted benthic macroinvertebrates with the following information. Use pencil or waterproof ink on paper with a high rag content for each label.

- Station number and location description
- Sample tracking number from logbook
- Date and time of collection
- Collection method (for example: Surber, snag, Ekman dredge)
- Container replicate number (for example: 1 of 3, 2 of 3, 3 of 3)
- Preservative used
- Name of collector(s)
- Name of person conducting subsampling procedure, if different from collector

Repeat this labeling process for each of the four replicate samples.

Affix a label with the sample tracking number and container replicate number to the outside of the container—*but never on the lid*. Make sure the container is dry, and wrap with clear tape to ensure the label will not come off.

Laboratory Procedures for Identification of Specimens Collected in Pools or Reservoirs using Ekman, Ponar, or Van Veen Dredge

Using appropriate reference(s), a stereo dissecting microscope, and compound phase contrast microscope, identify the organisms to the appropriate taxonomic level indicated on pages 5-9. *Maintain a separate count of individuals and list of taxa for each replicate dredge sample to allow an evaluation of variability between replicates*. Chapter 11 provides a complete listing of required and recommended freshwater benthic macroinvertebrate identification references.

Methodologies for assessing ALUs have not been developed for Texas freshwater depositional habitats including pools and reservoirs. Before conducting any biological monitoring activities on this type of water body, it is imperative to coordinate this work with the TCEQ and the TPWD. As methodologies and metrics are established, this manual will be updated to reflect those changes.

Records

In addition to sample labeling requirements as specified in this chapter, maintain the following records:

Field logbook. For each sample event, enter all relevant information in the field logbook, including the date and time of sample collection, location of the sample site (Station ID), collector's name(s), method of collection, number and type of samples collected, number of sample containers, and preservative used.

Sample tracking logbook. Maintain a sample tracking logbook that contains the information described in the QA chapter of this manual. This logbook documents when samples arrive at the laboratory or headquarters facility, when each sample enters each sample processing step, and who has custody or responsibility for the sample.

Laboratory bench sheets. Maintain laboratory bench sheets at the location where specimen identification and enumeration occurs. These bench sheets document the raw counts of individuals for each taxon and provide notes relevant to the identification and enumeration process.

Voucher Specimens

Retain at least one representative of each benthic macroinvertebrate taxon collected as a voucher specimen for a minimum of five years or until the conclusion of applicable regulatory decision (whichever is longer) to allow identification verification if necessary. Voucher specimens serve

as long-term physical representations that substantiate the names applied to organisms collected as part of the TCEQ SWQM Program. Voucher specimens ensure the credibility of TCEQ bioassessment data by documenting the identity of the organisms and making them available for review by the general scientific community.

Take the following into consideration when storing voucher specimens:

- Long-term maintenance of wet (alcohol-preserved) and mounted specimens
- Adequate quantity and quality of space to store specimens
- An effective mechanism for locating and retrieving specimens upon request
- Personnel experience in invertebrate taxonomy

The organization maintaining voucher specimens must have a history that indicates it will be able to preserve the specimens into the future (USGS 2000). This could include in-house provisions for maintenance of samples or archiving at a natural history collection at a university.