CHAPTER 5

FRESHWATER BENTHIC MACROINVERTEBRATES

Objective

This chapter describes the methods the TCEQ uses 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 combination with fish-community surveys (Chapter 3) and physical-habitat assessments (Chapter 9). These methods 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 in the biological assessments outlined in Chapter 1.

Scientific Collection Permit

Aquatic insects are not protected under state law; however, an 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.

Riffles, Runs, and Glides in Wadable Streams and Rivers

Rapid Bioassessment Protocols

5-minute Kicknet

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.

Snag Sampling

In deeper streams, or in shallow wadable streams with relatively unstable sand or silt bottoms, RBP samples can be collected from snag habitats. Snags are submerged pieces of woody debris (for example, sticks, logs, or roots), stems of emergent vegetation, and roots of riparian vegetation that are exposed to the current. 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.

Quantitative Protocols

Surber and Snag Sampling

Quantitative benthic macroinvertebrate samples may be collected using a Surber sampler or a quantitative snag-sampling protocol. The 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 that will allow an estimate of density. 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 not routinely used in TCEQ biological assessments. Detailed methods for each of these quantitative collection techniques can be found in Appendix F.

Lakes, Reservoirs, and Depositional Zones of Streams and Rivers (Pools)

Sediment Grabs

Ekman Dredge

The Ekman dredge is the preferred sampler for collecting samples of benthic macroinvertebrates from lentic or depositional habitats, such as pools or reservoirs whose bottom is primarily composed of mud, silt, or fine sand (or a combination of these). It is considered a quantitative sampling effort and should be collected and processed similarly to Surber samples.

Sediment grabs are not routinely used in TCEQ biological assessments. The detailed method for this quantitative collection technique can be found in Appendix F.

Equipment

Field equipment and materials necessary to conduct freshwater benthic macroinvertebrate sampling are listed in Appendix A. Forms required as part of a biological assessment appear in Appendix C. Technical terms are defined in Appendix E. Electronic copies of all the tables and forms in the appendixes are available online at <www.tceq.texas.gov/goto/biopacket>.

Records

The following records must be maintained for each sampling event.

Field Logbook

For each freshwater benthic macroinvertebrate sample event, record the following in a field logbook.

- date and time of sample collection

- location of sample site (Station ID)
- name of each collector
- method of collection
- number and type of samples collected
- number of sample containers
- preservative used

Sample-Tracking Logbook

Maintain a sample-tracking logbook that documents when samples arrive at the laboratory or headquarters, the sample-processing steps, and who has custody of, or responsibility for, the sample.

Upon return to the laboratory, assign a unique sample tracking number to each jar containing the fish specimens according to the sequence in the logbook. For example, an instance of numbering may look like *B 040 04*, where *B* refers to 'benthics,' *040* refers to sample number 40, and *13* refers to the year 2013.

Record the sample tracking number and related sample information in the sample tracking logbook, including:

- sample tracking number
- date and time of collection
- station number and location description
- name of each collector
- collection method (for example, kicknet or snag)
- preservative used
- number of containers in sample

Laboratory Bench Sheets

Laboratory bench sheets, as described in Chapter 11 of this manual, are maintained where specimen identification and counting occur. These sheets document the raw numbers of individuals for each taxon and notes relevant to identification and counting. See Appendix H for a sample benthic-macroinvertebrate laboratory bench sheet.

Wadable Streams and Rivers

The following procedures apply to benthic macroinvertebrate samples collected with the intent of using the data in conjunction with the RBP benthic macroinvertebrate IBI to make an ALU determination or evaluate an existing ALU. They may not apply for special studies with other objectives that do not involve determining an ALU, 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

A benthic macroinvertebrate biotic integrity assessment is typically based on a sample collected from a single habitat type within a stream reach. This differs from the multiple habitats sampled for fish assemblages. An exception would be a benthic snag sample collected across more than one habitat type. Benthic-macroinvertebrate assemblages can vary considerably in response to changes in the character and quality of the physical habitat. Careful consideration must be given to where a benthic macroinvertebrate sample is collected within a stream reach containing multiple habitat types. The overall objective is to collect the sample from optimal benthic macroinvertebrate habitat and physicochemical conditions within the reach. Once the habitat, fish, and benthic macroinvertebrate sampling crew leaders have agreed on the sampling location, 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, 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 is optimal 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 food source for filter-feeding macroinvertebrates, as well as nutrients for primary producers. The shallow depth typically allows the development of attached algae, which serves as 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 its length, 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—cobble and gravel are most desirable and bedrock substrate is least desirable.

1. cobble, gravel
2. debris jams
3. emergent vegetation
4. root wads
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 the 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 or riffles, making sure to spend most of the kick time in these microhabitats.

If the substrate of the riffles in a reach is essentially 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 habitats are areas of the stream with 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, giving cobble and gravel habitats the highest priority.

If no riffle, run, or glide habitat can be found that is appropriate, as described above, for collecting a kicknet sample, it may be necessary to collect a snag sample, as described in "Procedures for Collecting RBP Snag Samples."

Pools

Pool habitats are areas of the stream characterized by relatively slow water, 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 collecting benthic macroinvertebrate samples**.

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 slower current.

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.

Collecting RBP Kicknet Samples

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

If the count of individuals is low (< 100), the sample is inadequate for ALU assessments. Thus, it is important to inspect the RBP sample before leaving the site. If it appears that the sample

contains less than 140 individual benthic macroinvertebrates (175 – [0.2 × 175]), collect another sample.

If collecting an RBP kicknet sample is most appropriate, based on "Where to Collect Samples," proceed according to these guidelines.

Equipment

Use a standard D-frame kicknet with mesh size ≤ 590 µm to collect the RBP 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 it.

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.

Use the toe or heel of a boot to disturb the substrate in an area of 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 the procedure. Continue this technique for 5 minutes of actual kick time in a zigzag 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 possible.

Processing a Sample in the Field

To process the RBP kicknet sample in the field, place the contents of the net into a tray for sorting and sub-sampling. Carefully inspect the net. Use forceps to remove any remaining benthic macroinvertebrates and put them 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 removing all organisms from large pieces of snag or leaves, remove those pieces of detritus from the sorting pan. After determining that all organisms 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 "Processing Benthic Macroinvertebrate RBP Samples," later in this chapter. If it appears that there are fewer than 140 individuals in the sample pan, collect another 5-minute kicknet sample and combine it with the first sample before processing your field notes, recording that it was necessary to collect an additional kicknet sample.

Processing a Sample in the Lab

To process an 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 (or containers). If it appears that there are fewer 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 (or containers). Carefully follow the guidelines above to ensure that the collecting net and all large pieces of debris are carefully inspected, and preserve the sample according to guidelines in the "Preservation Procedures for RBP Samples," later in this chapter.

Collecting RBP Snag Samples

The snag sample-collection method is the primary method in riffles or runs when the predominant substrate type is sand or silt. The standard D-frame kicknet sample as described in "Collecting RBP Kicknet Samples" 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 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 "Where to Collect Samples" (earlier in this chapter).

Selecting Snags

Optimal snags for sampling are 0.5 to 2.5 cm in diameter and submerged in the stream for at least two weeks. Moss, algae, or fungal growth can be taken as evidence that a snag has been in the stream long enough to allow colonization by benthic macroinvertebrates.

Collecting a Sample

For RBP snag samples, collect woody debris accumulated in 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 the piece of woody debris to minimize loss of macroinvertebrates. Once the cut is made, place the snag immediately in a sorting tray, sieve bucket, or net with No. 30 or smaller mesh ($\leq$ 590 µm).

Alternatively, if snags are primarily found in debris jams, place a kicknet downstream of the snags in the debris jam and kick or disturb snags immediately upstream of the mouth of the net. Then place debris and organisms in a sorting tray, or a sieve bucket with No. 30 or smaller mesh net, before processing.

Emergent vegetation and root wads 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 before preserving the RBP sample, inspect it. If it appears to contain fewer than 140 individual benthic macroinvertebrates [175 – (0.2 × 175)], collect another sample. 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 all supplemental kicknet samples with all individuals from the snag sample in the 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 "Processing RBP or Snag Samples," below. Preserve according to guidelines in "Preservation of RBP Samples."

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 one or more sample containers. Preserve it according to guidelines in "Preservation Procedures for RBP Samples," below. Process the sample by following guidelines in "Processing RBP or Snag Samples," below. For either field or laboratory processing, if the inspection reveals fewer than 140 individuals in the sample, repeat the collection process for both supplemental 5-minute kicknet and snag samples and combine them with the first sample in either the sorting tray or one or more containers.

Preservation of 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. 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
- estimate of number of individuals in subsample

- name of each collector
- container replicate number if needed (for example, *1 of 2* or *2 of 2*)

Preservation for Processing a Sample in the Lab

To sort and subsample in the laboratory, transfer the entire sample from the net or sorting tray to one or more sample containers. 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 it 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.

Safety

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 material-safety data sheets for alcohol and formalin solutions for proper handling requirements. Follow your organization's hazardous waste disposal plan for formalin and alcohol waste.

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 each collector
- container replicate number if needed (for example, *1 of 2* or *2 of 2*)

Processing 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 easier to detect, 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 the 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 (± 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 ≤ 595 µm) to remove fine sediment. After rinsing large organic material (for example, whole leaves, twigs, algae, or macrophyte material), inspect the sample 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, a cookie cutter, or a similar device as a subsampler to decrease bias. Place the subsampling device in the tray containing the whole sample to isolate a small portion of the sample. Remove the portion isolated in the device and place it 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 at least 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 it 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 140 to 210 organisms are 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 Subsample Vials

Label each 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
- date and time of collection
- collection method (for example, 5-minute kicknet, or snag)
- preservative used
- name of each collector
- estimate of the number of individuals in the subsample
- name of person conducting subsampling procedure, if different from collector
- estimate of the number of individuals in the vial
- container replicate number if needed (for example, *1 of 2* or *2 of 2*)

Tracking Requirements for RBP Kicknet or RBP Snag Samples

Upon returning to the laboratory, assign a unique sample tracking number to each vial 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, an instance of numbering may look like *BM 040 04*, where *BM* refers to 'benthic macroinvertebrate,' *040* refers to sample number 40, and *13* refers to the year 2013.

The sample log will contain the following information.

- sample tracking number
- collection date and time
- station number and location description
- name of each collector
- collection methods
- 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 number to the outside of the container. Wrap the label with clear tape to ensure it will not come off. Do not affix the label to the container lid.

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 µm 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 "Field Processing RBP Kicknet or RBP Snag Samples," earlier in this chapter.

Safety

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 in Table 5.1. Chapter 11 lists required and recommended references on identifying freshwater macroinvertebrates.

Voucher Specimens

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

Table 5.1. Taxonomic levels for identification of organisms.

Taxon	**Identify to this level**
Insecta	genus, except leave Chironomidae at family
Oligochaeta	leave at Oligochaeta
Hirudinea	leave at Hirudinea
Hydracarina	leave at Hydracarina
Isopoda	genus
Amphipoda	genus
Nematoda	leave at Nematoda
Ostracoda	leave at Ostracoda
Palaemonidae	genus
Cambaridae	leave at Cambaridae
Gastropoda	genus
Turbellaria	family
Pelecypoda	genus

Voucher specimens serve as long-term physical proof that confirm the names applied to organisms stored in SWQMIS. Voucher specimens ensure the credibility of TCEQ biological data by documenting the identity of the organisms and making them available for review by the general scientific community.

Voucher Storage

Consider the following 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 demonstrates the ability to preserve the specimens into the future (USGS 2000). This could include in-house provisions for sample maintenance or archiving in a university or museum natural-history collection.

Data Evaluation 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 benthic index of biotic integrity (BIBI) metric criteria in Table B.11, Appendix B.

The BIBI 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 wadable 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 BIBI Metrics and Scoring for Kick Samples, Rapid Bioassessment Protocol-Benthic Macroinvertebrates (TCEQ-20152) in Appendix C or a similar form.

The criteria set includes the following 12 metrics.

1. **Total number of taxa.** 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. **Total number of EPT taxa.** This metric is the total number of distinct taxa (genera) within the orders of Ephemeroptera (mayflies), Plecoptera (stone flies), and Trichoptera (caddis flies). 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 taxon (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 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 percentage of 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.

   Sort aquatic macroinvertebrates into functional feeding groups (FFGs) according to Merritt and Cummins (1996). See Table B.6, 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 are:

   - **Scrapers (grazers).** Benthic macroinvertebrates morpho-behaviorally adapted to use the fungal-bacterial-algal complex (referred to as *periphyton*) closely attached to the substrata as their primary food resource.
   - **Collector-gatherers (deposit feeders).** Benthic macroinvertebrates morpho-behaviorally adapted to use fine particulate organic matter (FPOM) deposited either interstitially or on the surface of the substrata as their primary food resource.
   - **Filtering collectors (suspension feeders).** Benthic macroinvertebrates morpho-behaviorally adapted to use particulate organic matter (POM) suspended in the water column as their primary food resource.
   - **Predators (engulfers and piercers).** Benthic macroinvertebrates morpho-behaviorally adapted to use other living organisms (prey) as their 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 their primary food resource.

Note: The groups are not mutually exclusive—that is, one taxon may be considered both 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, with four individuals from the genus *Baetis*, which is a scraper and collector-gatherer genus, 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 reflect a balanced trophic structure, whereas 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 predators must be less correlated to resource base changes resulting from natural changes in habitat, and more attuned to changes that cause significant reduction or increase in prey items (toxicity effects, nutrient effects, and others). Further, most predators have relatively long aquatic life stages, usually greater than six months. This reflects the integration of physicochemical conditions over longer periods of time. Some groups, such as mayflies, complete their aquatic existence in less than two weeks in Texas streams. 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). It measures the relative contribution of tolerant and intolerant taxa to the composition of the community. The metric increases as the relative number of intolerant individuals increases; 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 percentage of total Trichoptera accounted for by the Hydropsychidae, or a complete lack of Trichoptera, likely reflects 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 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. Species of *Stenelmis*, perhaps the most commonly encountered genus, are relatively tolerant of 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.

Freshwater Mussels

Disclaimer

Methodologies for assessing ALU based on freshwater mussels have not been developed for Texas waters. Before conducting any biological monitoring activities using freshwater mussels, it is imperative to coordinate this work with the TCEQ and the TPWD. As methodologies and metrics become established, this manual will be updated to reflect those changes.

Objective

The objective of this section is to describe methods recommended by the TCEQ for the collection and assessment of mussels in freshwater systems.

The purpose of this sampling is to document the kinds and total number—or relative abundances—of mussels present through the collection of dead shells.

Scientific Collection Permit

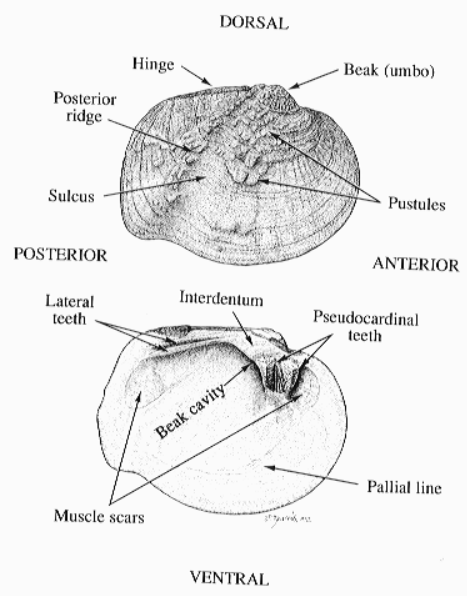
Anyone conducting mussel surveys in Texas must possess or be listed on a valid TPWD SCP. An SCP is required even for the collection of dead shells. All TCEQ regional-office and WQST personnel are included on the SWQM SCP. Any TCEQ employee that needs to be added to this permit should contact the central-office SWQM team. Details of applying for an SCP and SCP reporting requirements are in Chapter 3, “Freshwater Fish.”

Sample Collection

Sampling of dead shells should be associated with a measure of time, area, or effort. For instance, numbers can be documented by employee-hours spent searching, number collected per unit area sampled, or number collected over a linear distance searched. The minimum effort required is a thorough visual inspection of the stream bank for shells with an associated recorded effort.

Shells should be enumerated by species and placed into one of the six following shell-condition classes.

Very recently dead. Soft tissue remains attached to the shell; shell in good condition essentially as it would be in a living specimen; internal and external colors are not faded.



Recently dead. No soft tissue remains, but shell otherwise in good condition (looking like a living specimen that had been killed and cleaned); internal nacre (inner shell layer commonly known as *mother of pearl*) is glossy and without evidence of algal staining, calcium deposition, or external erosive effects; internal and external colors are not faded.

Relatively recently dead. Shell in good condition, but internal nacre is losing its gloss; algal staining, calcium deposition, or external erosive effects (or some combination of these) is evident on the nacre; internal and external colors often somewhat faded.

Long dead. Shell shows early signs of internal and external erosion, staining, calcium deposition, or some combination of these; most or all of the internal coloration and glossy nature has faded (especially in species with colored nacre); shell epidermis with major sections absent, or, if present, clearly aged and flaking.

Very long dead. Shell shows significant signs of erosion, staining, and calcium deposition more widely pronounced than above; color often faded white or nearly so; relatively little intact epidermis left; for specimens in erosive environments, internal features (for example, pseudocardinal teeth) and external features (for example, pustules) often weathered and smoothed, or otherwise exfoliated; shells often chalky, brittle, and crumbling.

Subfossil. Shells with little or no epidermis; nacre faded white and entire shell often white; sometimes with signs of erosion, staining, or calcium deposition; typically chalky and powdery to the touch; shells often brittle and crumbling.

As with other biological collections, retain voucher specimens from each species. Quality digital images suffice for this purpose.

Field Preservation

Dead shells can be stored in plastic storage bags with no fixative.

Labeling a Field Sample

Place in each sample container 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

- name of each collector
- container replicate number if needed (for example, *1 of 2* or *2 of 2*)

Identification of Mussel Samples

The identification of mussels to the species level requires taxonomic training and a familiarity with appropriate keys and literature. Consequently, species identifications must be performed by personnel with appropriate taxonomic training.

For identifying Texas freshwater mussels, one primary reference is Howells et al. 1996.