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Surface Water Quality Monitoring Procedures, Volume 2:

Methods for Collecting and Analyzing Biological Assemblage and Habitat Data

Water Quality Planning Division

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Surface Water Quality Monitoring Procedures, Volume 2:

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Prepared by
Monitoring and Assessment Section
Water Quality Planning Division
Texas Commission on Environmental Quality

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CHAPTER 1

INTRODUCTION

Purpose

This publication is intended for use with its companion volume *Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods*, RG-415 (TCEQ 2012).

This publication contains comprehensive information on conducting biological and habitat assessments, including proper documentation, standardized methods, and data-collection and assessment requirements. The Surface Water Quality Monitoring (SWQM) Program of the TCEQ generated these procedures in coordination with other water programs of the TCEQ and the Texas Parks and Wildlife Department through an established biological work group.

The procedures in this manual are used by the TCEQ, as well as by other monitoring personnel who collect data on behalf of the TCEQ's various water programs such as the Water Quality Standards Group (WQSG), the Standards Implementation Team (SIT), the Total Maximum Daily Load Program, and the Texas Clean Rivers Program. Monitoring authorities, such as the Clean Rivers Program planning agencies and other state and federal agencies submitting water quality data to the TCEQ, are required to follow these procedures.

Working together, these programs gather the data our state needs to develop water quality standards and perform assessments to ensure the quality of surface water in Texas.

Biological Assessments

There are four categories for biological monitoring in freshwater. Each is designed to serve a specific regulatory purpose.

Aquatic-Life Use-Attainability Analyses. UAAs are assessments of the physical, chemical, biological, and economic factors affecting attainment of a use. UAAs are used to determine if existing criteria and uses described in the Texas Surface Water Quality Standards (TSWQS) are appropriate, if the uses and criteria are being maintained, or to determine causes of the use or criteria not being attained (30 TAC 2010).

Receiving-Water Assessments. RWAs are used to assess characteristics on **unclassified** streams, primarily to obtain data so that the appropriate aquatic-life uses (ALUs) can be assigned.

Aquatic-Life Monitoring. ALM is applicable for routine monitoring sites and is conducted to provide baseline data on environmental conditions and to determine if criteria for ALU and dissolved oxygen criteria are being attained. This category also includes reference condition and ecoregion monitoring.

Aquatic-Life Assessments. ALAs are conducted on **unclassified** water bodies that are not included in Appendix D of the TSWQS and have been previously assessed and found not to support the presumed ALU.

How SWQM Procedures Are Used

The guidelines outlined in *SWQM Procedures* are important because they document the quality-assurance procedures that must be used to demonstrate that SWQM data collected by monitoring personnel are of known and comparable quality across the state.

The SWQM Program and the CRP are the programs primarily responsible for the collection of data that accurately describe the physical, chemical, and biological characteristics of state waters. Data collected as part of the statewide monitoring program and for special projects are used to achieve the following goals:

- Characterize existing water quality and emerging problems.
- Define long-term trends.
- Determine compliance with water quality standards.
- Describe seasonal variation and frequency of occurrence of selected water quality constituents.
- Produce the *State of Texas Integrated Report*, which is required by Sections 305(b) and 303(d) of the federal Clean Water Act (CWA). This assessment enables the public, local governments, state agencies, the Texas Legislature, the U.S. Environmental Protection Agency (EPA), and Congress to make water quality management decisions.

Legal Authority

Texas law requires monitoring personnel who collect and analyze water samples for the SWQM Program to follow procedures outlined in a TCEQ manual on SWQM. The rule is in Title 30 of the Texas Administrative Code (30 TAC), Section 307.9.

This revision of the manual is to be used with the companion publication, *Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods* (RG-415).

Contact Information

For questions or comments about this manual or SWQM, you can contact the SWQM Program at the TCEQ. A list of substantive changes to this manual will be proposed and discussed, as needed, at the TCEQ's annual SWQM workshop.

You can reach the SWQM Team in the following ways—

By e-mail:	swqm@tceq.texas.gov
By mail:	SWQM Program, MC 234 TCEQ PO Box 13087 Austin TX 78711-3087
By fax:	512-239-4410
On the Web:	Go to <www.tceq.texas.gov/waterquality/monitoring/>

Getting Resources

Volumes 1 and 2 of the *SWQM Procedures* are available in print and electronically. To order a print copy, call TCEQ Publications at 512-239-0028, or fax your request to 512-239-4488. These manuals and other SWQM publications and resources can be found at the TCEQ website. See Appendix A in SWQM Procedures, Volume 1.

CHAPTER 2

BIOLOGICAL MONITORING REQUIREMENTS

Biological organisms are collected and identified in a manner that, in most cases, permits an assessment of community composition and integrity. Most of this manual focuses on the collection and assessment methods for habitat (Chapter 9), freshwater benthic macroinvertebrates (Chapter 3) and freshwater fish (Chapter 5). It also addresses collection and assessment methods for saltwater nekton (Chapter 4), saltwater benthic macroinvertebrates (Chapter 6), benthic algae and aquatic macrophytes (Chapter 7), and plankton (Chapter 8); however, assessment methods for saltwater, lakes, and reservoirs are not as developed as those for freshwater streams. As estuarine, lake, and reservoir methods are developed or expanded to include assessment tools, they will appear in later revisions of this manual.

Index Period

In order to determine ALUs or to evaluate support of existing ALUs, the TCEQ has established an *index period* during which most bioassessments of aquatic assemblages in freshwater river and stream (lotic) systems should be conducted.

The index period was established to:

- Minimize year-to-year variability resulting from natural events.
- Maximize gear efficiency.
- Maximize accessibility of targeted assemblages.
- Allow adequate time for completion, considering sampling requirements and potential environmental and logistical constraints.
- Make the most efficient use of available resources.
- Ensure that a portion of the samples is collected during critical low-flow and temperature conditions.

The index period represents the warmer seasons of the year from March 15−October 15 (see Figure 2.1). The index period is further broken down into the *critical period*, July 1−September 30. The critical period is the time of year when minimum streamflows, maximum temperatures, and minimum dissolved oxygen concentrations typically occur in Texas.

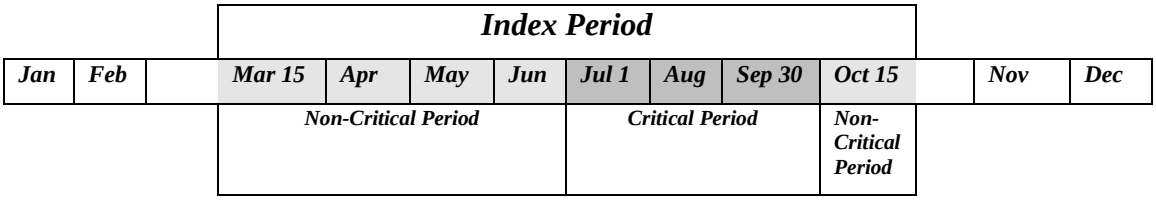


Figure 2.1. The index period.

Scheduling Biological Monitor Events

The TSWQS establishes the criteria for water quality conditions that need to be met in order to support and protect designated uses (30 TAC, Chapter 307).

All bioassessment sampling for freshwater streams must be conducted during the index period of March 15 to October 15. **Note:** Two exceptions are RWAs, which are carried out as needed, and special studies, which are completed with specific seasonal objectives.

Collecting a portion of the samples during critical conditions (when water temperature approximates critical summer values) helps determine if the criteria set for the designated uses are being met and maintained when streamflow is at or above critical low flow.

Note: The assumption is that criteria met under these conditions would be met during other seasons when expected streamflow is greater and water temperatures are lower.

General Sampling Guidelines

Before planning any specific biological monitoring event such as an RWA or UAA, refer to Appendix D, "Biological Fact Sheets," for detailed information on the required number of samples for that type of study.

When collecting only one sample, schedule the event during the critical period. If that is not possible, submit a written justification of why that objective was not met.

When collecting two samples at the same site during the same year, both samples must be collected during the index period—one in the noncritical period, and one in the critical period.

When collecting more than two samples at the same site, the study must be at least two years long with at least two samples collected per year—one event in the noncritical period and one in the critical period. At least half but not more than two-thirds of the events must occur during the critical period. No more than two-thirds of the total number of samples in the data set may be from any one year. Sampling events must be separated by at least one month.

Exceptions

When the intent is to use sample results to assess use support (ALM, ALA) or to establish the appropriate use (UAA, RWA), bioassessment events should fall within the critical and noncritical portions of the index period.

However, strict adherence to these temporal guidelines may not always be feasible as a result of either normal or unusual variability of local flow and temperature. For example, during a year with abnormally high flows, critical low-flow and temperature conditions may not begin exactly on July 1, so the noncritical period might extend into early July. In situations such as these, when conditions preclude meeting exact calendar guidelines, consult the TCEQ SWQM Team or WQSG before adjusting sample regimes and explain any deviation from temporal guidelines in writing when submitting results.

Representativeness of Sites

Select monitoring sites that best represent conditions of an entire water body for both biological and water quality. The reach must have a good variety of microhabitats to sample, such as a mixture of riffles, runs, and pools. Avoid selecting a reach where water quality and hydrology

change dramatically over the reach, such as areas with a major tributary or contaminant source. RWAs are the only category of biological sampling that requires the reach to be located specifically in relation to the existing outfall or proposed outfall of a permitted discharge. Refer to the RWA section in this chapter for details on locating RWA reaches.

Site Reconnaissance

Perform a reconnaissance of the water body and surrounding watershed before biological sampling begins at a site. Include an assessment of stream access, appropriate reaches for biological sampling, and site stability. Mark potential sites on a topographic map (7.5-minute series) before a reconnaissance trip. Determine stream reaches based on biological collection sites and habitat-assessment requirements. Adequate representation of the ecological community requires that a large enough distance of a stream site be evaluated. See Chapter 9, "Physical Habitat of Aquatic Systems," for details on selecting a stream reach.

Make an effort to collect the sample at least 30 to 100 m upstream from any road or bridge crossing (depending on the size of the bridge and crossing) to minimize its effect on stream velocity, depth, and overall habitat quality.

There are situations in which the best sampling reach can only be accessed through private property. Obtain landowner permission before accessing any private property.

Sampling Conditions

Collect all biological samples during stable, unscoured flow conditions, ideally when flow is at, or just above, the 7Q2 of a stream—the seven-day, two-year low flow, or the lowest average streamflow for seven consecutive days with a recurrence interval of two years, as statistically determined from historical data. If sampling a stream that is intermittent with perennial pools, the 7Q2 rules do not apply and sampling should proceed in the pools.

If stream conditions are not stable and do not reflect baseline conditions, reschedule the sampling event. Allow a minimum of two weeks of normal flow after a significant scouring event before collecting biological samples. If extreme weather conditions occur, such as significant drought or heavy rains, or if the stream has been dry, allow at least one month of normal flow before collecting biological samples. Use your best professional judgment to determine the appropriate sampling condition, since the return of the stream to normal conditions may depend on recruitment sources.

Low-Flow Monitoring

In order to maintain continuity for TCEQ SWQM activities during periods of drought, guidance is available online to facilitate meeting monitoring commitments specified in the coordinated monitoring schedule (CMS):

<www.tceq.texas.gov/goto/swqm-procedures>

Other Monitoring Requirements

Documentation and Field Notes

Use a bound field-data logbook to record biological information in the field. Record general information, field measurements, and other field observations. General information includes:

- station ID
- location
- sampling date, time, and depth
- collector's initials and employer

Field measurements include physicochemical parameters and other measurements, such as flow. Field observations include:

Water appearance. Note color; unusual amounts of suspended matter, debris, or foam; and other similar observations.

Water odors. Note unusual odors, such as hydrogen sulfide, musty odor, sewage odor, and others.

Weather. Document meteorological events that may have affected water quality, such as heavy rains or cold fronts. Record the number of days since the last precipitation that was significant enough to influence water quality.

Biological activity. Excessive macrophyte, phytoplankton, or periphyton growth may be present. The observation of water color and excessive algal growth is very important in explaining high chlorophyll *a* values. Note other observations, such as the presence of fish, birds, amphibians, reptiles, and mammals.

Stream uses. Note stream uses such as swimming, wading, boating, fishing, irrigation pumps, navigation, and others.

Watershed activities. Note activities or events in the watershed that have the potential to affect water quality. These may include bridge construction, shoreline mowing, and livestock watering upstream.

Sample information. Make specific comments about the sample itself, such as number of sediment grabs or type and number of fish in a tissue sample—these comments may be useful in interpreting the results of the analysis. If the sample was collected for a complaint or fish kill, make a note of this in the observation section.

Missing parameters. If a scheduled parameter, or group of parameters, is not collected, note this in the comments.

A field-data logbook must indicate whether data recorded in the logbook have been transcribed onto data forms.

Creating New Monitoring Stations

Sites where biological data are collected should have a station number associated with the data. The TCEQ prefers that the station location be set at the point in the reach where the multiprobe for 24-hour data collection is deployed.

Procedures for generating a new monitoring station are found in Chapter 3 of the *SWQM Data Management Reference Guide* (*SWQM DMRG*), available online. The *SWQM DMRG* contains detailed instructions and information necessary to complete a SLOC form. SLOC forms can be found in the *SWQM DMRG* or online (see Appendix A). Check the list of existing stations before submitting a station location (SLOC) form for a new Station ID. A list of existing stations, arranged by basin, can be found online:

<www.tceq.texas.gov/goto/dmrg>

Note: Station ID numbers are not assigned sequentially. A review of the entire list may be necessary. Unclassified water bodies appear first on the list.

Additional Latitude and Longitude Coordinates

In addition to the station coordinates, collecting the coordinates of the ends of the reach is strongly encouraged as well. Collect latitude and longitude coordinates using a global positioning system or geographic information system (GIS). For RWAs, collect the GPS coordinates at the existing or proposed wastewater discharge point as well. Specific GPS requirements for geolocational data appear in Chapter 3 of the *SWQM DMRG.*

Non-Biological Parameters

Non-biological parameters such as flow, 24-hour DO, and water chemistry are integral parts of any biological assessment. Flow is always required with a biological assessment, whereas water chemistry samples and 24-hour DO measurements are required in some assessments and are strongly encouraged in others. Methods for these parameters may be found in *Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods,* RG-415 (TCEQ 2012).

Monitoring Categories for Wadable Freshwater Streams

Data collection requirements for ALM, ALA, RWA, and UAA categories are similar. The main differences are the frequency and duration of sample collection. Detailed requirements regarding sampling effort and required parameters for these four categories are found in Appendix D.

ALM

ALM, a SWQM Program and CRP activity, is for selected routine monitoring sites. It is conducted to provide baseline data on environmental conditions or to determine if an ALU is being attained. Sites selected for ALM must be appropriate for biological monitoring as described in the "Site Representativeness" section of this chapter. Therefore, if a site historically monitored for routine water chemistry is chosen for ALM, every effort must be made to locate the best possible reach around that station for biological and habitat data collection. Data collected as part of an ALM are used for the State of Texas IR. Detailed ALM sampling requirements outlined in Appendix D.

Ecoregion ALM

In the early to mid-1980s, the TCEQ and TPWD undertook to develop a more effective approach to establishing attainable conditions for aquatic life in Texas streams. Studies, such as *An Assessment of Six Least Disturbed Unclassified Texas Streams* (Twidwell and Davis 1989) and the *Texas Aquatic Ecoregion Project* (Bayer et al. 1992), established the utility of the ecoregion approach, which uses carefully selected, least-disturbed streams within the same ecoregion as water quality reference sites to estimate attainable conditions. Ecoregions are geographic regions of relative ecological uniformity and may be delineated at varying levels (Omernik, 1985). These studies identified minimally impacted reference streams in 11 of the ecoregions found in Texas.

Identifying Minimally Impacted Ecoregion Reference Streams

The process used to identify minimally impacted reference streams begins with the professional knowledge of TCEQ central-office and regional biologists, as well as information from other sources, such as river authorities, TPWD, and academia, to identify candidate reference streams in each ecoregion whose watersheds meet the following criteria:

- No, or very little, urban development.
- No significant or atypical point sources of pollution.
- No channelization.
- Characterized by perennial flow or perennial pools.

Mapping the watershed also allows determination of areal coverage and provides the information necessary to ensure that selected streams represent a range of potential watershed sizes. To this end, the TCEQ has identified three relatively broad drainage-basin-size categories, small (< 100 sq mi), medium (100–200 sq mi), and large (> 200 sq mi). Biological characteristics, such as species richness and trophic organization, vary according to watershed size, especially in the fish community (Karr et al. 1986; Vannote et al. 1980).

Conduct ground-truthing for candidate sites, where possible, across several access points within each watershed to verify conformity with the criteria described above, to confirm GIS land-use data, to identify local and unmapped disturbances within the watershed, and to ensure the availability of appropriate habitat for sampling the target group (for example, benthic macroinvertebrates and fish). The goal of the SWQM Program is to continue to revisit a subset of the population of minimally impacted ecoregion reference streams to refine biological and water-chemistry criteria and to document variability in biological and physicochemical characteristics over time.

Aquatic-Life Assessments

ALAs are conducted on unclassified water bodies, not included in Appendix D of the TSWQS, that have previously been assessed and found not to support the presumed ALU. Unclassified waters are those smaller water bodies—such as small rivers, streams, and ditches—that are not designated in the TSWQS as segments with specific uses and criteria.

The presumed ALU for unclassified streams, not in Appendix D of the TSWQS, with the following flow types are:

- Perennial—High
- Intermittent with perennial pools—Limited
- Intermitent—Minimal

Classified water bodies refer to water bodies that are protected by site-specific criteria. The classified segments are listed and described in Appendix A and C of Chapter 307.10 in the TSWQS. The site-specific uses and criteria are described in Appendix A. Classified waters include most rivers and their major tributaries, major reservoirs, and estuaries. The purpose of an ALA is to confirm if indications of nonsupport are appropriate, and if necessary to identify more appropriate ALU and DO criteria. Appendix D details ALA sampling requirements.

Site and reach selection must ensure that adequate data are generated to accurately characterize biotic integrity through the entire study area. This will require at least one site, depending on the size of the water body. The number of sites needed to adequately characterize the water body must be negotiated with the WQSG. Sampling of multiple sites and reaches may be necessary for most water bodies.

Data collected as part of an ALA are used by the WQSG to determine if the water body is meeting its presumed high ALU designation. The result of this type of monitoring may lead to the development of a UAA to propose the appropriate site-specific ALU designation and DO criterion in the next revision of the TSWQS.

Receiving-Water Assessments

RWAs are conducted on unclassified water bodies with existing or proposed wastewater discharges during a single study, on a specific reach of a stream, to assess their physical, chemical, and biological characteristics. Unclassified waters are those smaller bodies—such as small rivers, streams, and ditches—that are not designated in the TSWQS as segments with specific uses and criteria. RWAs are requested by the Water Quality Standards Implementation Team when the applicable ALU category for an unclassified stream has not been determined and cannot be adequately established from existing information. Generally, RWAs are conducted in response to a proposed amendment to an existing wastewater permit or before a new permit is issued. Data collected during an RWA are used to determine the appropriate ALU and DO criterion. RWAs are conducted on freshwater streams only.

RWA data are used primarily by the WQSIT for two TCEQ objectives—reviewing wastewater-permit applications and establishing site-specific standards. Within the Texas Pollutant Discharge Elimination System (TPDES), wastewater-permit applications are reviewed for potential water quality impacts on surface waters of the state. RWA data help the WQSIT assign appropriate ALUs and standards to water bodies potentially affected by proposed or existing wastewater discharges. By studying the area upstream of an existing discharge or downstream of a proposed discharge, it is possible to determine the appropriate ALU for a stream receiving wastewater effluent.

For discharges from existing wastewater treatment plants (WWTPs), the RWA must be conducted upstream from the discharge. A reach beginning approximately 30 m upstream of the discharge point is mandatory. If this area is not accessible, or is not representative of conditions downstream of the discharge, the reach may be further upstream where access is possible or conditions are more representative of the stream downstream of the wastewater discharge. For

new wastewater permits for treatment plants that have not yet discharged, the RWA must be conducted on a reach immediately downstream of the proposed discharge point.

In addition, for WWTP dischargers that could potentially affect DO on other larger tributaries downstream of the discharge, RWA data must be collected on selected reaches downstream of those tributary confluences. Use Table B.1 in Appendix B to determine if other downstream tributaries need to be assessed. Typically, with larger wastewater plants, downstream tributaries will require assessment. In some cases, the receiving stream may be dry or have limited uses upstream of the outfall, but the impact zone may extend to the next Strahler stream order unclassified stream. In those cases, for an existing wastewater discharge, an additional RWA reach must be assessed upstream of the confluence of the secondary receiving stream. For new wastewater permits for treatment plants that have not yet discharged, an additional RWA reach must be assessed downstream of the confluence of the secondary receiving stream. Additional RWA reaches must be assessed if the impact zone extends into even larger unclassified streams. Figure B.8 (Appendix B) illustrates the RWA reach for an existing discharge of 3.6 million gallons per day into an intermittent and perennial stream. Figure B.9 illustrates the RWA reach for a proposed discharge of 3.6 MGD discharge into an intermittent and perennial stream. RWAs should be planned in consultation with the TCEQ's WQSIT to ensure that all necessary data are collected.

Ideally, RWAs should be conducted during summer low-flow conditions or the critical period (July 1 through September 30), but may be performed anytime during the index period. Occasionally, RWAs may have to be performed outside the index period because action on a permit is necessary. Whenever possible, RWAs must be completed six months before the wastewater-permit renewal or amendment. RWAs must be coordinated with representatives from other interested parties, such as wastewater permittees, TCEQ central and regional offices, the TPWD, and any other entities associated with the permit action. RWAs may serve as the basis for the development of a UAA on the unclassified water body at a future time. Detailed RWA sampling requirements are outlined in Appendix D.

Use-Attainability Analyses

As part of the triennial revision of the TSWQS, UAAs are primarily used by the WQSG to review and set site-specific standards for water bodies. The purpose is to determine if the existing designated or presumed ALU and associated DO criterion are appropriate and, if not, to develop information for adjusting designated uses or criteria. UAAs require coordination with the WQSG. A UAA considers the physical, chemical, and biological characteristics of a water body, as well as economic factors to determine the existing and attainable uses. Completed UAAs are submitted to the EPA for technical approval. If approved, the changes are incorporated into the next triennial review of the TSWQS after public notice and full public participation.

Site and reach selection must ensure that sufficient sites and reaches are monitored to derive adequate data to accurately characterize the ALU for the entire study area. Sampling of multiple sites or reaches will be required for most water bodies. Land use–land cover analysis of the proposed sites is strongly recommended before selection of the sites. As each water body differs in the number of sites necessary to adequately characterize it, coordinate with the WQST to determine the appropriate number. Detailed requirements for UAA sampling appear in Appendix D.

Monitoring and Assessment of Large Rivers

Collecting and assessing fish assemblages in predominantly large, runoff-dominated streams and rivers present substantially greater complexity and potential for problems than work in wadable streams. The scale of the systems and corresponding fauna and habitats can be quite different. Most major drainages in Texas begin within the state or just outside its borders, and drain into the Gulf of Mexico. Depending on the reach surveyed, large rivers and streams in Texas may be similar to wadable streams in terms of discharge and scale. However, most have significant reaches that are not primarily wadable, have substantial flow, and may pass through multiple ecoregions. Unlike smaller water bodies, which are normally replicated across a given region or basin, large rivers are typically unique (Emery et al. 2003).

The summer index period may not be appropriate in large rivers, depending on issues such as system hydrology including seasonal releases from reservoirs and irrigation withdrawals. Instead, sampling periods should be specific to the sites and collection methods and meet the objectives of the study. Before adjusting the index-period sampling strategy to better fit the system where work is being conducted, consult with TCEQ central-office SWQM personnel or the WQSG (or both). Reference streams may not be available, given human-induced modifications to larger waterways and the lack of streams of similar size and faunal composition. Aside from issues associated with establishing a comparative baseline, large streams and rivers require different equipment or application of equipment than wadable streams to adequately assess assemblages, and may require different assessment tools. Obtaining a representative sample can be difficult given the scale and distribution of habitat patches within large rivers, making reach selection extremely important.

Collection technologies appropriate for large rivers have varying limitations with regard to how each type of gear can thoroughly sample a single habitat or be uniformly applied to multiple habitats (Emery et al. 2003). In general, multiple types of collection gear must be employed to obtain a representative sample.

When analyzing biological data collected in large stream and rivers, consider that the assessment tools and regionalized indices of biotic integrity described by Linam et al. (2002) for nekton assemblages, and Harrison (1996) for benthic macroinvertebrate assemblages, are a starting point, but were designed for wadable streams (≤ 4th order). Thus, they may not apply directly in all situations and depend on the system being sampled. One potential test of the adequacy of wadable-stream sampling methods is whether at least 50 percent of the reach can be sampled by wading methods. Example methods include backpack electrofishing, seines, benthic kicknet, and Surber samplers.

Many reaches may be marginally wadable, whereas others are predominantly deep except for the stream margins. The former might be adequately sampled using a combination of a backpack electrofisher and seines for fish, and a kicknet for benthic macroinvertebrates as in the wadable-stream protocols, whereas boat electrofishing equipment for fish and snag sampling and near-bank sweep-net samples for benthic macroinvertebrates would be more appropriate in non-wadable sites. In the latter case, seines must still be used as a complementary tool for sampling.

Other kinds of gear may be required, depending on the objectives of the study and stream conditions. Example gear includes gill nets, hoop nets for fish, and artificial substrates, dredges, or snag samplers. Sampling duration may vary depending upon the system scale. The EPA has

proposed 40 to 100 times the wetted stream width as a reach length for sampling in large streams and rivers. Simon and Sanders (1999) observed that 500 m was long enough to capture sufficient numbers of species to characterize biological integrity but not biological diversity in great rivers. The study objectives will influence the number of reaches sampled and sampling duration. Given the aforementioned complexity in sampling and assessment, personnel from the TCEQ must be consulted to determine the proper sampling regime and method for evaluating the samples if a study is anticipated on large, nonwadable streams and rivers.

Monitoring and Assessment of Lakes and Reservoirs

The index period for sampling freshwater streams, as described above, may not be appropriate for lakes and reservoirs. In these types of habitat, the appropriate sampling period should be specific to the study and collection method. In general, the period of summer stratification—when water temperature is highest, the volume of suitable, well-oxygenated habitat is reduced, and inflows are usually lowest—will be considered the critical period. In these situations, a written explanation of how appropriate sample windows were established must be included with the results. For example, the TPWD procedures for assessment of inland fisheries allow for collection methods (boat-mounted elecrofishing, gill netting, and trap netting) during optimum conditions based on surface water temperature, fish ecology, and assessment needs. Electrofishing has a preferred surface water temperature range of 15.5°–23° C. This occurs in the fall (September through December) and in the spring (March through May). Gill netting is conducted from January through June. The gill-netting sampling period is based on fish ecology and assessment needs more than water temperature. Trap netting has a preferred surface water temperature range of 10°–18° C.

To date, there is limited guidance on assessing the biological and habitat integrity of lakes and reservoirs. The artificial nature of reservoirs complicates regulatory processes, as it may be difficult to determine the specific biological communities that correspond with designated ALUs. The TCEQ has well-developed guidance for assessing the biology and habitat in freshwater streams. There is a growing need for the same guidance in lakes and reservoirs. TPWD (2002) began preliminary work on developing procedures for assessing biological and habitat integrity of lakes and reservoirs. This work was prompted by concerns that some reservoirs or portions of reservoirs were not meeting designated ALUs based on DO concentrations. Reservoirs will continue to be a growing concern and a uniform approach to assessment of these water bodies will be an important regulatory tool.

In addition to the preliminary work in Texas, a few other states and the EPA have developed methodologies for assessing the biological integrity of lakes or reservoirs. The Ohio EPA developed a multimetric assessment for inland lakes or reservoirs—the Ohio Lake Condition Index (Davic and DeShon 1989). The Tennessee Valley Authority developed biological assessment methods for its reservoirs that use a similar approach to what has been developed for stream assessment (Dycus and Baker 2001). The EPA (1998) has also published a technical guide for the development of lake and reservoir bioassessment and biocriteria programs.

Before conducting any biological monitoring at a lake or reservoir, 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.

Categories of Saltwater Biological Monitoring

The three categories of saltwater biological monitoring are ALM, ALA, and UAA. Each is designed to serve the same regulatory purpose as those for freshwater.

While the purposes for conducting these assessments in saltwater are the same as for freshwater, the protocols used to collect the data are quite different and, in many cases, are still under development. Additionally, standardized metrics for evaluating aquatic-life uses for saltwater bodies do not exist at this time. Before conducting any biological monitoring activities on a saltwater or tidally influenced water body, it is imperative to coordinate this work with the TCEQ WQST. As methodologies and metrics are established, this manual will be updated to reflect those changes.

Tidal Streams and Estuaries

The biological monitoring process of tidal streams and estuaries for regulatory purposes is not clearly defined in Texas. When the water quality standards were originally formulated and codified, state environmental professionals ranked aquatic-life uses of tidal streams as "high" or "exceptional," based on their best professional judgment. As development occurs along the coast, UAAs have begun for tidal streams. Additionally, a number of tidal streams being assessed are not meeting DO criteria. Important considerations for UAAs on tidal streams and estuaries include:

Water quality sampling. Instantaneous field measurements must be collected, including profiles, since the water column is often stratified due to temperature and salinity. Samples can be collected for analysis of routine water chemistry and carbonaceous biochemical oxygen demand, five-day ($CBOD_5$). Profiles and grab samples should be collected at depths specified in Volume 1 (RG-415).

Flow. Tidal-stream hydrology is very different from freshwater-stream hydrology. The multi-directional nature of these flows is critical to tidal stream and estuary communities. Technologies to measure multidirectional streamflows may be considered in order to derive information about the hydrology in these systems.

Biological. Important biological components of tidal streams and estuaries include nekton, benthic macroinvertebrates, zooplankton, phytoplankton, and macrophytes.

Habitat. Both instream habitat and riparian habitat must be considered for tidal streams. In estuaries, bottom structure and sediment must be sampled.

Dissolved oxygen. DO measurements, collected over a minimum of 24 hours, are important in tidal streams. The nature of the hydrology in these streams makes low DO concentrations more likely. Refer to Volume 1 (RG-415) for details on collecting 24-hour DO.

Land-use and land-cover analysis. This type of analysis derives valuable information about potential sources of pollution in a watershed and must be considered when doing biological assessments.

Sampling Index Period

Marine and tidal systems may require adjustment of the temporal guidelines mentioned above to ensure that bioassessment events are conducted during an index period that meets the objectives of the study.

In general, the critical period for most tidal and marine systems is similar to that set out for freshwater streams—in late summer, when water temperatures are highest, inflows are lowest, and many tidal systems tend to stratify at times of greatest stress for estuarine biotic assemblages.

The noncritical portion of the index period may not be so easily defined and may be related to fish migration patterns, and periods of high runoff and inflow, as well as tidal patterns and temperature. Consult the TCEQ SWQM Program or WQSG before establishing the sample regime in these systems. Include a written explanation of how appropriate sample windows were established with results.

CHAPTER 3

FRESHWATER FISH

Objective

The goal of fish sampling is to collect a representative sample of the species present in their relative abundances. Given the variability of habitats, flow regimes, and water chemistry, the individual biologist's judgment is important in assessing the sampling effort necessary for an adequate characterization of the fish community. Sampling includes all available habitats and combinations of habitats in a reach until no additional species are collected. Be prepared to preserve voucher specimens for later identification and verification with a 10 percent formalin solution (one part full-strength formalin and nine parts water).

Scientific Collection Permit

A Scientific Permit for Research is required to collect, salvage, band, or hold native Texas wildlife for scientific purposes. Scientific purposes include activities aimed at enhancing, protecting, conserving, or managing protected wildlife, or furthering scientific understanding of a resource or the environment. Refer to the TPWD website for application requirements and reporting forms: <www.tpwd.state.tx.us/business/permits/land/wildlife/research/>.

Notify each TPWD Law Enforcement Office in each region of your field activities by telephone at least 24 hours, but not more than 72 hours, before collection if collection techniques or devices being used are ordinarily illegal (e.g., using gill nets or electroshocking devices to collect fish, or hunting or collecting along public roads and rights-of-way). A confirmed response from the local game warden is required before collection if the sampling activities being conducted involve methods of capture ordinarily classified as illegal. In addition, please be advised that collecting in a wildlife management area is not authorized without prior written permission from the area manager.

For regional office location and telephone numbers, see <www.tpwd.state.tx.us/warden/connect/offices> or call a Parks and Wildlife Communication Center: Austin—512-389-4848; Houston—281-842-8100.

Permit holders are required to carry this permit when conducting authorized activities. Sub-permittees may carry a copy in lieu of the original permit. It is also advisable to carry additional corroborative identification such as a driver's license. A permittee engaging unpermitted assistants must keep on file at his or her office and on his or her person in the field a signed and dated list of all unpermitted persons assisting in permitted activities. TCEQ regional office staff and central office SWQM and WQS staff are listed as sub-permittees on the permit currently held by the TCEQ SWQM team. Any TCEQ employee that needs to be added to this permit should contact the central-office SWQM team.

Records

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

Field Logbook

For each sample event record the following information in a field logbook.

- date and time of sample collection
- name of water body
- location of sample site (Station ID)
- name of each collector
- collection methods and equipment
- number and type of samples collected
- number of sample containers
- preservative used
- time spent electrofishing
- number of seine hauls and length of each haul
- a description of habitats sampled
- unusual site characteristics
- field measurements (flow, DO, pH, temperature, specific conductance)

Sample-Tracking Logbook

Maintain a sample-tracking logbook containing the information described in Chapter 11. This logbook documents when samples arrive at the laboratory or headquarters, the sample-processing steps, and who has custody of, or responsibility for, the sample.

Laboratory Bench Sheets

Laboratory bench sheets must be maintained where specimen identification and counting occurs. These bench sheets document the raw counts of individuals for each taxon and notes relevant to identification and enumeration.

Sample Collection

The method used to collect nekton samples will depend on several factors, including water-body characteristics, the number of sampling personnel, and available sampling equipment. The field equipment and materials necessary to collect fish are listed in Appendix A. Forms needed for biological assessments appear in Appendix C. Examples of laboratory bench sheets are in Appendix H. Electronic copies of all the tables in the appendixes are available at the TCEQ website. Definitions of technical terms are in Appendix E.

In most streams, fish are collected using multiple gear types, with fishes counted separately. Both electrofishing and seining are required for collecting fish samples. If unable to employ multiple types of gear, indicate the reason in the field logbook and increase effort with the gear used. For example, if electrofishing is not possible because of elevated conductivity, increase your seining effort by conducting additional seine hauls to ensure all possible habitat types are sampled. Collections at each site in the study must be comparable. Consequently, collectors must

ensure that the sampling procedures, level of effort expended, and types of habitat sampled are similar in succeeding years.

Once the leaders of crews for sampling habitat, fish, and benthic macroinvertebrates have agreed where sampling will be conducted, the habitat crew marks the ends of the reach with bright survey flagging. The TCEQ discourages sampling from areas outside those boundaries.

All fish must be reported by collection method so data from each sampling method can be stored in SWQMIS as unique sample sets. Details on submitting biological data to SWQMIS can be found in Chapter 12 of the *SWQM DRMG*.

Electrofishing

Electrofisher capabilities vary by manufacturer and model. Each model is effective under certain ranges of specific conductance. For example, the Smith-Root Type 12 model is most effective at specific conductance levels less than 1,000 µS/cm, though it is rated to 1,600 µS/cm (Smith-Root, Inc. 2003). Check the manufacturer's specifications for optimal operating procedures and consult with the manufacturer's user manual if the unit can work effectively at higher conductivities.

Collection Procedures

Since the objective of the nekton sample is to obtain information on the composition and integrity of the fish community, collectors must net, identify, and enumerate all fishes possible (Murphy and Willis 1996).

Backpack Electrofisher

Safety

Safety is of the utmost importance. Use only commercially produced electrofishers with adequate safety devices, such as tilt switches, overload devices, and kill switches. At least two persons are required when electrofishing (one to carry the backpack and the other to net fishes), though three make an optimum crew. Always be cautious while using electrofishing equipment. All participants must wear rubber lineman gloves rated for at least 1000 volts and rubber or neoprene waders. Breathable waders **must not** be worn, as electric current can pass through them.

Adjusting a Backpack Electrofisher

Use a backpack electrofisher in wadable streams, where conductivity falls within the range specified in the equipment's user manual. In waters near the upper range of conductivity (based on specific conductance) for a given backpack unit, a smaller ring anode may be an option. Alternately, a standard ring may be covered with electrical tape in a candy-cane pattern. Note that using electrofishers at higher conductivities shortens battery life.

After reaching the stream,

- power up the unit and set controls for ambient stream conditions
- set the initial frequency at 60 Hz at 6 milliseconds (setting I5 on the newer Smith-Root backpacks) and the voltage at 100 volts

- engage the unit and check the output

Since the goal is to generate the optimum duty cycle for the water conditions, disengage the electrofisher and adjust the voltage to the next setting. Power up the unit again and test the output. Repeat this procedure until the voltage is maximized. The electrofisher will automatically reset when the output is beyond specifications. In general, lower voltages are used in high-conductivity waters and higher voltages in low-conductivity waters. Smith-Root makes general recommendations for voltage in waters of differing conductance: 100 to 300 volts for conductance of 400 to 1,600 µS/cm, 400 to 700 volts for 200 to 400 µS/cm, and 800 to 1,100 volts for < 200 µS/cm.

Collection Method

Once the controls are adjusted, reset the timer according to the instructions for that model of backpack. The collector carrying the backpack wades upstream to eliminate the effects of turbidity caused by disturbing bottom sediment. To maximize collection, do not apply electricity continuously. For example, electrical current could be applied along the length of an undercut bank and then turned off until another discrete habitat type is encountered. This allows the netters time to attempt capture of all stunned fishes and records a more accurate shocking time. Polarized sunglasses facilitate spotting stunned organisms. In particularly turbid water, a small seine may be employed behind the electrofisher to capture stunned fishes that are difficult to observe.

Where to Sample

Sample all available habitat and instream cover types within the delineated reach length—normally 40 times the wetted width. In contrast to routine sampling for benthic macroinvertebrates, during which only one habitat type is usually sampled, attempt to sample as many different habitat and cover types as possible. Habitats include riffles, runs, glides, pools, brush piles, undercut banks, boulders, snags, midstream bars, current breaks, and others. See Chapter 9 for detailed information on habitat types.

Sampling Time

Actual shocking (trigger) time as recorded by the backpack timer must not be less than 900 seconds, but that is a minimum. Record time and distance on data forms. **Always** continue shocking as long as additional species are being collected. Note all species observed but not captured.

Boat-Mounted Electrofisher

Safety

As with backpack equipment, safety is extremely important when using boat-mounted electrofisher equipment. Use only commercially produced electrofishing equipment. At least three persons are required when electrofishing from a boat. Everyone on the boat must wear rubber, nonconductive gloves, and knee boots and make every effort to keep the gloves dry. Everyone on the boat must wear a personal flotation device.

Adjusting the Output

The procedures for setting output are similar to those for backpack electrofishers. Set the unit to pulsed direct current with an initial voltage of 100 volts and an initial pulse rate of 60–120 Hz. Once the controls are adjusted, reset the timer, then apply electricity discontinuously. As with the backpack electrofisher, catches can usually be increased in areas of submerged cover by moving in with the power on but the circuit off and then energizing electrodes for an element of surprise. If fishing success is poor, increase the voltage. If mortalities occur, decrease the voltage. In areas of elevated specific conductance observe the equipment limits recommended by the manufacturer.

Collecting

In larger, non-wadable streams, or in reservoirs and lakes, use boat-mounted electrofishers. The minimum sampling effort is 900 seconds of actual shock time, though more is normally required in non-wadable systems. All habitat and cover types must be sampled within the delineated reach length, normally 40 times (or more) the wetted width of the stream. (The TCEQ recommends sampling habitats in at least one meander wavelength.) See Chapter 9 for details on determining the reach length.

When sampling in streams and rivers, boat-mounted electrofishers will normally be used with the boat moving downstream. However, there may be times when upstream sampling is warranted (e.g., backwaters, slow current velocity, safe approach to cover). When electrofishing downstream, the boat speed should be slightly slower than the flow, increasing the chances that fish will float to the surface and stay close enough to the boat for capture.

Seine

Seining is a required collection technique in all sampling habitats. Seining is often more successful where electrofishing may not be as effective, such as deep pools where wading with a backpack electrofisher would be difficult, or shallow riffles where staking out a seine and kicking the substrate efficiently captures organisms washing downstream.

Seining is an active method of fish capture mainly for smaller fish and juveniles. Seines can be hard to use in stands of emergent vegetation or areas with a lot of woody debris, stumps, or cypress knees.

Seine Types

Several different seines are used, depending on the habitats. Deep pools may be sampled with a 30 ft × 6 ft × ¼ in mesh seine, whereas riffles, runs, and small pools are usually sampled using a 15 ft or 6 ft × 6 ft × $^{3}/_{16}$ in mesh seine. All are straight seines constructed of delta-weave mesh with double lead weights on the bottom line. Seines must be inspected for any holes and repaired or replaced prior to each use.

Collecting

A seining crew consists of at least two persons, but is more effective with three. Attempt at least six effective seine hauls covering at least 60 m. Use a 6, 15, or 30 ft straight seine, depending on stream size, current, and depth. One end of the seine is positioned near the bank. The seine is positioned perpendicular to the bank. With the net fully extended or rolled to make it taut (in areas were the sampling habitat is smaller or the stream channel narrows), two persons pull the

net parallel to the bank with a person on bank slightly behind a person in the channel. The person in the channel proceeds to the bank with seine extended. Both persons pull the seine onto the bank. Be sure the lead line remains on the bottom until the seine is pulled out of the water.

Given moderate velocity, seining may be most effective in a downstream direction, with fishers moving slightly faster than the current. Staking out the seine in swift water and having others kick into the net can also be effective in riffles.

Repeat this process until at least six replicates are collected, covering 60 m. One unit effort for each seine haul would average 10 m, but several short hauls may be required to make up one unit level of effort (e.g., riffle kicks). For a seine haul to be considered effective, evaluate whether the haul was negatively affected in any way. If the seine gets caught on woody debris or the net is lifted in a manner that may allow fish to escape, the haul must be considered ineffective and not counted as viable. Capturing no fish would not necessarily constitute an ineffective haul. Keep any fish collected even if the haul is ineffective.

As in backpack electrofishing, continue sampling until no new species are noted.

Count and record all organisms collected by the seine or put them in a container with preservative and attach a label. Often the organisms are so small and numerous that it is preferable to bring the entire catch back to the laboratory for identification and enumeration.

Sample Preservation and Processing

Field Processing

Maintain the fishes in some type of holding bucket or tank with adequate aeration until ready to process them.

Do not combine data from electrofishing and seining into one sample. The catch from each method constitutes a separate sample. Use a separate biological reporting form for each collection method.

Other than voucher specimens, easily identified fishes may be counted in the field after all collection activity at a sampling location has been completed. ***Do not*** **release any fish caught using either method back into the stream where additional sampling may occur.**

Retain two individuals of each species collected (either seining or electrofishing) for positive identification in the laboratory. Do not retain any fishes greater than 0.3 m total length. These specimens are photo vouchered. Retain all but the most easily identifiable fishes for laboratory identification.

Use of Digital Photos as Fish Vouchers

An exception to the voucher requirement is the use of photographs as vouchers for fish greater than 0.3 m total length. **This is acceptable only when the photograph clearly shows the characters necessary for identification of the specimen to species.** It is also permissible to photo-voucher fish smaller than 0.3 m under certain circumstances, such as when they belong to endangered or threatened species.

For large or protected fish, photographic vouchering is an economical method that reduces the overall volume of hazardous chemicals needed for preservation and eliminates the need for storage containers and space to maintain large specimens.

Stauffer et al. (2001) give detailed guidance for producing photographic vouchers, including discussions of photographing specimens and understanding the rules for capturing voucher images of fishes. This document is the primary reference for the discussion of photographic vouchers.

A digital camera is required to produce satisfactory photographic vouchers and the following camera capabilities are required:

- color photographs
- high pixel density (8 megapixels or greater)
- macro capability
- built-in or external flash

Even on large fish it may be necessary to photograph small characters such as fins, gills, and spines. Thus, the camera should be capable of focusing on objects that are very close to the lens. Stauffer et al. (2001) recommend that the camera be able to focus on images as close as 4 cm.

It is not unusual to conduct fish assessments in low light. If possible, move specimens from shade to full sun. However, where it is not possible to sense natural light, the flash allows fast shutter speeds that produce crisp photos.

The primary considerations for image collection and data handling are:

- field of view
- size referencing
- identification of individuals
- saving files for vouchers

Fill the field of view with the specimen or the part of the anatomy being photographed. The macro option for the camera will be useful for photographing particular characters or areas of the specimen.

Size is a key piece of information about the specimen and can be helpful in the identification or verification of vouchers. In each photograph, include a means of estimating size such as a tape measure, meter stick, or calibrated device.

Some type of text label should be included in the image of the specimen. This might be a small dry-erase or magnetic board that includes, at minimum:

- species
- location of collection
- date of collection
- sample or specimen ID number

- name of each collector
- name of identifier, if different from collector

Cameras can usually store images in compressed JPEG format. When selecting the degree of compression or size of the captured image, choose the physically largest image available (in horizontal and vertical resolution). Also, it is best to choose medium- to high-quality JPEG formats in order to preserve image quality.

The camera automatically assigns a filename, typically consisting of alphanumeric characters, to an image when captured. This automatically assigned name gives no indication of the file contents. Therefore, develop a system to name photographic voucher images as files that can be saved and subsequently retrieved as efficiently as possible. The filename should include:

- species scientific name
- individual identifying number
- collection site description or collector's reference code (for example: TCEQ Station ID)
- date of capture

This information facilitates searching files for a particular photographic voucher. For example, including the species name enables all voucher images for that species to be found on a computer by searching filenames.

Rules for Capturing Voucher Images of Fishes

For the voucher images to convey the most information and enable identification or verification of the specimen, it is especially important that the viewing aspect is appropriate for each type of fish. By following the guidelines discussed below, Stauffer et al. (2001) suggest that most fish species can be successfully identified from digital images.

Physical work on preserved fishes is done on the right side, often damaging tissues and blemishing the specimen's appearance. Therefore, the convention is to photograph the left side. Also, if more than one species are expected to co-occur, photographs should clearly show the characters that allow identification of each species.

Table 3.1 summarizes guidelines for the appropriate view for the photographic image of fish in common freshwater families. It assumes that the collector can use the photograph to identify a fish to family level. Table 3.1 includes families where individuals are expected to reach at least 12 inches in total length.

Field Preservation

The standard preservative is 10 percent formalin—one part full-strength formalin and nine parts water. Place specimens in this fixative while still alive, as those that die before preservation normally do not retain distinctive markings. To allow proper preservation, do not crowd fishes into bottles.

Slit larger specimens on the right side of the abdominal cavity to allow proper preservation. Avoid all contact with formalin.

Table 3.1. Guidelines for photographing the appropriate view of fish specimens.

Acipenseridae (sturgeons)	lateral view
Amiidae (bowfin)	lateral
Anguillidae (freshwater eels)	lateral
Catostomidae (suckers)	lateral; ventral (head and jaw)
Centrarchidae (sunfish)	lateral
Clupeidae (herrings)	lateral
Cyprinidae (minnows)	lateral; ventral (head and jaw)
Esocidae (pikes) adults	lateral
Gobiidae (gobies)	lateral
Hiodontidae (mooneyes)	lateral
Ictaluridae (bullhead catfishes)	lateral (clear view of dorsal and caudal fins);ventral (head and chin)
Lepisosteidae (gars)	lateral
Percichthyidae(temperate bass)	lateral with anal fin flared; close-up of flared anal fin
Percidae (perches)	lateral
Petromyzontidae (lampreys)	(adult) lateral and of oral disk
Polyodontidae (paddlefish)	lateral
Salmonidae (trouts)	lateral

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, requiring special care in storage and handling.

Check the material-safety data sheets for alcohol and formalin for proper handling requirements.

Labeling a Field Sample

Place inside each sample container a label that includes, at minimum, the following information. Use pencil or waterproof ink on paper with a high rag content (not recycled paper) for each label.

- the station number and location description
- the date and time of collection
- the collection method (for example, seine or electrofishing)

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

Laboratory Processing

Identification of Fish-Assemblage Samples

The identification of fish-assemblage samples to the species level requires taxonomic training and a familiarity with appropriate keys and literature. The validity of identifications affects the quality of community analyses and, frequently, the ALU designated for a stream. Consequently, species identifications must be performed by personnel with appropriate taxonomic training.

Appropriate equipment must be available for laboratory determinations of biological specimens, including a dissecting microscope, an assortment of probes, dividers, a ruler, forceps, and appropriate taxonomic references. For identifying Texas freshwater fishes, the primary reference is Hubbs et al. (2008), with complementary sources used as necessary.

Sample-Tracking Log

Upon return to the laboratory, assign a unique sample tracking number to each jar containing the fish specimens according to the sequence in the fish-sample-tracking logbook. For example, an instance of numbering may look like *F 040 14*, where F refers to 'fish,' *040* refers to sample number 40, and *14* refers to the year 2014.

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

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

Laboratory Sample Processing

Keep specimens in 10 percent formalin for at least one week and then soak in water for three days, changing the water each day. Take care to avoid breathing or exposing yourself to the formalin. Transfer the specimens to 50 percent isopropyl alcohol or 75 percent ethanol before examination.

If the intent is to archive specimens in a museum, the preservative must match the individual museum's requirements—normally non-denatured ethyl alcohol. When samples are rinsed and transferred from formalin to alcohol, take care to examine each internal label to ensure that it remains in legible condition. Labels are often destroyed during the rinsing process when samples are agitated heavily. Procedures for disposing of formalin must follow your

organization's chemical-disposal plan. Detailed information on storing specimens is outlined in Fink et al. (1979).

Sorting and Identification

When sorting and identification begins, handle collections individually with each staff person working up one sample at a time. For quality assurance, maintain a record of who identified specimens from each sample. Chapter 11 provides a complete listing of required and recommended references for identifying freshwater fish.

Place samples in a sorting tray, grouped by species. Keep specimens moist to prevent deterioration or desiccation. Again, do not combine samples collected using different methods. Once sorted and identified, place each species into a separate jar, by gear type, with appropriate labels inside the jar that include the following:

- station number and location description
- state
- county
- river basin
- name of each collector
- collecting method (for example: seine, electrofishing)
- species name (not common name)
- number of specimens
- range of total length
- preservative used
- number of containers in sample

Label those that are not identifiable with a similar label noting either no species name or *possibly*—for example, "possibly *Cyprinella venusta*."

Affix a separate label to the outside of the container with the sample tracking number and container replicate number. Make sure the container is dry, and wrap it with clear tape to ensure the label will not come off. Do not put the label on the container lid.

Laboratory Bench Sheets

Prepare a laboratory bench sheet listing the species, numbers of specimens, disease presence, and sample identifiers. Sample identifiers must include information from the collecting label, such as location, date, and collector. Once species counts are completed, double check the laboratory bench sheet against the sample bottles to ensure the counts are correct.

Quality-Control Checks

At least 5 percent of all identifications should be subjected to a blind recheck by another biological expert. Selection of samples for rechecking must be random. A record of rechecks must be kept for quality control. If identifications done by a particular individual have an error

rate of more than 10 percent, reidentify all specimens. Laboratories must be aware of the potential for systematic or consistent errors in identification of a particular family, genus, or species.

Voucher Specimens

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

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 fish 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.

Data Evaluation

The primary tools required for analyzing fish data for wadable freshwater streams are described in Linam et al. (2002). The report outlines regional indices of biotic integrity and their application for assessing aquatic-life uses, and explains in detail the individual metrics for the various regions. As noted in the section on large rivers, these indices may be suitable for evaluating fish assemblages in those water bodies as well, but that should be discussed with TCEQ personnel. The full report is online at <https://www.tpwd.state.tx.us/publications/pwdpubs/media/pwd_rp_t3200_1086.pdf>.

See Tables B.3 through B.9 in Appendix B for the complete metric sets. Figure B.8 in Appendix B is a map of the Level IV ecoregions. It identifies the correct ecoregion in which the data were collected and determines the correct IBI metric set to use.

Large-River Monitoring and Assessment

Collecting and assessing fish assemblages in large, non-wadable streams and rivers typically present more challenges and are more resource intensive than sampling wadable systems. The scale and associated fauna and habitats can be quite different. In addition, large rivers are complex systems often influenced by multiple anthropogenic disturbances. Most major drainages in Texas begin within the state or just outside its borders and drain into the Gulf of Mexico. Depending on the reach surveyed, large rivers and streams in Texas may be similar to wadable streams in terms of discharge and scale. However, they also have significant reaches that are not primarily wadable, have substantial flow, and may pass through multiple ecoregions. Unlike

smaller water bodies, which are normally replicated across a given region or basin, large rivers are typically unique (Emery et al. 2003).

The summer index period may not be appropriate in large rivers depending on issues such as system hydrology including seasonal releases from reservoirs and irrigation withdrawals. Instead, sampling periods should be specific to the site and collection method, and meet the objectives of the study. Reference streams used for comparison may not be available given modifications by humans to larger waterways and the lack of streams of similar size and with similar faunal composition. Aside from issues associated with establishing a comparative baseline, large streams and rivers require different equipment or application of equipment than wadable streams to adequately assess assemblages, and may require different assessment tools. Obtaining a representative sample can be difficult, given the scale and distribution of habitat patches within large rivers, making reach selection extremely important. Collection technologies appropriate for large rivers have varying limitations with regard to how each type of gear can thoroughly sample a single habitat or be uniformly applied to multiple habitats (Emery et al. 2003). In general, multiple kinds of collection gear are necessary to obtain a representative sample. In reaches that are marginally wadable, the river may be adequately sampled using a combination of a backpack electrofisher and seines as in the wadable-stream protocols. Boat electrofishing equipment will be required in most river systems. As with wadable streams, seining must still be used to complement sampling.

Other kinds of gear, such as gill and hoop nets, may be required, depending on the objectives of the study and stream conditions. Sampling duration and reach length may vary depending upon the system scale. The EPA has proposed 40 to 100 times the wetted stream width as a reach length when sampling in large streams and rivers. Simon and Sanders (1999) observed that 500 m was long enough to capture sufficient numbers of species to characterize biological integrity but not biological diversity in great rivers. The study objectives will influence the number of reaches sampled and sampling duration.

Before starting a large river or stream project, consult with TCEQ personnel to determine a sample plan and method for evaluating the data.

Before sampling large rivers and streams, consider the tools available for biological data analysis. Assessment tools and regionalized indices of biotic integrity described by Linam et al. (2002) were designed for wadable streams and may not be directly applicable in all situations or the system being sampled. A simple question may determine if methods for sampling wadable streams are appropriate—can at least 50 percent of the reach be sampled by wading methods such as backpack electrofishing or seining? In general, the development of multimetric approaches for assessing larger systems has progressed more slowly than for wadable systems because of issues of sampling representativeness and efficiency and the lack of large reference streams (Bergstedt et al. 2004).

Recently, more focus has been directed toward sampling large rivers and nationwide, several projects have focused on protocols. Lazorchak et al. (2000) published a methods manual for non-wadable rivers and streams as part of the EPA's Environmental Monitoring and Assessment Program. Subsequently, EMAP produced a manual (Angradi 2006) directed at sampling methods for the "great" rivers of the Central Basin of the United States—the Missouri, Upper Mississippi, and Ohio. In 2007, the EPA initiated the National River and Stream Assessment, which included detailed methods for sampling both wadable and nonwadable streams (U.S. EPA 2007).

Within Texas, the Texas Instream Flow Program and its basin partners have developed considerable biological data on nonwadable systems—the Sabine, Trinity, San Antonio, Guadalupe, and Brazos rivers—that will be used to evaluate sampling and assessment methods. In addition, the TPWD and the TCEQ sampled data from nonwadable streams through participation in the National Stream and River Assessment Program. Data from these and other efforts will be used to evaluate the sensitivity of various biological indices that have been developed at both the regional level and within basins for assessing large rivers.

Reservoir Monitoring and Assessment

Chapter 2 discusses the index period or preferred fish-sampling conditions for lakes and reservoirs.

To date, there is limited guidance on methods of assessing the biological and habitat integrity of lakes and reservoirs. The artificial nature of reservoirs complicates regulatory processes, as it may be difficult to determine the specific biological communities that correspond with designated ALUs. The TCEQ has well-developed guidance for assessing the biology and habitat in freshwater streams. There is a growing need for the same guidance in lakes and reservoirs. Development of procedures for assessing the biological and habitat integrity of lakes and reservoirs was initiated by a concern that some reservoirs or portions of reservoirs were not meeting designated ALUs (based on DO concentrations). It is foreseeable that reservoirs will continue to be a concern and a uniform approach of assessing these water bodies will be an important regulatory tool.

In addition to the preliminary work in Texas, a few other states and the EPA have developed methodologies for data collection and assessment of the biological integrity of lakes or reservoirs (U.S. EPA 1997). The Ohio EPA developed a multimetric assessment for inland lakes or reservoirs, the Ohio Lake Condition Index (Davic and DeShon 1989). The Tennessee Valley Authority developed biological assessment methods for its reservoirs that use a similar approach to what has been developed for stream assessment (Dycus and Baker 2001). The EPA has also published a technical guidance document (1998) for the development of lake and reservoir bioassessment and biocriteria programs.

Before any biological monitoring of a lake or reservoir, 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.

In general, the same level of effort used per sample site for seining and electrofishing freshwater streams can be applied to sampling lakes and reservoirs. In reservoirs, electrofishing is often most productive at night or twilight as predators move inshore to feed. One lap of the shoreline of a small lake cove is a complete unit. In addition to seining and electrofishing, gill netting, hook and line, and trap netting may be incorporated in the sampling effort. The TPWD (2002) has assessment procedures for inland fisheries specifically designed to estimate abundance and population structure for game and forage fish species. These procedures are not designed to assess the ALU for reservoirs, but may be used as a guide for effective methods for collecting fish in reservoirs. Refer to “Boat-Mounted Electrofisher” for more detailed instructions on fish collection in lakes and reservoirs.

CHAPTER 4

SALTWATER NEKTON

Disclaimer

Methodologies for assessing ALUs have not been developed for Texas saltwater habitats—including Gulf waters, bays, estuaries, the Intracoastal Waterway, and tidal streams. Before conducting any biological monitoring activities on a saltwater 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.

Objective

A common goal in fish sampling is to collect a representative sample of the species present and their relative abundances. This chapter describes standard fish-collection techniques for saltwater bodies in Texas. Years of data collection and a large data set may be needed to develop a reliable estimate of relative abundance for marine and estuarine species. However, if all data from similar estuarine habitats are collected using comparable kinds of gear and techniques, the data will be valuable not only for the given study, but also to address the development of assessment methodologies for these saltwater bodies.

Any study employing saltwater fish collection must have clearly defined objectives. Careful consideration of the end uses of the data is essential. Choose specific methods, kinds of gear, and level of effort so that the study objectives can be met. Collections at each site in the study must be comparable. Consequently, collectors must ensure that the sampling procedures, level of effort expended, and types of habitat sampled are similar at each station and in succeeding sample events. At a minimum, collections that are intended to obtain fish-community data must include at least one active gear type, generally seines or trawls. Passive gear, such as gill nets or trap nets, must be used in conjunction with active gear.

Scientific Collection Permit

A Scientific Permit for Research is required to collect, salvage, band, or hold native Texas wildlife for scientific purposes. Scientific purposes include activities aimed at enhancing, protecting, conserving, or managing protected wildlife, or furthering scientific understanding of a resource or the environment. Refer to the TPWD website for application requirements and reporting forms: <www.tpwd.state.tx.us/business/permits/land/wildlife/research/>.

Notify each TPWD Law Enforcement Office in each region of your field activities by telephone at least 24 hours, but not more than 72 hours before collection if collection techniques or devices being used are ordinarily illegal (e.g., using gill nets or electroshocking devices to collect fish, or hunting or collecting along public roads and rights-of-way). A confirmed response from the local game warden is required before collection if the sampling activities being conducted involve methods of capture ordinarily classified as illegal. In addition, please be advised that collecting in a wildlife-management area is not authorized without prior written permission from the area manager.

For regional office location and telephone numbers, see <www.tpwd.state.tx.us/warden/office_locations> or call a Parks and Wildlife Communication Center: Austin—512-389-4848; Houston—281-842-8100.

Permit holders are required to carry this permit when conducting authorized activities. Sub-permittees may carry a copy in lieu of the original permit. It is also advisable to carry additional corroborative identification such as a driver's license. A permittee engaging unpermitted assistants must keep on file at his or her office and on his or her person in the field a signed and dated list of all unpermitted persons assisting in permitted activities. TCEQ regional office staff and central office SWQM and WQS staff are listed as sub-permittees on the permit currently held by the TCEQ SWQM team. Any TCEQ employee that needs to be added to this permit should contact the central-office SWQM team.

Records

In addition to sample labeling requirements as specified in this chapter, the following records must be maintained by the collector.

Field Logbook

For each sample event record the following information in a field logbook.

- date and time of sample collection
- name of water body
- location of sample site (Station ID)
- name of each collector
- collection methods and equipment
- number and type of samples collected
- number of sample containers
- preservative used
- time spent electrofishing
- number of seine hauls and length of each haul
- description of habitats sampled
- unusual site characteristics
- field measurements (flow, DO, pH, temperature, specific conductance)

Sample-Tracking Logbook

A sample-tracking logbook, containing the information described in Chapter 11 of this manual, must be maintained. The logbook documents when samples arrive at the laboratory or headquarters, when each sample enters each processing step, and who has custody or responsibility for it.

Laboratory Bench Sheets

Laboratory bench sheets must be maintained where specimen identification and enumeration occur. These bench sheets document the raw counts of individuals for each taxon and notes relevant to their identification and enumeration. Examples appear in Appendix H.

Sample Collection

The method used to collect nekton samples will depend on the type of habitat. Collections of saltwater fish will generally be conducted in one of three major habitat types—tidal streams, open bay, or Gulf waters. Each requires a slightly different approach. The coastal bays and nearshore Gulf waters are sampled extensively by the TPWD Coastal Fisheries Division to understand status and trends of selected finfish and shellfish species, within the realm of fisheries management. Any fish collections in these areas must follow TPWD methods (TPWD 2002) unless there is a compelling reason to do otherwise. Fish collections in tidal streams must follow the methods outlined in this manual. Because of the limited guidance available for assessing fish data from tidal streams for regulatory purposes, it is important to consult with the TCEQ WQSG staff before sampling.

The concept of critical and index periods used for freshwater streams may not directly apply to bays, estuaries, and tidal streams. The same is true for level of effort in using collection gear. Thus any assessment must be planned in coordination with TPWD and TCEQ personnel to ensure that the timing and level of effort are appropriate for the type of assessment.

The field equipment and materials necessary to collect fish are listed in Appendix A. Forms needed for biological assessments appear in Appendix C. Electronic copies of all the tables in the appendixes are available at <www.tceq.texas.gov/goto/biopacket>. Definitions of technical terms are in the glossary (Appendix E).

Collection Procedures

Because the objective of the nekton sample is to obtain information on the composition and integrity of the nekton community, collectors must net, identify, and enumerate all organisms possible or selectivity (bias) may occur (Murphy and Willis 1996). The amount of effort must be recorded. Catch per unit effort is used as a way of measuring and comparing fish data when the same methods and gear types are employed. Do not combine fish from different kinds of gear.

Fish Collection in Tidal Streams and Bayous

In general, one must use smaller gear in a tidal stream compared to what is used in open bays or Gulf waters—for example, a 10 ft trawl in tidal streams instead of the 20 ft trawl used in open bays and 100 ft gill nets instead of 600 ft gill nets. A 15 ft straight seine is used in tidal streams, rather than the larger bag or beach seines used in more open habitats. In bayous where the salinity may be low, such as in the upper coast, it may also be possible to use electrofishing techniques.

Trawl

Trawling is an active fish-capture method because it uses moving gear (a towed net) to collect organisms in open water. Trawls sample a discrete area or volume over a specified time, thus making quantitative sampling possible. This method captures pelagic (water-column) and bottom-dwelling organisms. As the net (trawl) is dragged along the bottom, fish enter it and are captured. They collect in the end of the net (cod end), which is tied shut. After retrieval of the net from the water, the cod end is untied to easily remove the fish for identification. Otter trawls use heavy wooden "doors" or "otter boards" to spread the mouth of the net open and keep the net on the bottom by applying lateral pressure on it as it is towed forward. It may not be possible to use a trawl in environments with abundant rock or woody debris on the bottom.

In tidal streams, use a 10 ft otter trawl.

Appropriate methods for deploying and collecting biological specimens with trawls are outlined in Murphy and Willis (1996).

Usually four replicates, composited, are required for a complete trawl sample. All stations must receive a similar level of effort.

Do not trawl in marked navigation channels.

Attach a tail buoy to the end of the trawl when collecting bay trawl samples to ensure its retrievability.

Seine

Seining is an active fish-capture method used near shore to capture mainly smaller fish and juveniles. Seines can be hard to use in stands of emergent vegetation or areas with a lot of woody debris, stumps, or cypress knees.

Seines must be inspected for any holes and repaired or replaced before each use.

In tidal streams, use a 15 ft straight seine. In some cases, the banks of a stream drop off too steeply to use a 15 ft straight seine. One alternative is for one worker to hold a 30 ft seine from the bow of a boat with another worker standing near shore holding the other end of the seine. The boat is then maneuvered to pull the seine along shore and back to shore while the worker on shore holds the other end of the seine steady. Choose a section of shoreline to seine that will allow the net to be pulled for approximately 8 m at a time. Shoreline is considered to be the edge of the emergent vegetation if vegetation extends out from shore. One end of the seine is positioned near the shore. The other end of the seine is positioned perpendicularly offshore. With the net fully extended, both persons pull the net parallel to shore with the person onshore slightly behind the person offshore. At 8 m, the person at the shore remains stationary. The person offshore proceeds to shore with the seine extended. Both persons pull the seine onto shore. Be sure the lead line remains on the bottom until the seine is pulled out of the water.

Count and record all organisms collected by the seine or put them in a container with a label and fixative. Often the organisms are so small and numerous that it is preferable to bring the entire catch back to the laboratory for identification and enumeration.

Repeat this process until six or more replicates are collected.

Boat-Mounted Electrofisher

Electrofishing uses electricity to temporarily stun fish so they may be collected with a dip net. This method is typically used by moving the boat slowly along the shore.

Safety

Safety is extremely important. Use only commercially produced electrofishing equipment. At least three people are required when electrofishing out of a boat. All persons on the boat must wear rubber, nonconductive gloves and knee boots or waders. Keep the gloves dry. Everyone in the boat must wear a personal flotation device.

Collecting

Electrofishing is only effective for collecting nekton in areas where the salinity is low, such as in the upper end of tidal streams or bayous. The duration of sampling is 900 seconds of actual shock time.

Frequency and voltage are set to maximize output for the water conditions. Once the controls are adjusted, the samplers reset the timer. Electricity is discontinuously applied. Catches usually can be increased in areas of submerged cover by moving in with the power on but the circuit off and then energizing electrodes for an element of surprise. All habitat and cover types must be electrofished. Record the distance and the actual shocking time. A slow, deliberate style of capture is safer than fish chasing, and also yields more representative samples.

If conductivities are elevated, observe manufacturer recommendations about equipment limitations. Electrofisher capabilities vary by manufacturer and model. Each model is effective under certain ranges of specific conductance. Check manufacturer specifications for optimal operating procedures and consult with the manufacturer if the unit is consistently operated at higher conductivities. Electrofishing gear does exist for use in higher salinity waters (Smith-Root 2003)—for example, the Smith-Root 7.5 GPP Electrofisher, 10–11,000 µS/cm, and the Smith-Root 9.0 GPP Electrofisher, 100–25,000 µS/cm; however, there are not enough data to determine their effectiveness. See Chapter 3 for detailed information about boat-mounted electrofishing.

Gill Netting

Gill netting is a passive fish-capture method since the gear is stationary and fish become entangled in the gear while it is deployed, usually for several hours or overnight. Experimental gill nets contain panels of different mesh sizes, which are able to capture different sizes of fish.

Weights attached to each end of the net and lead weights at the bottom of the net (lead line) keep the net near the bottom of the water column, while floats and flotation material in the top line of the net keep the net stretched open and suspended in the water column or near the surface (depending on the water depth where the net is set). These nets target pelagic species as they move upstream and downstream or along the shore. See Figure 4.1.

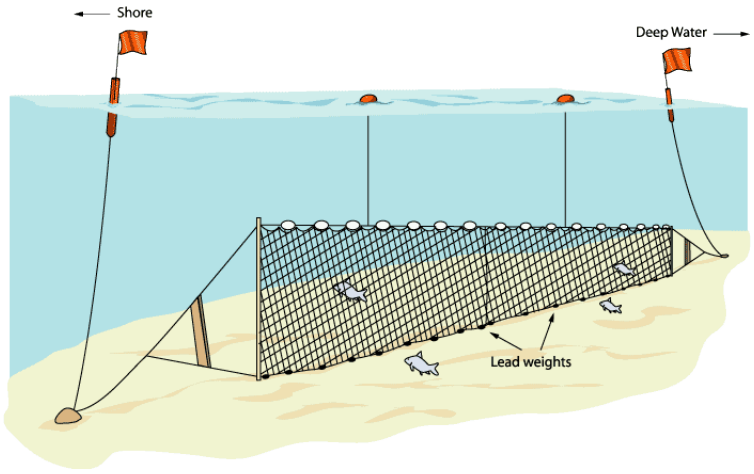


Figure 4.1. A bottom gill net. (Michigan Sea Grant.)

Use experimental gill nets and set them one hour before to four hours after sunset. The first gill-net pickup is to begin no sooner than sunrise, and within the first hour after sunrise. Start time is when the nets are fully deployed and end time is when pickup begins.

Appropriate methods for deploying gill nets and collecting the fish are outlined in Murphy and Willis (1996).

Save all edible dead organisms and make them available to local charities, or other needy organizations or individuals, if possible. Retain written records and receipts.

Sample Preservation and Processing

Field Processing

Maintain the fish in some type of holding bucket or tank with adequate aeration until ready to process.

Do not combine data from electrofishing and seining into one sample. The catch from each method constitutes a separate sample. Use a separate biological reporting form for each collection method.

Other than voucher specimens, easily identified fish may be counted in the field after all collection activity at a sampling location has been completed. **Do not** release any fish caught using either method until all sampling is completed!

Retain two individuals of each species collected for positive identification in the laboratory. Do not retain any fish greater than 0.3 m in total length. These specimens are photo-vouchered. Retain all but the most easily identifiable fish for laboratory identification. See Chapter 3 for detailed information on retaining voucher specimens and photographing fish as vouchers.

Field Preservation

The standard preservative consists of 10 percent formalin—one part full-strength formalin and nine parts water. Place specimens in this fixative while still alive, as those that die before preservation normally do not retain distinctive markings. To allow proper preservation, do not crowd fishes into bottles.

Slit larger specimens on the right side of the abdominal cavity to allow proper preservation. Avoid contact with formalin.

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 for proper handling requirements.

Labeling a Field Sample

Label each field container with an internal label that includes the following information. Use pencil or waterproof ink on paper with a high rag content for each label. Chapter 11 outlines the details of the container label requirements.

- station number and location description
- date and time of collection
- collection method (for example, trawl, gill net, or seine)
- preservative used
- name of each collector
- container replicate number if needed (for example, *1 of 2* or *2 of 2*)

Laboratory Processing

Identification of Fish-Assemblage Samples

The identification of fish-assemblage samples to the species level requires taxonomic training and a familiarity with appropriate keys and literature. The validity of identifications affects the quality of community analyses and, frequently, the ALU designated for a stream. Consequently, species must be identified by staffers with appropriate taxonomic training.

Appropriate equipment must be available for laboratory determinations of biological specimens, including a dissecting microscope, an assortment of probes, dividers, a ruler, forceps, and taxonomic references.

The primary reference for identifying Texas saltwater fishes is Hoese and Moore (1998) with complementary sources as necessary. Many estuarine and freshwater fishes, often collected in tidal streams, can be identified using Hubbs et al. (1991). Chapter 11 contains complete required and recommended references for identifying saltwater fish.

Sample-Tracking Log

Upon return to the laboratory, assign a unique sample tracking number to each jar containing the fish specimens according to the sequence in the fish-sample-tracking logbook. For example, an

instance of numbering may look like *F 040 13*, where F refers to 'fish,' *040* refers to sample number 40, and *13* refers to the year 2013.

Record the number and related 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, trawl, gill net, seine)
- preservative used
- number of containers in sample

Laboratory Sample Preservation

Specimens remain in 10 percent formalin solution for at least one week and then soak in water for three days with a change of water each day. Transfer the specimens to 45 percent isopropyl alcohol or 70 percent ethanol before examination.

If the intent is to archive specimens in a museum, the preservative must match the individual museum's requirement, normally non-denatured ethyl alcohol. When samples are rinsed and transferred from formalin to alcohol, examine each internal label and ensure that it remains legible. Labels are often destroyed during rinsing. Procedures for disposing of formalin must follow your organization's chemical disposal plan. Detailed information on storing specimens is outlined in Fink et al. (1979).

Sample Sorting and Identification

When sorting and identification begins, handle collections individually with each staff person working up one sample at a time. For QA purposes, maintain a record of who identified specimens from each sample.

Place samples in a sorting tray, grouped by species. Keep specimens moist to prevent deterioration or desiccation. *Do not* combine samples collected using different methods. Once sorted and identified, place each species into a separate jar by type of gear with appropriate labels that include the following:

- station number and location description
- state
- county
- river basin
- name of each collector
- collection method (for example, trawl, gill net, seine)
- species name (not common name)

- number of specimens in container
- range of total lengths
- preservative used
- number of containers in sample

Label those that are not identifiable with a similar label noting either "no species name" or "possibly"—for example, "possibly *Opisthonema oglinum*."

Affix a label with the sample tracking number and container replicate number to the outside of the container. Make sure the container is dry, and wrap it with clear tape to ensure the label will not come off. Do not affix the label to the container lid.

Laboratory Bench Sheets

Prepare a laboratory bench sheet listing the species, number of specimens, disease presence, and sample identifiers. Sample identifiers must include information from the collecting label, such as location, date, and collector. Once species enumerations are completed, double check the bench sheet against the sample bottles to ensure the counts are correct. Examples of laboratory bench sheets appear in Appendix H.

Quality-Control Checks

A minimum of five percent of all identifications are subject to a blind recheck by another biological expert. Selection of samples for rechecking must be random. A record of rechecks must be kept for QC purposes. If identifications by a particular individual have an error rate of more than 10 percent, reidentify all specimens. Laboratories must be aware of the potential for systematic or consistent errors in identification of a particular family, genus, or species.

Voucher Specimens

Retain at least one representative of each fish 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 verification of identification if necessary. 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 bioassessment data by documenting the identity of the organisms and making them available for review by the general scientific community.

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 fish 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.

Field Notes

Field notes must describe the collection methods employed, equipment used, areas sampled, the way equipment was used, time spent sampling, a description of all sampled habitats, and any unusual site characteristics.

Data Evaluation

There are no currently accepted criteria for analyzing saltwater fish data. Consult personnel of the TCEQ WQSG, the SWQM team, or the TPWD for guidance in interpreting fish data collected from saltwater bodies.

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

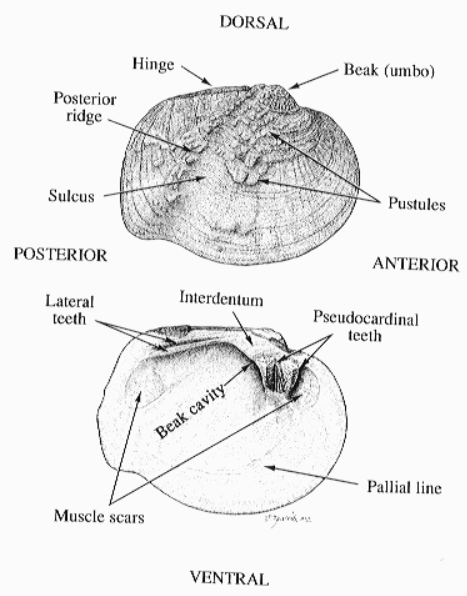
Anyone conducting mussel surveys in Texas must possess or be listed on a valid TPWD SCP. An SCP is required even for the collection of dead shells. All TCEQ regional-office and WQST personnel are included on the SWQM SCP. Any TCEQ employee that needs to be added to this permit should contact the central-office 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.

CHAPTER 6

SALTWATER BENTHIC MACROINVERTEBRATES

Disclaimer

Methodologies for assessing ALU and other regulatory bioassessments have not been developed for Texas saltwater habitats, including Gulf waters, bays, estuaries, the Intracoastal Waterway, and tidal streams. Before any biological monitoring of a saltwater 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.

Objective

This chapter describes the methods used by the TCEQ for the collection and assessment of benthic macroinvertebrate samples from saltwater systems. In general, the TCEQ will use samples collected according to these methods in conjunction with fish-community surveys and physical habitat assessments to holistically evaluate the health of biological assemblages and to develop future indices of aquatic-life use for these waters. Sampling saltwater benthic macroinvertebrates derives data that can be used for assessing water quality trends and comparing water quality differences between sites (USGS 1977).

Years of data collection and a large data set may be needed to develop a reliable estimate of relative abundance for marine and estuarine species. However, if all data from similar saltwater habitats are collected using comparable kinds of gear and techniques, the data will be valuable, not only for the given study, but also to the development of assessment methodologies for saltwater bodies.

Any study employing the collection of saltwater benthic macroinvertebrates must have clearly defined objectives. Careful consideration of the data uses is essential. Choose methods, kinds of gear, and levels of effort with the goal of meeting study objectives. Sample collections at each site in a study must be comparable. Consequently, collectors must ensure that the sampling procedures, effort, and types of habitat sampled are similar at each station and in succeeding sampling events.

Scientific Collection Permit

An SCP is required for the collection of marine benthic macroinvertebrates. This requirement applies to oysters, shrimp, clams, mussels, and crabs that are subject to license requirements, possession limits, means and methods of take, and size restrictions.

Equipment

Field equipment and materials necessary to conduct freshwater benthic macroinvertebrate sampling appear in Appendix A.

Selecting the Sampling Site

Marine benthic macroinvertebrates are collected from a soft-sediment bottom, rather than from an area with sand, shell litter, oyster reef, or grass flats, unless there is a compelling reason to sample those habitats. In choosing a sample location, some trial dredge hauls will help determine if an area has suitable silt or mud sediments.

Sampling Procedure

The Ekman dredge is the preferred sampler for collecting benthic macroinvertebrate samples from estuarine habitats. In estuarine areas with large amounts of shell hash or hard sand, a heavier dredge may be necessary, such as a Ponar or Van Veen dredge. Use the same type of dredge at a station to ensure consistency in the data set over time. Before using any of these devices, inspect it 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 other dredges as well.

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 dredge in ambient water. Once the Ekman has been cleaned, use the line (or pole in shallower areas) to lower the dredge to the bottom. Avoid lowering the sampler too rapidly, as that 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.

Sample Washing

To minimize damage to organisms, homogenize the sample by hand. Wash the sediments overboard through a No. 30 (mesh size ≤ 595 µm) or No. 35 (mesh size = 500 µm) sieve bucket by dunking the bucket gently, or gently washing it with a deck pump. If using a deck pump, screen the water through a slightly finer mesh than the benthic sieve to inhibit contamination of the sample with plankton.

Narcotizing Sample

Narcotizing the sample relaxes the soft-bodied organisms and may make identification easier. If the sample is to be narcotized, wash the material retained on the bucket screen onto a 0.5 mm sieve, and place the sieve in a suitable bucket with narcotizer to a depth of about 3 cm. The narcotizing solution must cover the sample without washing the sample out of the sieve. Narcotizing solution is 7 percent magnesium chloride in seawater—75 g of $MgCl_2$ per liter.

After 0.5 to 1.0 hour the sample is narcotized and the sieve can be removed from the bucket. The narcotizer can then be reused for subsequent samples.

Preserving in the Field

Wash the sample from the sieve or bucket with ambient water into a wide-mouth jar. **It is very important *not* to use freshwater to rinse newly collected benthos.** Preserve it in 10 percent formalin—one part full-strength formalin to nine parts seawater. Add several grams of borax to buffer the formalin solution. Do not use alcohol as a fixative with marine organisms.

Use enough preservative to cover the sample. To ensure adequate preservation of benthic macroinvertebrate collections, fill sample containers no more than 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 and limit the amount of water when transferring sample material to the jar. Too much organic matter in the jar or not enough preservative can cause samples to start decomposing before they can be sorted and identified.

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 for proper handling requirements.

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

Sample Processing

Preserving in the Lab

When a preserved sample is brought into the laboratory, it will be assigned a sample tracking number according to the sequence in the benthic macroinvertebrate sample log and logged with pertinent information, including:

- sample tracking number
- collection date and time

- station number and location description
- name of each collector
- collection method (for example, Ekman dredge)
- sieve type
- number of containers in each replicate sample

Within two weeks of their collection, transfer the samples from the formalin mixture used in the field to a solution of 70 percent ethanol or isopropyl alcohol. To do this, rinse the field-preserved sample with water in a sieve and return it to its original (rinsed) jar, and add 70 percent ethanol or isopropyl alcohol as the new preservative. You may add Rose Bengal vital stain (or another appropriate stain) to the samples to aid in their sorting. The stain can be added to the formalin-preserved sample in the field or to the alcohol-preserved sample in the lab. Add a small amount (about 0.25 g) of Rose Bengal vital stain and swirl the jar to mix the stain and preservative with the sample.

Safety

To reduce formalin exposure, rinse the sample with water in a sieve with mesh size ≤ 595µm to remove preservative and fine sediment and transfer to alcohol before sorting. Do this under a ventilation hood or, if a hood is not available, in an area with good ventilation. Follow your organization's hazardous waste disposal plan for formalin waste.

Sorting in the Lab

The objective of processing an estuarine benthic macroinvertebrate sample in the laboratory is to count and identify every individual collected.

Process each of the four replicate samples individually. Place all of the individuals from each replicate grab sample in a separate vial.

Once sorting is complete, there will be four separate vials if four replicate samples were collected, each containing all of the specimens from each individual replicate. Processing each replicate sample separately is important, since it allows the variability between replicates to be evaluated.

Thoroughly rinse the sample using a No. 30 or smaller (≤ 595 µm) sieve to remove preservative and fine sediments. Place the 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. Using a lighted magnifying device (2×), pick **all visible macroinvertebrates** and place them in a sample bottle or vial containing 70 percent ethanol or isopropyl alcohol and a label. It is usually necessary to place small portions of the sample in the pan to ensure that no organisms are missed.

Repeat this process until the entire replicate sample has been inspected under magnification. Most of the organisms will be stained red or pink, but some may be very dark or light red, and some mollusks will not stain well at all.

After thoroughly inspecting the sample and removing all macroinvertebrates, either replace the sample in alcohol for later checking or have another investigator check to ensure that no

organisms were missed. Check at least 10 percent of samples for missed organisms. Record the date and identity of each sorter in the sample-tracking logbook.

Labeling a Lab Sample

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
- date and time of collection
- collection method (for example, Ekman dredge)
- container replicate number (for example, *1 of 3, 2 of 3, 3 of 3*)
- preservative used
- name of each collector
- name of person conducting sorting procedure, if different from collector

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

Additionally, affix a label with the sample tracking number and container replicate number to the outside of the container. Make sure the container is dry, and wrap it with clear tape to ensure the label will not come off. Do not put the label on the container lid.

Identifying Specimens in the Laboratory

Identification and enumeration of estuarine benthic macroinvertebrates must be conducted by persons with appropriate expertise, training, and knowledge of the literature. Using appropriate references, a stereo dissecting microscope, and a compound microscope, identify the organisms to the lowest practical taxonomic level, **species** in most cases. Chapter 11 gives a complete list of required and recommended references for identifying saltwater benthic macroinvertebrates. Record the species names and counts for each replicate on a laboratory bench sheet that contains the sample information recorded on the label. **Maintain a separate count of individuals and list of taxa for each replicate grab sample to allow an evaluation of variability between replicates.**

Records

Besides the labeling of samples specified in this chapter, maintain the following records.

Field Logbook

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

- date and time of sample collection
- the location of the 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 logbook that documents when samples arrive at the laboratory or headquarters, the steps in processing samples, and who has custody or responsibility for each sample.

Upon returning to the laboratory, assign a unique sample tracking number to each jar containing the fish specimens according to the sequence in the fish sample–tracking logbook. For example, an instance of numbering may look like *BM 040 14*, where *BM* refers to 'benthic macroinvertebrate,' *040* refers to sample number 40, and *14* refers to the year 2014.

Record the tracking number and related information on the sample in the logbook. This information includes:

- 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

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

Voucher Specimens

Retain the benthic macroinvertebrates collected as voucher specimens for at least five years or until the conclusion of any applicable regulatory decision (whichever is longer) to allow verification of identification if necessary. 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.

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 sample maintenance of samples or archiving in a university or museum natural-history collection.

Tidal Streams

In tidal streams or estuaries with sandy bottom sediments, a Van Veen or Ponar dredge might be necessary to collect benthic macroinvertebrates. A suction-coring device is another alternative for collecting a good sample from some locations.

CHAPTER 7

BENTHIC ALGAE AND AQUATIC MACROPHYTES

Disclaimer

Methodologies for assessing ALU and other regulatory bioassessments based on benthic algae or macrophytes have not been developed for Texas waters. Before conducting any biological monitoring activities using benthic algae or macrophytes, 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 chapter is to describe methods recommended by the TCEQ for the collection and assessment of benthic algal assemblages in wadable freshwater systems. Benthic algae are an important component of the periphytic community. Periphyton is best described as the community of microscopic organisms associated with submerged surfaces of any type or depth, including bacteria, algae, protozoa, and other microscopic animals (U.S. EPA 1976).

Overview of Methods for Collecting Benthic Algal Samples

The TCEQ recommends different sampling techniques for algae depending upon the habitat being sampled and the purpose of the study. This chapter outlines methods for sample collection, processing, preservation, and evaluation for visual assessments, qualitative, or quantitative benthic algal samples. Aquatic macrophyte sample collection methods are included at the end of the chapter.

Sampling of benthic algae and aquatic macrophytes is not part of routine monitoring; however, special studies may require qualitative analyses. Special studies require an approved quality-assurance project plan or quality-assurance plan before sampling. If a study is in progress, refer to the study's QAPP or QAP for details. If you are developing a QAPP or QAP for a special study, contact appropriate SWQM personnel for assistance.

Equipment

Equipment for collecting benthic algal samples is minimal. Visual assessment requires a transect line or quadrat, a ruler marked with centimeters and millimeters, and a field notebook for recording observations. Qualitative sample collection requires a sample collection jar, a pocketknife or similar device for scraping algae from hard substrates, a pipette for suctioning algae from soft substrates, and the proper preservatives. Quantitative sample collection requires a bit more equipment, as described in the section on quantitative sample collection. See Appendix A for a complete list of equipment needed for benthic algal sampling.

Records

In addition to sample labeling requirements as specified in this chapter, maintain the following records for algal sampling.

Field Notebook

For each algal sample event, record the following in a field logbook.

- date and time of sample collection
- location of the 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 logbook that documents when samples arrive at the laboratory or headquarters, the steps in processing samples, and who has custody or responsibility for each 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 fish sample–tracking logbook. For example, an instance of numbering may look like *BA 040 14*, where *BA* refers to 'benthic algae,' *040* refers to sample number 40, and *14* refers to the year 2014.

Record the tracking number and related information on the sample in the logbook. This information includes:

- 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

Maintain laboratory bench sheets where specimen identification and enumeration occur. These bench sheets document the raw counts of individuals for each taxon and notes relevant to their identification and counting. See Appendix H for a sample algae laboratory bench sheet.

Wadable Streams and Rivers

Visual Assessment of Algae

Visual assessment gives an estimate of percent cover of both macroscopic and microscopic benthic algae. It is adapted from published methods such as those used in the *Stream Periphyton Monitoring Manual* by Biggs and Kilroy (2000). Such information is useful in determining if algal abundance is great enough to indicate nutrient enrichment. Nuisance algal growths can affect recreational uses such as swimming, fishing, and general aesthetic enjoyment of a water body.

The visual assessment method is a relatively rapid qualitative estimate of biomass. This assessment can be done by an observer trained in algal identification who at a minimum must be able to distinguish moss from filamentous algae, and be able to distinguish diatom mats from bluegreen algal mats or other microalgae. It would be helpful for the observer to be able to identify various divisions of algae for best results. In addition, it is preferable for the same observer to assess all transects to minimize variability.

Required Equipment

- tape measure
- 0.25 meter^2 quadrat or viewing bucket with a known area.
    - depending on the water clarity, use either a viewing bucket with a clear bottom or a square 0.25 meter quadrat constructed of perforated 1″ PVC pipe for the observations; other quadrats of similar size are acceptable as long as the same size is used consistently
- small white plastic ruler marked in mm and cm
- field data sheet for visual assessment of benthic algae (Figure 7.1)
- clipboard
- pencil

Categories of Algae

Nine categories of algae are used in the visual assessment.

Long Filaments

This category includes filamentous green algae that are 30 mm in length or longer. Treat unattached filamentous algae that is entangled and not floating downstream as if it were attached to the substrate. Common filamentous green algae that may be encountered include *Cladophora, Stigeoclonium, Rhizoclonium, Hydrodictyon, Oedogonium, Spirogyra,* and *Mougeotia.* The yellow-green filamentous alga *Vaucheria* should be included in this category. It is not necessary to identify algae in the field. Samples may be returned to the laboratory and identified if desired.

Short Filaments

This category includes filamentous green algae that are shorter than 30 mm.

Thin Diatom Mat

The category includes mats less than 0.5 mm thick. This can range from a slimy surface to just visible cover, thickness not measurable. Color will range from dark brown to light brown to greenish brown. Mats on soft substrates such as silt and sand will be recognizable by color differences and occasionally small bubbles on the substrate, especially on sunny days.

Medium Diatom Mat

This category includes mats that are 0.5 to 3 mm thick. This will be a measurable thickness, up to the thickness of two pennies stacked together. Color will range from dark brown to light brown to greenish brown.

Thick Diatom Mat

This category includes diatom mats that are thicker than 3 mm—greater than two pennies stacked together. Color will range from dark brown to light brown to greenish brown. At times diatom mats may resemble filaments but will dissolve into individual cells if rubbed between the fingers. The nuisance alga *Didymosphenia* or "rock snot," which has been identified in Texas, can form thick mucilaginous mats or blobs.

Thin Blue-Green Mat

This category includes blue-green algal mats that are less than 0.5 mm thick. This can range from a slimy surface to just visible cover, thickness not measurable. Their color will range from dark green to bluish green to dark brown or black.

Medium Blue-Green Mat

The category includes blue-green algal mats that are 0.5 to 3 mm thick. This will be a measurable thickness, up to the thickness of two pennies stacked together. Their color will range from dark green to bluish green to dark brown or black.

Thick Blue-Green Mat

This category includes blue-green algal mats that are thicker than 3 mm—greater than two pennies stacked together. Their color will range from dark green to bluish green to dark brown or black. Short intertwined filaments may be observed within the mat.

Other

This category includes other groups that are not classified as green or blue-green algae or diatoms and are attached to the substrate. Examples include reddish or brown filaments, *Chara*, *Nitella*, sewage fungus (*Sphaerotilus*), or moss. Identify these taxa to division or genus level if known, or provide description. Do not record attached or floating macrophytes, including duckweed or rooted emergent or submergent plants. If they are abundant, note that in the "Observations" section at the bottom of the data sheet.

Procedure

1. Establish four transects across the stream in riffle, run, or glide habitats, in that order of preference, avoiding pools if possible. Lay out transects from downstream to upstream to avoid disturbing the substrate and making the water too turbid for viewing. On the field data sheet, circle the type of habitat—"riffle," "run," "glide," or "pool"—for each transect. See Chapter 9 for more information on these habitat types. Along each transect choose five equally spaced observation points—right bank (RB), mid-right (MR), center (C), mid-left (ML), and left bank (LB).

   Left-Bank and Right-Bank Orientation—to be consistent and to help orient others to the location of observations, the convention *left bank–right bank* is used. "Left" and "right" refer to the banks to those sides of an observer when facing downstream.

2. Starting at the right bank (facing downstream), place the quadrat or viewing bucket one quadrat width from the wetted edge. This viewing area is "RB" on the field data sheet.

3. Estimate and record the percentage cover of each category of algae listed on a field data sheet for visual assessment of benthic algae (see Figure 7.2 for example). If a particular algae type is not present, record a zero for that category. Record percentage cover to the nearest 5 percent. If the water is too turbid to see clearly, it may be necessary to pick up a piece of substrate to make the field measurements, or carefully feel the substrate with your fingers to estimate the percent cover.

4. If filamentous algae are present, record the length (mm) of the longest filament at each observation point to the nearest 5 mm. If no filamentous algae are present, record a zero.

5. Move to the next observation point, MR, which should be midway between the first point and the center of the stream. Repeat steps 3 and 4.

6. Continue across the transect to observation points C, ML and LB, repeating steps 3 and 4 at each observation point.

7. Repeat this pattern for the next three transects, always starting at the right bank. That way, the field data sheet will serve as a "map" of algal distribution.

8. After all transects are completed, circle the length of the longest filament (max) on the bottom row of the field data sheet. Record "*n* =" at the top of the last column as the total number of observation points. Normally $n = 20$ unless any number of transects other than four are used.

Observations

Record any pertinent information, such as type of taxa observed, abundant macrophyte types, or unusual observations at the bottom of the field data sheet.

Note: If samples are returned to the laboratory for identification, label and preserve as described in the "Benthic Algal Qualitative Sample Collection Procedures" section of this chapter. Useful identification resources include Biggs and Kilroy (2000) and Prescott (1978).

Figure 7.1. Example field data sheet for visual assessment of benthic algae—percent cover.

Station ID: _____ Site Name: _____ Date: _____ Sampler: _____

Record percent cover to nearest 5%	Transect 1 riffle run glide pool					Transect 2 riffle run glide pool					Transect 3 riffle run glide pool					Transect 4 riffle run glide pool					$\overline{X}$
	RB	MR	C	ML	LB	RB	MR	C	ML	LB	RB	MR	C	ML	LB	RB	MR	C	ML	LB	***n* =**
Long filaments (> 20 mm)																					
Short filaments (< 20 mm)																					
Thin diatom mat (< 0.5 mm)																					
Medium diatom mat (0.5–3 mm)																					
Thick diatom mat (> 3 mm)																					
Thin blue-green mat (< 0.5 mm)																					
Medium blue-green mat (0.5–3 mm)																					
Thick blue-green mat (> 3 mm)																					
Other (describe):																					
Total % cover (∑ all categories)																					
Longest filament (mm)—Circle Max																					
Observations:																					

Data Evaluation

Calculate and record the mean percentage cover for each algal category and total mean percentage in the last column of the field data sheet. Include zeros in the calculation.

Visual assessment data can be used alone as an estimate of the stream or river bed that is covered with algae and the relative lengths and thickness of the algae present. It can also be used in conjunction with quantitative analyses of the algal community. Data that are reported include

1. mean percent cover of each category of algae
2. mean total length of longest filament
3. mean percent cover of the stream or river bed

Mean percent cover, longest filament length, percent cover of long filaments, and percent cover of thick diatom or thick blue-green mats may be useful metrics in assessing nutrient enrichment.

Collecting Qualitative Benthic Algal Samples

For a synoptic analysis of the benthic algal community, collect a qualitative composite sample from each available habitat. Sample those habitats in approximately the same proportion they appear in the sample reach. For example, if the reach is approximately 60 percent riffle by area, then 60 percent of the sample volume must be from riffle areas, and the other 40 percent from other habitats, such as snags, depositional areas, and aquatic vegetation. The algal sample must contain any macroalgae, green and bluegreen algal mats, and diatom mats in the sample reach.

Collect macroalgae with forceps and place them in a separate sample jar for later identification. This also keeps macroalgae from being lost during diatom sample processing. Continue sampling until 20 to 50 mL of algal material has been collected.

Habitat Types

Hard Substrates

Algae living on hard substrates are called *epilithic* algae. Sample hard substrates, such as rocks, boulders, turtle shells, or mollusk shells by scraping with a knife or stiff brush and rinsing into the sample jar.

Woody Debris

Algae living on hard substrates are called *epidendric* algae. Collect samples by brushing, scraping, or picking algae from submerged snags. If possible, move the snags from beneath the water surface before scraping to avoid losing algae.

Sand or Silt

Algae living on hard substrates are called *epipsammic* or *epipelic* algae. In depositional areas with no current, sample algal mats growing on top of fine sediments using the sharp edge of a pocketknife or microspatula and gently lifting the top layer into the sample jar. You can also use a pipette to suction algae from the surface of fine sediments.

Macrophytes, Root Wads, Mosses

Algae living on hard substrates are called *epiphytic* algae. Rub algae from plant material with fingertips and place it in the sample jar. Squeeze water and algae from mosses into the sample jar. Place bits of plant material into the jar and shake vigorously to remove attached algae. Some plant material can be left in the sample jar to be examined later for tightly adhered diatoms and other epiphytic algae.

Preserving Benthic Algal Samples

The preferred preservative for algal samples is 2 percent glutaraldehyde. If glutaraldehyde is not available, preserve them in 3 to 5 percent formalin—three to five parts full-strength formalin and 97 to 95 parts water. Place algal samples in a dark container and cool it until analysis. Samples in glutaraldehyde that are refrigerated and kept in the dark tend to maintain their natural pigmentation longer than samples preserved in formalin.

Safety

Avoid breathing formalin fumes! Glutaraldehyde and formalin are corrosive to the eyes, skin, and respiratory tract. Wear safety glasses and latex when working with these chemicals. **Formalin is a suspected carcinogen.** Always work in a well-ventilated area or under a hood when preparing glutaraldehyde or formalin solutions.

Check the material-safety data sheets for formalin solution and glutaraldehyde for proper handling requirements.

Labeling the Container

Attach a label to the outside of the container, making sure the container is dry, and wrap with clear tape. Do not place labels for algal samples inside the sample container because the algae will discolor them and make them illegible. Label the container with the following information.

- station number and location description
- date and time of collection
- collection method (for example, hard substrate, snags, or macrophytes)
- preservative used
- name of each collector
- sample type
- container replicate number if needed (for example, *1 of 2* or *2 of 2*)

If two or more samples are collected at a site, label them accordingly; for example, one may be labeled as macroalgae and the other as composite.

Field Notes

Record the following information in a field notebook.

- type of macroalgae present
- percentage of the substrate covered by algae
- any extensive growths of filamentous algae or surface algal blooms
- evidence of recent scouring
- any other pertinent observations

Processing and Identification of Qualitative Benthic Algal Samples in the Laboratory

Process benthic algal samples in a laboratory for microscopic examination. Process samples in two parts.

1. **Non-diatom.** Examine samples to inventory the algal community on a generic level. These include the macroscopic and microscopic algae, except for the diatoms.
2. **Diatom.** Clean and mount samples on slides for identification to species level. Species-level identification allows calculation of several metrics for more in-depth analysis of biotic integrity. Metric calculations are described in the "Multimetric Analysis" section of this chapter.

Chapter 11 contains a complete list of required and recommended references on algal identification.

Microscopic Taxonomic Analysis of Non-Diatom Samples

The purpose of microscopic examination of the non-diatom algae sample is to inventory the algal community. Examine samples within 24 hours of collection unless they have been chemically preserved in formalin or another chemical fixative. Preserved samples can be stored indefinitely; however, pigmentation that may aid in identification will fade quickly, making it preferable to analyze them as soon as possible upon return to the laboratory.

1. Thoroughly shake the sample to dislodge epiphytes from filamentous algae. Using fine-tipped forceps, pick representative macroalgae filaments from the mixture and place them on a microscope slide with a few drops of water. Place a cover slip over the filaments and identify them under a compound microscope equipped with 10×, 20×, and 40× objectives. Do not attempt to examine a wet mount using oil and an oil-immersion objective.
2. Shake the sample again and pipette a few drops onto a new slide with a cover slip to identify non-filamentous algae. If there are many diatoms present, it may be useful to clean them as described below to aid in their identification. For non-diatom algae, examine at least three slides, continuing to scan the slides until no new taxa are encountered.
3. Identify the algae to the lowest possible taxonomic level. Most taxa must be identifiable to genus. Record the observed taxa on a laboratory bench sheet along with estimated relative abundance, such as abundant, common, or rare.
4. If identifying and counting algae to get a numerical estimate of taxa richness and relative abundance, count colonies as individual units, and filaments in 10 µm segments. For example, a *Pediastrum* colony would be counted as 1 unit, while a 100 µm filament of

Cladophora sp. would be counted as 10 units. Count at least 300 units, continuing to scan the slide until no new taxa are encountered.

Microscopic Taxonomic Analysis of Diatom Samples

For some studies, it may be desirable to analyze only the diatoms in a sample of algae. This may be especially advisable if analytical time or resources are limited. Diatoms are most easily identified if the cells are cleaned and mounted in a permanent medium as described below.

Cleaning Method for Diatoms

1. Shake the sample jar thoroughly to homogenize the sample. Pour a small subsample, about 5 to 10 mL, into a 2000 mL Erlenmeyer flask.
2. **Working under a fume hood**, pour approximately 50 mL of concentrated nitric acid into the flask.

   Safety Note: This will produce an exothermic reaction and fumes. Make sure to wear eye protection and gloves that are resistant to acid. Avoid breathing fumes. Always add acid to water. Do not attempt this procedure without use of a fume hood.
3. Allow the sample to oxidize overnight. To reduce the oxidation time, gently boil the sample for a few minutes on a hot plate under the fume hood; delicately silicified diatom frustules may be damaged by this procedure, however.

   Safety Note: Use extreme caution if you boil the sample, as additional fumes will be produced. Use insulated gloves to handle the hot flask. Always use a 2000 mL flask or larger to prevent acid from boiling over.
4. After oxidation overnight, or after the sample is cooled after boiling, fill the flask with distilled water. Allow the sample to settle overnight.
5. Decant or siphon off the supernatant, and refill the flask. Allow it to settle overnight again.
6. Siphon off the supernatant and pour the cleaned sample into a 1000 mL glass cylinder. Fill it with distilled water and allow it to settle overnight, or at least four hours, until all the diatom frustules have settled to the bottom of the cylinder. Siphon off the supernatant and pour the diatom sample into a small vial. Scintillation vials with polyethlene cap liners work well for storing cleaned diatom samples. Add one drop of preservative (formalin or glutaraldehyde) to prevent bacterial growth in the stored sample.

Slide-Preparation Method for Diatoms

1. Shake the diatom sample for at least 60 seconds.
2. Pipette two to three drops of sample onto a cover slip on a cool hot plate under a fume hood. Immediately pipette enough distilled water (approximately 1 mL) onto the cover slip to dilute the diatom solution without breaking the surface tension over the cover slip. This may take practice to learn but will aid in making slide mounts with evenly distributed diatom frustules for identification and counting.

3. Let the cover slip dry, then place a microscope slide on the hot plate next to it. Put a drop of Naphrax or another highly refractive index-mounting medium onto the slide and invert the cover slip onto it. Turn the hot plate on low and heat it until the slide begins to bubble.
4. Remove the hot slide from the hot plate with flat-bladed forceps and set it to cool on a heat-resistant surface (a piece of corrugated cardboard is suitable).
5. After the slide is cool and hardened, scrape any excess mounting medium from it.
6. Permanently label the slide. Slides with a frosted end are preferable, as information can be written directly on the slide; however, adhesive labels are acceptable.

Taxonomic Analysis of the Diatom Sample

Examine the diatom slide on a compound microscope equipped with a 100× oil-immersion objective. **Quality optics and lighting are critical for identification of diatoms to species.**

1. Before counting, scan the slide and record the taxa encountered until no new species is observed for at least three transects across the slide. This method of identifying diatoms will speed up counting.
2. To begin counting, select a random spot on the slide and scan across the slide in transects. Be careful not to scan the same area of the slide twice.
3. Identify and count the first 500 diatom frustules encountered. A tally counter will help to keep track of the most numerous taxa. Record any new taxa encountered. Identify diatoms to species, if possible, using the references in Chapter 11.

Evaluation of Benthic Algae from Qualitative Samples

Non-Diatom Benthic Algal Samples

The following metrics may be useful in evaluating the non-diatom algal community.

Number of Algal Divisions Present

The number will be higher in sites with good water quality and high biotic integrity. Dominance by filamentous green algae (for example, *Cladophora*) may indicate nutrient enrichment.

Generic Taxa Richness

This is generally higher in reference sites and lower in impaired sites. Total number of genera, diatoms, soft algae, or both, provides a robust measure of diversity (Barbour et al. 1999).

Indicator Taxa

Certain genera of non-diatom algae can be used as indicators of different levels and causes of pollution (Bahls 1992; Palmer 1969, 1977).

Diatom Assemblages

Diatom assemblages are especially well suited as biological indicators of environmental impacts in streams and have been used extensively for this purpose. Round (1991) has published a

thorough review of the use of diatoms in studies of river-water monitoring. Diatoms have historically been used as environmental indicators because of the following qualities.

- Since they are attached to the substrate, they are subjected to immediate, intermittent, or prolonged disturbances.
- Diatoms are ubiquitous, with at least a few species found under almost any aquatic environmental condition.
- The taxa and individuals found at any given site are usually sufficiently numerous for use in metric calculation.
- Most diatoms can be identified to species level by trained phycologists.
- Tolerance of, or sensitivity to, pollutants is understood for many species or assemblages of diatoms.
- Diatom populations rapidly respond and recover times because of their relatively short life cycle (compared to fish or macroinvertebrates) and their ability to quickly recolonize formerly disturbed sites (Dixit et al. 1992).

Multimetric Analysis

The diatom community lends itself to multimetric analysis due to its historical use as a water quality indicator, the many species found in the benthic algae, and the known ecological tolerances of many species. At the time of this publication, a diatom IBI has not been developed for Texas. However, the following metrics can be calculated and, as data are collected, regional scoring criteria could be developed to aid in assessment of the algal community. Other potential diatom metrics and IBIs are described in the *USEPA Rapid Bioassessment Protocols for Periphyton* (Barbour et al. 1999), *Methods for Assessing Biological Integrity of Surface Waters in Kentucky* (Kentucky Division of Water 2002), and *Montana Water Quality Monitoring Standard Operating Procedures* (Bahls 1992). The following list of example metrics is not inclusive; other metrics can and should be calculated and evaluated.

1. **Richness of taxa.** High species richness is assumed in an unimpaired site and species richness is expected to decrease with increasing perturbation. Slight levels of nutrient enrichment may increase species richness in naturally unproductive, nutrient-poor streams. In general, however, higher values for this metric indicate higher water quality.
2. **Diversity.** The diversity index has been used in water pollution surveys extensively in the past as an indicator of organic pollution (Weber 1973, Weitzel 1979). While higher values for this metric have historically been assumed to indicate higher water quality, this interpretation can be misleading if richness of taxa is extremely low due to toxicity and the few individuals present are evenly distributed among a few tolerant taxa (Stevenson 1984). Compare values to those from a reference stream (Pontasch and Brusven 1988). Use caution in comparing diversity-index values to those published in the literature unless you are confident they are calculated using the same formula you have used. Different formulas exist.
3. **Percent dominance.** Recently, the diversity index has been replaced by indices that more directly measure the two components of the original index, richness of taxa (above) and evenness of distribution. Since biological assemblages are naturally not evenly distributed, a better metric measures the amount of unevenness. Percent dominance of one or a few

taxa indicates an unbalanced community. The relative abundance of the three most common taxa can be a useful replacement for the Shannon index. Higher values indicate lower water quality.

4. **Pollution-tolerance index (PTI).**

$$\text{PTI} = \sum n_i t_i / N$$

—where n_i is the number of individuals of a particular species, t_i is the tolerance value of that species, and N is the number of organisms in the sample.

This diatom index is modeled after the HBI for benthic macroinvertebrates (Hilsenhoff 1987), with the exception that tolerance values range from 1 to 4, and increasing numbers signify increased sensitivity. While tolerance values for Texas have not been published, values have been generated for a Kentucky database from a literature review including Lowe (1974), Patrick and Reimer (1966, 1975), Patrick (1977), Lange-Bertalot (1979), Descy (1979), Sabater et al. (1988), and Bahls (1992). An extensive Kentucky Division of Water database (1977–93) and data collections by the Kentucky Nature Preserves Commission (1979–86) were also instrumental in assigning tolerance values. General tolerances of the most common species are fairly well-understood. If no information is available for a given species, do not include individuals of that species in the PTI calculation. Higher values for this metric would indicate higher water quality.

5. **Richness of *Cymbella* group taxa.** The *Cymbella* group of diatoms contains many intolerant species. This metric is calculated as the number of *Cymbella*-group taxa identified in the sample. This metric can be especially important in headwater streams, where diversity and richness may be naturally lower, causing the other metrics to underestimate water quality. Higher values for this metric indicate higher water quality.

6. **Percent motile diatoms.** The combined relative abundance of motile diatoms able to glide to the surface of sediments (*Nitzschia, Navicula*, and *Surirella*) has been used as a siltation indicator (Bahls 1992). Other genera may be added as their silt tolerances become known. Higher values of this index indicate decreased habitat quality or increased siltation.

7. **Percent community similarity.** The percent community similarity index (PCSI) discussed by Whittaker (1952) and Whittaker and Fairbanks (1958) can be used to compare the diatom community of a reference site and one or more test sites. It can be used with relative abundance data, therefore giving more weight to dominant taxa than rare ones without disregarding the rare taxa altogether. Higher percent similarity to the reference site may indicate higher water quality, assuming the reference site is of high quality.

Collecting Quantitative Benthic Algal Samples

Sampling methods for quantitative analysis depend on the type of study and should follow general guidelines described in this section. Modifications are acceptable as long as they are detailed in the QAPP or QAP.

For example, quantitative sampling of benthic algae may be necessary to determine if nuisance levels of periphyton are present. While no screening criteria are yet established, periphyton chlorophyll *a* biomass of > 200 mg/m^2 is at or above nuisance levels (Dodds and Welch 2000). Future studies on nutrient enrichment and algal biomass may require quantitative sampling of the benthic algal community. These samples may include estimates of chlorophyll *a* and other biomass as well as qualitative counts of algal abundance and distribution. It may be desirable to collect quantitative samples for algal biomass in conjunction with the qualitative visual assessment described earlier in this chapter.

Collecting Quantitative Samples in Streams with Bedrock or Cobble Substrate

Setting up the Transect

Collect and analyze a minimum of five replicate samples separately for chlorophyll *a* biomass estimates or other quantitative analyses. The number of transects will depend on such factors as the objectives of the study, the size of the stream, the size of the sampling device, and the patchiness of the algae within the stream. Refer to the study's QAPP for specific guidance on setting up transects.

Use the following method to collect replicate samples from a riffle or run along each transect.

1. Select an undisturbed spot in the middle of the site—one that has not been walked over during sample collection procedures.
2. Drive a stake into the ground on one bank.
3. Attach a tape measure to the stake and stretch it across the stream. Secure it with another stake.
4. Divide the width of the stream into a predesignated number of intervals. Start sampling at the midpoint of the first interval.
5. Move to the first midpoint and, without looking, reach down and select the first rock you touch for a cobble sample, or sample other substrate types using appropriate collection methods as outlined below.
6. Move to the midpoint of the next interval and collect the second replicate. Repeat until all replicates are collected.
7. Move to the next transect and continue until all replicates from all transects are collected.
8. Label and preserve samples as outlined below in "Sample Preservation," later in this chapter. **Do not** preserve samples collected for biomass (chlorophyll *a*, ash-free dry mass, etc.). Wrap those samples in aluminum foil to exclude light and keep them on ice until transport to the laboratory.

Sample Collection

Use the sample-collection method that is most appropriate for the habitat. In all cases, accurately measure the surface area of the bedrock or cobble sampled and attempt to collect all the algal material within that selected area. For detailed instructions on how to measure the area

of the rock surface, see USGS (2002). If you use the syringe–PVC pipe method to collect the sample, accurately measure the area collected by the sample device. Collect all replicates from the same habitat type, as biomass will vary greatly between habitat types. Collect samples from riffles or runs if possible. These methods do not apply to collecting samples from a pool or depositional area.

Bedrock, Boulder, or Other Large-Substrate Habitats

After setting up the transect use the following method to sample areas of rock, bedrock, or other large substrates (such as logs in low-gradient streams without rocky substrates) with a brush and suction device. See Figure 7.2 for images of the following procedures.

1. Press a 60 mL syringe with the end cut off against the substrate tightly enough that water and dislodged algae does not leak out of the enclosed area. If a sampling area with a larger diameter is desired, it is possible to use a 3″ to 4″ section of PVC pipe with a rubber gasket glued to the end that is pressed against the substrate.
2. Scrape and remove as much filamentous algae as possible from within the enclosed area and place it in a sample jar. If the algal filaments are particularly long, it helps to cut around the outside of the syringe with small scissors.
3. Brush the remaining algal material off the substrate with a stiff brush. A toothbrush bent at the head at a 90-degree angle or a stiff artist's paint brush are suitable.
4. Keeping the syringe or PVC pipe section firmly pressed against the substrate. Suction the algal material and associated stream water into the sample jar using a syringe, turkey baster, or hand-operated vacuum pump.
5. Repeat this process until at least five replicates are collected from each transect.
6. Keep replicate samples separate.

Cobble Habitats

After setting up the transect, use the following method to sample cobble riffle habitat.

1. From each transect interval, carefully remove a rock from the stream, disturbing as little algae as possible, and place it in a white pan.
2. Using a combination of scraping, brushing, and rinsing with stream water, collect all the algal material from the top surface of the rock. Use as little rinse water as possible. The sample size should not exceed 500 mL.
3. Pour the sample from the pan into a 500 mL wide-mouth sample jar.
4. Measure the sampled surface area of the rock as accurately as possible, using appropriate formulas. Record the surface area and sample volume for later biomass calculations.
5. Repeat this process along the transect until at least five replicates are collected from each transect.
6. Keep replicate samples separate.

Figure 7.2. How to remove algae from rock surface and how to measure rock surface area. (USGS 2002.)

Collecting Quantitative Samples in Streams with Clay, Silt, or Sandy Substrates, or Non-Wadable Streams

If the stream has a clay, silt, or sandy substrate, and does not contain any large substrates that can be sampled, the above methods are not appropriate. In this case, the following collection methods may apply.

Macrophytes and Snags

Cut sections of submerged plant material or woody snags and wash the algal material into a sample jar. Measure the surface area of the plant material sampled. This may be difficult if the plant material is highly dissected. Make sure the plant material sampled has been submerged in the stream long enough to have developed a natural algal community and not material recently washed into the stream. In the case of woody snags, look for evidence of biological colonization, such as filamentous algae, macroinvertebrate cases, or aquatic insect larvae burrowing into the wood. Plant material should have observable attached filamentous algae or diatom growth.

Artificial Substrates

Use artificial substrates if there is no other way to collect a sample. While the benthic algal community that colonizes artificial substrates is usually not representative of the community that colonizes a natural substrate, artificial substrates can be used to assess water quality (Patrick 1973; Stevenson and Lowe 1986). Artificial substrates include rocks, clay tiles, glass slides mounted in commercially available trays, and nutrient-diffusing substrates. Deploy artificial substrates for three to four weeks to allow sufficient time for algal colonization (Aloi 1990). If substrates are disturbed, either by natural causes (flood, drought) or vandalism, redeploy fresh ones.

Sample Preservation

Samples for chlorophyll *a* analysis must not be treated with chemical preservatives. They must be wrapped in aluminum foil to exclude light, placed on ice, and transported to

the laboratory for immediate subsampling and analysis. Samples for chlorophyll *a* analysis must be processed and filtered within 24 hours of collection. If frozen and kept in dark containers, filters can be retained for 28 days before extraction. See the "Laboratory Procedures for Quantitative Benthic Algal Sample Processing and Identification" section for details on processing chlorophyll *a* samples.

If both chlorophyll *a* and algal-identification analyses are to be performed from the same samples, they can be subsampled in the laboratory before preservation and processing. Samples for identification and counting must be preserved in 2 percent glutaraldehyde or 3 to 5 percent formalin—three to five parts full-strength formalin and 97 to 95 parts water. Preserved algal samples must be placed in a dark container and kept cool until analysis. Keep samples in glutaraldehyde refrigerated in the dark to maintain their natural pigmentation longer than samples preserved in formalin. This may aid in identification.

Safety

Avoid breathing formalin fumes! Glutaraldehyde and formalin are corrosive to the eyes, skin, and respiratory tract. Wear safety glasses and latex when working with these chemicals. Formalin is a suspected carcinogen. Always work in a well-ventilated area or under a hood when preparing glutaraldehyde or formalin solutions. Check the material-safety data sheets for formalin solution and glutaraldehyde for proper handling requirements.

Labeling the Sample Container

Attach a label to the outside of the container making sure the container is dry, and wrap it with clear tape to ensure the label stays on the container. Do not place labels for algal samples in the sample container because the algae will discolor them and make them illegible. Labels must contain the following information.

- station number and location description
- date and time of collection
- collection method (for example: hard substrate, snags, or macrophytes)
- preservative used
- name of each collector
- sample type
- container replicate number if needed (for example, *1 of 2* or *2 of 2*)

If two or more replicate samples or sample types are collected at a site, label them accordingly.

Field Notes

Field notes comprising at least the following information must be recorded in a field notebook.

- type of macro-algae present
- percent of the substrate covered by algae
- presence of any extensive growths of filamentous algae or surface algal blooms

- evidence of recent scouring
- any other pertinent observations

For quantitative samples from natural substrates, record method of sampling, number of replicates, and area sampled. For artificial substrate samples, record time of deployment and substrate type (for example, periphytometer, clay tile) in the field data log.

Processing and Identification of Quantitative Benthic Algal Samples in the Laboratory

Estimating Biomass

Quantitative samples collected for biomass estimation must be processed by the laboratory as outlined in the latest version of *Standard Methods for the Analysis of Water and Wastewater* (APHA 2012). Samples for chlorophyll *a* analysis must be processed and filtered within 24 hours of collection. Filters can then be kept frozen in a dark container for 28 days before extraction. While the *Standard Methods* are written for samples from artificial substrates, they can be easily adapted for qualitative samples from natural substrates. Content-analysis for ash-free weight and chlorophyll is described in methods 10030.C.5 and 10030.C.6. If fluorometric analysis of chlorophyll is to be performed, use EPA method 445.0 (U.S. EPA 1997).

Taxonomic Analysis

Chapter 11 gives a complete list of required and recommended references on algal identification.

The person initially performing the taxonomic identification of diatoms will seek verification by outside experts of those diatoms for which identification is unsure. The person seeking the verification will circle those diatoms that need verification on the slide with a diamond pencil and will number each specimen circled with an accompanying reference to the taxon name.

If a special study requires quantitative analysis of the algal community, samples must be processed in the same way as qualitative samples, except that calculations are needed to report the data as cells per mm^2. Using a laboratory bench sheet record the original sample volume, sample area, subsample volume, and, if algal density is high, any serial dilutions.

When counting, use a Sedgewick Rafter or Palmer counting chamber filled with exactly 1 mL of sample. Use of an inverted microscope and volumetric counting chambers is acceptable, as well. Allow a short period of time for algal cells to settle to the bottom of the chamber and then proceed to count strips or fields. See *Standard Methods* (APHA 2012) for details on using counting chambers.

Identify algae to genus whenever possible. Count unicellular algae and colonies as individual units, and filaments in 10 µm segments (one 10 µm segment = one unit). Calculate and report benthic algal data as cells/mm^2 using the following formula (APHA 2012).

$$\text{Organisms/mm}^2 = \frac{N \times A_t \times V_t}{A_c \times V_s \times A_s}$$

Where: N = number of organisms counted
A_t = total area of chamber bottom

V_t = total volume of original sample suspension (mL)
A_c = area counted (strips or fields) (mm^2)
V_s = sample volume used in chamber (mL)
A_s = surface area of substrate (mm^2)

Evaluating Benthic Algal Data for Quantitative Samples

Evaluating Periphyton Biomass

Two common measurements of biomass are chlorophyll *a* and ash-free dry mass (AFDM). A ratio of these measurements can be used to calculate an autotrophic index (AI) (Weber 1973).

Chlorophyll *a* gives an estimate of the autotrophic component (photosynthetic) of the periphyton sample. While there are no screening criteria established yet, current information indicates that periphyton chlorophyll *a* biomass of > 200 mg/m^2 is at or above nuisance levels (Dodds and Welch 2000).

AFDM gives an estimate of the entire amount of organic material in the sample, including autotrophs (algae, cyanobacteria, and moss) and heterotrophs (bacteria, fungi, and living microinvertebrates), as well as dead algae, other organisms, and organic litter.

The AI is calculated as the ratio of the AFDM to chlorophyll *a*. This index is indicative of the relative proportions of autotrophic to heterotrophic components of the benthic periphyton community. Values of 50 to 100 are characteristic of non-polluted conditions with little organic detritus (Biggs and Kilroy 2000); whereas, values greater than 400 may indicate assemblages affected by organic pollution (Collins and Weber 1978).

Evaluating Benthic Algal Assemblages

Quantitative samples can be analyzed for density and biovolume. Conversion of algal density information into biovolume enables a more accurate analysis of the biomass dominance of different taxa. By calculating representative biovolumes for a sample of each of the main taxa, the data can be corrected for the contribution of each taxon to the total amount of organic matter at the site (Biggs and Kilroy 2000).

Aquatic Macrophytes

Macrophyte sampling is not a routine SWQM activity; however, special studies of aquatic macrophytes in specific areas may be desirable. The purpose of macrophyte sampling may be to illustrate short- and long-term changes in the environment, or simply to inventory the types of macrophytes present in a water body.

Contact the SWQM central-office staff for assistance when developing a macrophyte sampling project. Know the specific objective of the monitoring so that a QAPP can be written to address its purpose and how the data will be used.

Data may be used to describe presence or absence of nuisance growths of aquatic plants, can be expressed as percent cover or abundance of individual plants or taxa, or can be an estimate of total biomass of macrophytes, depending on the study objectives.

Seagrass

Seagrass communities serve as critical nursery habitat for estuarine fisheries and wildlife. Additionally, seagrasses serve as food for fish, waterfowl and sea turtles; contribute organic material to estuarine and marine food webs; cycle nutrients; and stabilize sediments. They are economically important, based on their function in maintaining Gulf fisheries, and were identified as a critical area by the Coastal Coordination Act in 1977.

Three state agencies with primary responsibility for conserving coastal natural resources—the Texas General Land Office, the TCEQ, and the Texas Parks and Wildlife Department—signed the Seagrass Conservation Plan for Texas in 1999. One component of the Seagrass Conservation Plan, the Texas Seagrass Monitoring Plan, provided for the formation of a stakeholder work group. Since that time members of the seagrass-monitoring work group have been developing a monitoring plan for seagrass on the Texas coast; the work group proposed this plan in 2010 (Dunton, Pulich and Mutcher, 2010).

At this time, methodologies for assessing ALU and other regulatory bioassessments based on seagrass have not been established for Texas waters. Currently, the SWQM program is developing seagrass monitoring procedures to be used by agency personnel when sampling seagrass. Before conducting any biological monitoring involving seagrass, with the intention of submitting data for inclusion in the TCEQ SWQMIS database, it is important 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.

CHAPTER 8

PLANKTON

Disclaimer

Methodologies for assessing ALU and other regulatory bioassessments based on plankton have not been developed for Texas waters. Before conducting any biological monitoring activities using plankton, 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.

Non-Wadable Streams, Rivers, Lakes, Reservoirs, and Bays

This chapter describes methods the TCEQ recommends for the collection and assessment of plankton assemblages. Plankton are free floating, mostly microscopic, plants, animals and bacteria. They generally cannot swim; instead, plankton are transported by tides and currents.

Plankton assemblages may include either phytoplankton (algae)—tiny single-celled plants—or zooplankton—free-floating animals. This chapter describes sampling methods for each assemblage.

The purpose of plankton sampling is to collect data that can be used to assess water quality trends and compare water quality between sites. Phytoplankton may also be sampled during a harmful algal bloom (HAB) such as one that resulted in a fish kill. Special handling may be required for red-tide, golden-alga, or cyanobacteria blooms. For HAB monitoring, contact the TPWD before collecting any samples. The TPWD—<www.tpwd.state.tx.us/landwater/water/environconcerns/hab/>—is responsible for investigating and researching the causes of HABs.

Plankton sampling is not part of routine monitoring and would only be done as part of a special study. Special studies require an approved QAPP or QAP prior to sampling. If a study is in progress, refer to the study's QAPP or QAP for details. If you are developing a QAPP or QAP for a special study, contact appropriate SWQM personnel for assistance.

Phytoplankton-Collection Methods

Phytoplankton can be collected either by a grab sample or with a plankton net. Surface grab samples are generally sufficient when sampling an algal bloom for presence of HABs. When the purpose of the sample collection is to document the entire phytoplankton community, collect integrated samples through the euphotic zone. For certain studies, phytoplankton net tows may be desired. When sampling phytoplankton with a net, its size and mesh size are important.

Collecting Samples from a Harmful Algal Bloom

For algal-bloom samples (red tide, golden alga, cyanobacteria, or unknown blooms) surface grab samples are often sufficient when the sampler comes across a bloom unexpectedly in the field. If this happens, collect a surface sample (0.3 m depth) in a clean 500 mL container. Some algal

blooms may be harmful or contain skin irritants. Wear gloves to collect the sample and avoid skin and eye contact or ingestion. Contact the TWPD for instructions on preserving the sample, or refer to the section below on sample preservation.

Collecting Grab Samples of Phytoplankton

Phytoplankton grab samples can be collected using methods developed by the EPA for the 2007 and 2012 National Lakes Assessment (U.S. EPA 2012). This method uses an integrated sampler based on a design by the Minnesota Pollution Control Agency; any similar device that allows a quantitative sample to be collected evenly through the water column of the euphotic zone should provide acceptable results.

The integrated water sampler is a PVC tube 2 m long with an inside diameter of 1.24 inches (3.2 cm) fitted with a stopper plug at the upper end and a valve that opens and closes the bottom end. The tube can be made in two sections with a screw coupler in the middle for easier storage and transportation. This device allows collection of a 1 L integrated grab sample from the upper two meters of the water column within the euphotic zone.

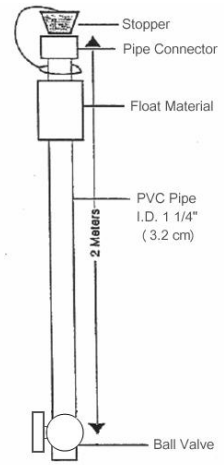


Figure 8.1. Integrated water sampler.

1. Estimate the depth of the euphotic zone by doubling the Secchi-disk depth at the site.
2. If the euphotic zone is greater than 2 m, sample the top 2 meters of the water body. If the euphotic zone is less than 2 meters, sample only to the depth of the euphotic zone—two times the Secchi-disk measurement. See Chapter 3 of Volume 1 (RG-415) for details on Secchi-disk measurements.
3. Rinse the sampler by opening both ends and submerging it in the lake three times.
4. With the valve open and the stopper off, slowly lower the sampler into the water as vertically as possible until the upper end is just below the surface or you have reached the depth of the euphotic zone if it is less than 2 meters.
5. Cap the sampler and slowly raise it to the surface, closing the valve before you pull it out of the water.
6. Empty the sample into a clean 4 L container by removing the stopper and slowly opening the valve. A large funnel, rinsed three times with ambient water, simplifies the sample transfer.
7. Repeat four times, each time dispensing the sample into the 4L container.
8. Once the four grab samples are composited into the 4L container, mix it thoroughly and pour 1 L into a clean container for phytoplankton processing.
9. Preserve samples as described in "Sample Preservation," below.

The remainder of the composite sample can be used for accompanying chlorophyll *a*, nutrient, or other analyses if required by the study plan. If the euphotic zone is less than 2 m, more than four

grabs may be required, depending on the total volume needed for the phytoplankton sample and accompanying sample analyses.

Collecting Phytoplankton Samples with a Net

Two types of net samples can be collected—either vertical or horizontal tows. Vertical tows can be made from a pier or other fixed location or from a stationary boat. Horizontal tows are made by slowly towing the plankton net from a moving boat or towing the length of a pier or bulkhead. Plankton net samples are not as quantitative as integrated or composite grab samples, but can be used to sample the phytoplankton community that is captured. Plankton nets are available in many different sizes and mesh sizes. Use one with a mesh size less than or equal to the size of the smallest phytoplankton cells expected in the sample.

1. Using a plankton net with a mesh size appropriate for the desired community, collect at least two net tows per station. In general, use a mesh size of 20 to 80 µm for microplankton, and 2 to 20 µm for nanoplankton.
2. Vertical tows:
    - Estimate the euphotic zone by calculating a depth of two times the Secchi-disk measurement.
    - Lower a clean plankton net with bucket attached to the lower end of the euphotic zone. In a shallow water body where the euphotic zone is equal to the depth, sample 0.5 m from the bottom to avoid disturbing bottom sediments.
    - Keeping the net line as vertical as possible, raise the net through the water column at about 0.3 m/sec. Proceed to step 4.
3. Horizontal tows:
    - Lower the net until it is completely submerged.
    - Let out a sufficient amount of line to allow the net to be towed beneath the surface.
    - Tow the net slowly for the desired distance. All samples in the study should be collected over the same distance. Once that distance is covered, pull the net slowly out of the water so that water flows out through the net mesh and not out the mouth of the net. Proceed to step 4.
4. Rinse the plankton on the net surface down into the bucket. Either:
    - hold the net upright and dunk it several times into the water, up to the mouth, or
    - splash water on the outside of the net and the plankton will be washed down to the bucket.
5. Disconnect the bucket and rinse it into a clean, waterproof container using a rinse bottle filled with ambient water that has been filtered through the plankton net.

High Phytoplankton Abundance—Composite Grab Samples

Some water bodies may be eutrophic and have very high phytoplankton abundance. If plankton are too abundant for the sample to drain freely through the net, but a net sample is preferred over

an integrated grab sample, it is acceptable to discard the net sample and collect a composite from water samples collected from specified depths with a Kemmerer or Van Dorn sampler.

1. Collect samples from the following 3 depths.
   a. 0.3 m below the surface
   b. midway between the surface and the lower end of the euphotic zone *or* midway between the surface and the bottom of the water body
   c. the lower end of the euphotic zone *or* 0.5 m above the bottom

   Additional samples may be collected as specified in the study plan, for example, every 3 meters in deeper water bodies.
2. Pour each water sample through the plankton net into the attached bucket.
3. Rinse the outside of the net with ambient water to wash the organisms down into the attached bucket.
4. Rinse the bucket into a clean container using a rinse bottle filled with ambient water that has been filtered through the plankton net.
5. Disconnect the bucket and rinse the sample into a clean waterproof container using a rinse bottle filled with the prefiltered ambient water.

Sample Preservation

Do not use preservatives for samples that are going to be analyzed immediately unless instructed otherwise by the laboratory that will be analyzing them. Preservatives tend to distort the organisms and make them more difficult to identify. Instead, collect samples in an amber bottle (if available), wrap a bottle in foil, or use whatever method is available to exclude as much light as possible. Wrap the sample in wet paper to keep it as cool as possible, but do not place it directly on ice.

Several algal preservatives are available, each having its advantages and disadvantages. If in doubt, contact the laboratory or person responsible for identifying the plankton for guidance. The most commonly used preservative for phytoplankton is Lugol's solution (5 to 10 percent); however, formaldehyde (3 to 5 percent) or glutaraldehyde (2 percent) may be used.

Add approximately 0.5 to 1.0 mL of Lugol's for every 100 mL of sample. The fixed sample will be light brownish, the color of weak tea or brandy. Lugol's solution is degraded by light, so both its container and preserved water samples should be stored in dark containers or using some other method to exclude light.

Recipe for Lugol's Solution

Prepare Lugol's solution by dissolving 20 g potassium iodide and 10 g iodine crystals in 200 mL distilled water containing 20 mL glacial acetic acid.

Safety

Label the secondary container with solution components. Be sure to add the acid to water. Wear safety glasses and latex gloves when preparing this solution.

Labeling Sample Containers

Attach a label to the outside of the container making sure the container is dry and wrap with clear tape to ensure that the label stays on the container. Labels must contain the following information.

- station number and location description
- date and time of collection
- preservative used
- name of each collector
- sample type (grab, integrated, net, net composite)
- sample volume
- container replicate number if needed (for example, *1 of 2* or *2 of 2*)

Additional labeling is necessary depending on the method of collection.

For net hauls include:

- number of hauls
- depth of hauls
- diameter of net mouth
- net mesh size

For Kemmerer (or Van Dorn) samples, subsequently filtered with a net and composited, include:

- volume of sampler (liters)
- sampling depths
- number of samples

Sample Storage

Samples must be stored at a moderate temperature in the dark until analysis.

Sample Processing

The purpose of samples collected for HAB or other blooms is to identify the bloom organisms and estimate the abundance of the organism under investigation—for example, *Karenia brevis* (red tide) or *Prymnesium parvum* (golden alga). These samples will usually be examined within 24 hours of collection in the event of an accompanying red-tide outbreak or fish kill unrelated to a planned study of the algal community. In those cases it is acceptable to only identify and enumerate the organisms of interest.

For other types of sampling, the purpose of microscopic examination of the phytoplankton sample is to inventory the algal community. For these samples as well as HAB samples, it is important to examine them within 24 hours of collection unless they have been chemically preserved in Lugol's solution or another chemical fixative. Preserved samples can be stored

indefinitely; however, pigmentation that may aid in identification will fade quickly, making it preferable to analyze them as soon as possible upon return to the laboratory.

Quantitative microscopic analyses of grab samples should follow the methods outlined in APHA 2012 (or the latest edition), Section 10200 F. "Phytoplankton Counting Techniques."

Zooplankton Collection Method

Using a plankton net with a mesh size appropriate for the desired community, collect at least two vertical net tows per station. Use a Wisconsin-style plankton net with a mesh size of 50 to 243 µm, depending on the size of organisms to be collected. Unless another study plan is proposed, sampling should follow protocols similar to those used in the 2007 and 2012 EPA National Lakes Studies as outlined below.

1. Determine the number of tows required to achieve a standard cumulative 5 m tow.
   - For lakes deeper than 7 m, take a 5 m tow.
   - For lakes with a depth less than 7 m, determine the number of tows that will be required to achieve the standard cumulative 5 m tow. For example, if the lake is 6 m deep, take two 2.5 m tows. Refer to Table 8.1 for the number and depth of tows.

Table 8.1. Number and depth of zooplankton tows.

Water Body Depth (m)	Depth of Tow (m)	Number of Tows
7+	5.0	1
4–6	2.5	2
2–3	1.0	5
1–2	0.5	10

2. Slowly lower the net over the side of the boat keeping it as vertical as possible until the correct depth is reached. It is helpful to mark the line attached to the net in increments of 0.5 m.
3. Retrieve the net by pulling it back to the surface at a steady rate of 0.3 m/s without stopping.
4. Once at the surface, slowly dip the net up and down in the water without submerging the net mouth to rinse the organisms into the bucket attached to the cod end of the net.
5. Splash or squirt ambient lake water against the outside of the net to rinse remaining organisms into the bucket. If necessary, rinse the insides of the net with deionized (DI) water only to avoid introducing additional organisms into the sample.
6. Remove the bucket from the net and set it into a small pail containing lake water and two Alka-Seltzer (CO_2) tablets to narcotize the organisms in the sample. Be sure not to submerge the top of the collection bucket or sample loss will occur. Wait one minute or until all zooplankton movement has stopped.
7. Empty the collection bucket into a 125 mL sample container. Rinse the collection bucket with DI water until all zooplankton are rinsed into the sample container. Do not fill the container more than half full of sample and rinse water. If the sample and rinse water combined exceed half the sample container volume, use a second container to preserve the additional sample and label appropriately.

8. Fill the 125 mL sample container to the shoulder with 95 percent ethanol to preserve the zooplankton sample. To be effective, half the sample volume must contain preservative.

Labeling Sample Containers

Attach a label to the outside of the container, making sure the container is dry and wrap with clear tape. Label the sample container with the following information and also include it in the field logbook.

- station number and location description
- date and time of collection
- sampling depths
- number of tows
- preservative used
- name of each collector
- sample type
- container replicate number if needed (for example, *1 of 2* or *2 of 2*)

Sample Storage

The sample container must be stored in a cool, dark location until analyzed. It is not necessary to refrigerate preserved samples.

Sample Processing

Quantitative microscopic analyses of grab samples should follow the methods outlined in APHA 2012 (or the latest edition), Section 10200 G, "Zooplankton Counting Techniques."

CHAPTER 9

PHYSICAL HABITAT OF AQUATIC SYSTEMS

Objective

This chapter describes the methods used by the TCEQ for the collection and assessment of physical-habitat data primarily in wadable freshwater streams. A stream is considered wadable if most of its channel is accessible by wading during normal flow conditions. Generally, these streams are third order or less. Pool areas or high-flow conditions may make the stream inaccessible to wading in certain places or at certain times; however, the stream will still be considered wadable when determining reach length. Method modification for use of these protocols in non-wadable rivers and streams is described at the end of this chapter.

In general, the TCEQ will use aquatic habitat data collected according to these methods, in conjunction with fish and benthic macroinvertebrate community surveys, to holistically evaluate the health of biological assemblages and to develop future indices of aquatic-life use for these waters. A physical habitat evaluation of a stream is an integral and required part of all biological assessment activities. One of the main functions of a habitat assessment is to characterize the aquatic-life potential of a stream. Aquatic habitat quality is an important factor affecting the integrity of fish and benthic macroinvertebrate communities. Characteristics of physical stream habitat such as the presence or absence of instream cover, substrate characteristics, and riparian integrity have important effects on both benthic macroinvertebrate and fish assemblages. Habitat characterization, therefore, is important in interpreting results and determining the cause of decreasing biotic integrity. The data-collection protocols outlined below must be followed.

Habitat-Assessment Forms

Habitat assessment is recorded using forms broken into three parts. Use the Stream Physical Characteristics Worksheet—Part I to record required data in the field. The worksheet is divided into two portions. The upper portion is for general observations made over the entire evaluated reach, while the lower, or boxed, portions are for measurements and observations made at specific transect locations. After field work is complete, summarize and average data from the worksheets to complete the Summary of Physical Characteristics of Water Body—Part II. Then score and calculate the Habitat Quality Index (HQI) using the Habitat Quality Index—Part III form based on the values summarized in Part II. Mark transect locations on a USGS topographic quadrangle map and attach it to the forms. For RWAs, also locate each existing or proposed discharge point on the map. See Appendix C for Part I, II, and III forms.

Requirements for Habitat Assessments

Aquatic-Life Monitoring, Aquatic-Life Assessments, and Use-Attainability Analyses

A habitat assessment is a required part of any biological monitoring event. For ALM, ALA, and UAA monitoring, the placement requirements for habitat reach are the same and are outlined in

Chapter 2. It is very important that the habitat-assessment reach covers the areas where fish and benthic macroinvertebrate samples are collected. Ensure the reach is not close to a bridge overpass. Occasionally a bridge crossing is strewn with riprap or other debris that forms the only riffle in the reach. Sampling from these artificial riffles is discouraged and should only be conducted after exhausting all other sampling efforts outlined in Chapter 5. Once the leaders of the crews sampling habitat, fish, and benthic macroinvertebrates 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.

Habitat Assessment Requirements for UAA, ALA, and ALM during the Second Event in an Index Period

In any one year, most ALMs, ALAs, and UAAs will involve two sampling events within an index period. An HQI score must be part of every biological monitoring data set. A full habitat assessment must be conducted at the **first** biological monitoring event (within the index period) per year. Photographs must be included. A full habitat assessment must be conducted at the **second** biological monitoring event per year, unless conditions have demonstrably not changed appreciably since the first habitat assessment that year. The following evidence must be gathered to demonstrate similar conditions between events.

1. flow
2. wetted-channel width
3. photographs of reach
4. description of bank conditions in relation to first event
5. description of canopy conditions in relation to first event

If best professional judgment determines that conditions have not changed significantly based on these five pieces of evidence, then the HQI from the first event may be used in the second data set. The same lead field staff must assess habitat at both events. These allowances apply only to two habitat events within one index period.

Habitat-Assessment Requirements for RWAs

For new permit applications or for WWTPs that have not yet discharged, conduct the habitat assessment beginning at the proposed location of the WWTP outfall and proceed downstream. Once the transect measurements are completed, make general observations over the reach while returning to the point of the proposed outfall.

For amendments or renewals of existing WWTP permits, an assessment upstream of the WWTP outfall is required. Make transect measurements starting from a point approximately 30 m upstream of the outfall and continuing upstream. Once the transect measurements are completed, make general observations over the reach while returning to the discharge point.

See Figures B.8 and B.9 in Appendix B for examples of where to locate sites for RWAs in relation to existing or proposed WWTP outfalls.

Determining Reach Lengths and Placing Transects

After site selection, the next step in conducting a stream habitat assessment is to determine the length of stream to be evaluated, or the "stream reach." Determine the stream reach by walking the stream for several hundred meters to locate the areas where biological collections will be made. Determine an average stream width during this initial reconnaissance by taking four to five width measurements at points along the stream channel that best represent the diversity of stream widths without biasing measurement toward very narrow or very wide sections of the channel. Take the width measurements and average them, then multiply that average by 40 and round to the nearest whole number using standard rounding rules. Forty times the average wetted stream width becomes the length of stream reach evaluated, with a minimum required reach length of 150 m. For most streams, this usually results in a reach length of approximately 200 to 300 m. The maximum reach length for wadable streams is 500 m. The calculated reach length is then divided into evenly spaced units depending on its length.

Once you have determined the reach length, locate the first transect far enough upstream from a bridge or road crossing so as not to influence the natural stream channel. Mark it with bright survey flagging and label it Transect "A" or "1." This becomes the downstream end of the reach and biological sampling should not be conducted downstream of that point unless absolutely necessary. Then locate and flag subsequent transects upstream of the first transect and evenly spaced to cover the entire reach.

The placement of transects within the reach is as follows:

For streams 150–300 m, place five equidistant transects along the reach, and include the ends of the reach. Then divide the reach length by four. The distance between transects is no greater than 75 m. See Figure 9.1.

For streams 301–500 m, place six equidistant transects along the reach and include the ends of the reach. Then divide the reach length by five. The distance between transects is no greater than 100 m. See Figure 9.2.

For example, if the average stream width is 9.7 m, the average will be rounded to 10 m. Then $10 \times 40 = 400$ m, so 400 m is the reach length. Divide 400 by 5 (to include one transect at each end of the reach) to determine the transect spacing—in this case, $400/5 = 80$, so each transect will be 80 m apart.

The stream reach encompasses the biological and chemical collection areas and includes as many different geomorphic channel units as possible, e.g., riffles, runs, glides, and pools.

Transect Area

For transect measurements, left- and right-bank orientation is determined by the investigator facing downstream.

Place transect lines perpendicular to the stream channel at five to six evenly spaced intervals along the reach, as shown in Figure 9.3.

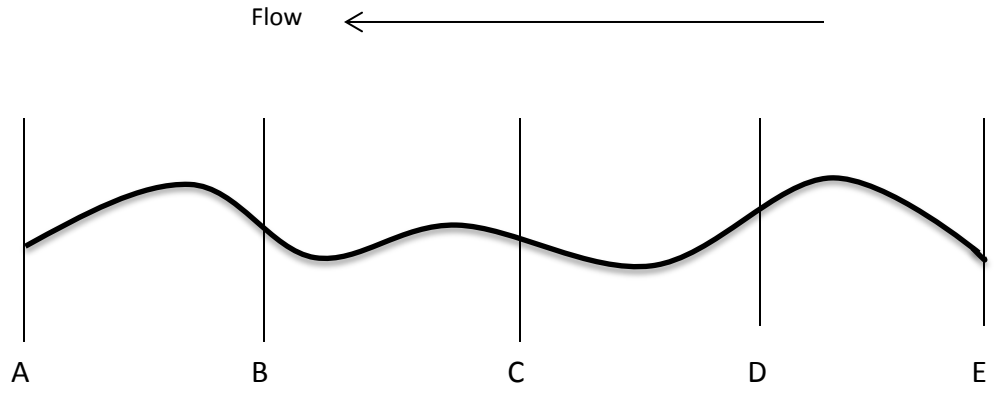


Figure 9.1. Transect placement for reach lengths of 150 to 300 m.

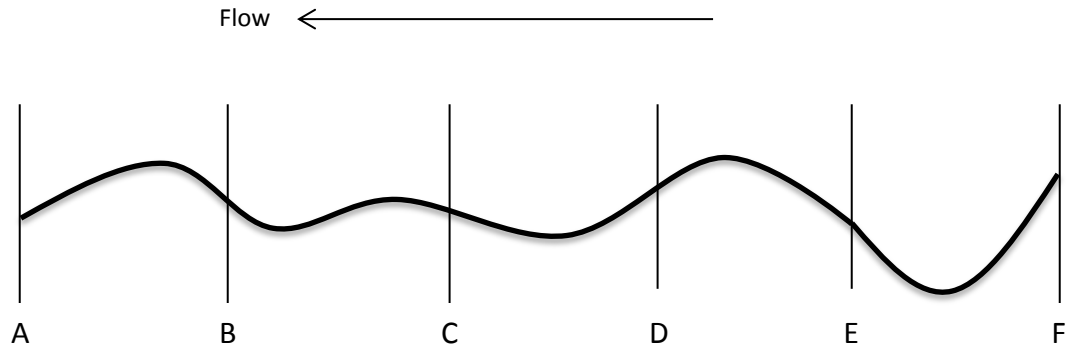


Figure 9.2. Transect placement for reach lengths of 301 to 500 m.

Measurements of some habitat attributes are made along the transect line and in the area 3 m on either side of the transect line defined as the transect area in Figure 9.3. Reach boundaries are included as transects. It is preferable to begin with the transect farthest downstream, as this allows biological sampling ahead of the habitat assessment, thereby minimizing disturbance to the biota. The distance between transects is uniform and must be measured with a measuring tape, hip chain, or range finder.

Low-Flow or Dry Conditions

A habitat assessment must accompany the collection of biological community samples in rivers and streams. If the stream is dry, do not conduct a habitat assessment. If the stream contains standing perennial pools in which aquatic life is found and sampled, conduct a habitat assessment as described in this document, with the following modifications.

Determine the reach length as stated above, using the channel width at base-flow conditions (as best as can be determined) as the wetted width. If the existing perennial pools cover most (> 50 percent) of the reach length, assess the habitat according to the procedures in this chapter. If a transect crosses a dry part of the channel, record any meaningful data from that transect, such as substrate characterization, bank angle under what appears to be normal base flow conditions,

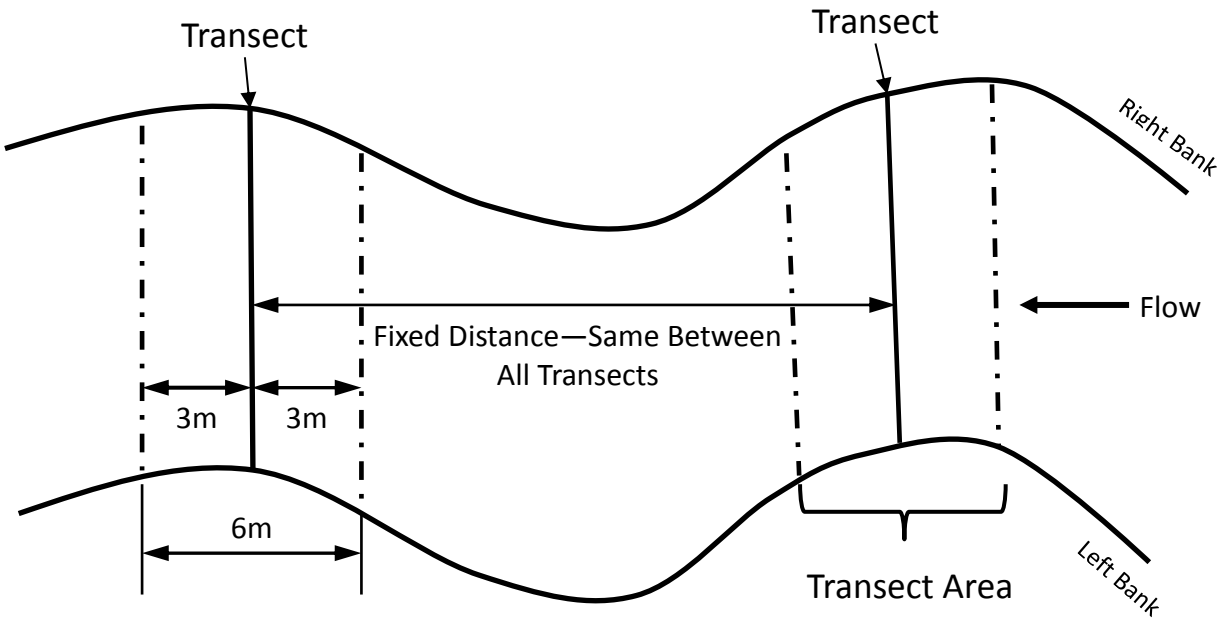


Figure 9.3. Transect area.

riparian information, etc. If the pools cover less than 50 percent of the reach length and are separated by exposed channel bed, place transects such that they best characterize the pools and available water.

Record the maximum pool length, depth, and width at each pool measured in the reach. Transect placement must still follow the minimum spacing requirements stated above; however, some transects may be spaced farther apart, depending on pool location. The overall objective is to characterize the pools where either benthic macroinvertebrates or fish are sampled and to assess the same number of transects that a typical reach of that length would have if there were water in the stream. Characterize only those pools in the reach that are at least 10 m in length and at least 0.4 m in depth.

Part I: Stream Physical Characteristics Worksheet

Use the Stream Physical Characteristics Worksheet to record primary, secondary, and tertiary attributes for each transect or for the entire reach. Record instream channel measurements (primary information), stream morphology (secondary information), and riparian environment (tertiary information) in the upper portion of the form for attributes describing the entire reach, and in the lower boxes for each transect.

Primary Attributes—Instream Channel Characteristics

Primary attributes of a stream's aquatic habitat are the in-channel aspects of habitat type, substrate quality, and food and cover availability for fish and benthic macroinvertebrates. Basically, primary attributes characterize the shelter and food quality for aquatic organisms.

Kaufmann and Robison (1998) provide the basis for many of the measurement protocols in this chapter.

Habitat Type (Geomorphic Units)

Identify the habitat type in the area where the transect falls. To be considered a discrete habitat type, the width of the geomorphic unit (riffle, run, glide, pool) must be greater than 50 percent of the width of the stream and the length of the geomorphic unit must be greater than or equal to the average stream width. If the transect falls in a transition area or on the border between two habitat types, identify both in the box marked "Habitat Type."

Riffle: A shallow portion of a stream extending across a stream bed characterized by relatively fast-moving turbulent water with a broken surface. The water column in a riffle is usually constricted and water velocity is high due to a change in surface gradient. The channel profile in a riffle is usually straight to convex.

Run: A relatively shallow portion of a stream characterized by relatively fast-moving, bank-to-bank, non-turbulent flow. A run is usually too deep to be considered a riffle, but the water velocity is too fast to be a glide. The channel profile under a run is usually a uniform flat plane.

Glide: A portion of a stream where the flow is slow moving and laminar, similar to that found in a shallow canal. Water surface gradient over a glide is nearly zero, so velocity is low, but flow is uniform across the channel without eddy development. A glide is too shallow to be a pool but the water velocity is too slow to be a run. The channel profile under a glide is usually a uniform flat plane.

Pool: A portion of a stream in which water velocity is low and the depth is greater than the riffle, run, or glide. Pools often contain eddies with varying directions of flow compared to riffles, runs, and glides where flow is almost exclusively downstream. The water surface gradient of pools is close to zero and their channel profile is usually concave.

Number of Riffles

Count the number of riffles in the entire habitat assessment reach, not just those that fall in transect areas. Riffles are considered discrete if they are separated by a run, glide, or pool that is at least as long as the average stream width. Otherwise, count one riffle.

Dominant Substrate Type

The channel substrate is the mineral or organic material that forms the bottom of the stream.

Substrate materials are usually classified by particle size. Identify the dominant substrate type that characterizes the stream bottom along and 3 m on either side of each transect according to the following guidelines.

- Bedrock: > 400 cm, size range—larger than a car
- Boulders: > 25 to 400 cm, size range—basketball to car
- Cobble: > 6 to 25 cm, size range—tennis ball to basketball
- Gravel: > 2 to 60 mm, size range—ladybug to tennis ball
- Sand: 0.06 to 2 mm, gritty between fingers

- Mud, clay, and silt: < 0.06 mm, not gritty between fingers

The size composition can be assessed visually or by obtaining one or more small samples by hand or grab. For situations where a thin layer of silt or organic material covers a dominant substrate type such as cobble or bedrock, ignore the overlying material. For areas where the overlying material is thick enough to alter the habitat for the types of organisms who would normally live there—i.e., all interstitial spaces are filled in, dense leaf pack, etc.—then use professional judgment to possibly call the overlying material the dominant substrate.

Percent Gravel or Larger

Estimate the percentage of the substrate that is > 2 mm in size along the transect, and 3 m on either side of it. The size composition can be assessed visually or by obtaining a small sample by hand.

Algae and Macrophytes

Determine if algae and macrophytes are present along and 3 m on either side of the transect. Visually estimate whether algae and macrophytes are abundant, common, rare, or absent. If only algae or only macrophytes are present, circle either "algae" or "macrophyte" on the form and estimate the abundance.

Instream Cover Types

Instream cover refers to physical structures that shelter fish and benthic macroinvertebrates. It includes, but is not limited to, logs, tree stumps, woody debris, root wads, leaf packs, gravel or larger substrates, boulders, artificial cover (for example: tires or cement slabs), undercut banks, macrophyte beds, and overhanging vegetation.

Percent Instream Cover

Visually estimate percentage instream cover along and 3 m on either side of the transect. This percentage represents an evaluation of the area of the stream bottom described above, as well as the water column and area immediately above the water surface along the stream banks. The cover must be at a depth suitable for use by aquatic organisms. For example, if leaf packs and logs are in 2 cm of water on a sand bar, they are not suitable for use by fish or most benthic macroinvertebrates and must not be counted.

Percent instream cover must be evaluated with a gradient of percentages from the lowest percentage, for bare bedrock or concrete, to the highest, for a highly heterogeneous mix of several categories, such as gravel, cobble, logs, macrophytes, and overhanging vegetation. Additional dimensions contribute to higher percentages, such as cover that extends from the substrate up though the water column and above the stream surface.

Secondary Attributes—Stream Morphology

Secondary attributes of a stream's aquatic habitat are characterized by the structure of the stream channel over the entire reach where the primary attributes are located. It is a broader look at the channel itself and the morphological characteristics that influence the quality of the primary attributes. Figure 9.4 depicts a typical stream channel with a well-developed stream pattern.

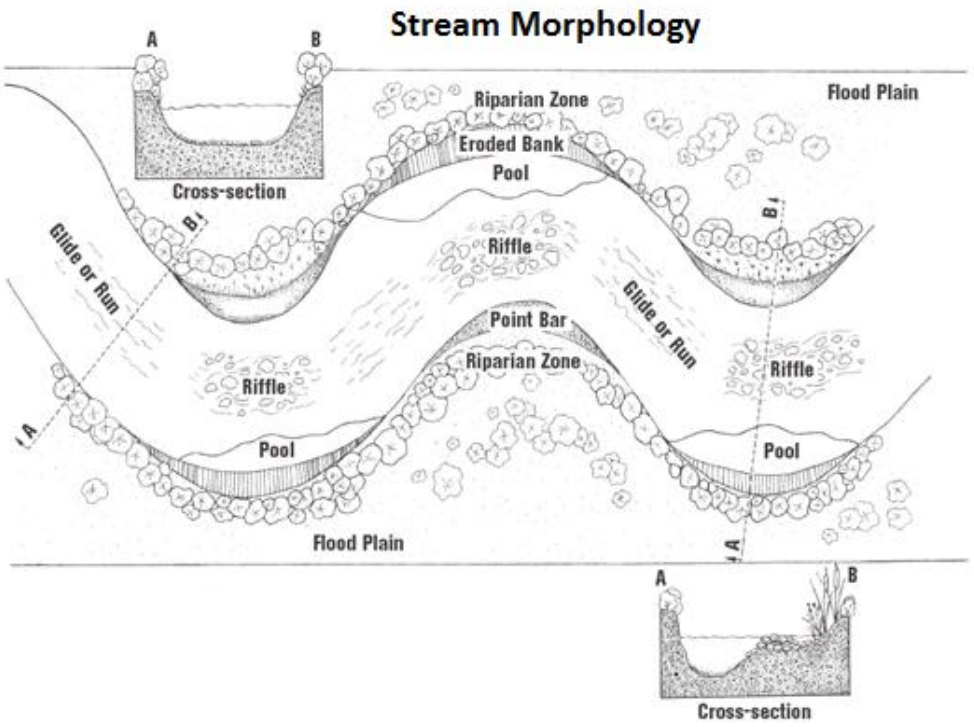


Figure 9.4. Stream morphology.

Stream Bends

Count the number of stream bends and determine their definition (well-defined, moderately defined, poorly defined). Figure 9.5 illustrates stream-bend classifications.

High sinuosity produces diverse habitat and fauna, and such a stream is better able to handle surges during storm fluctuations. The absorption of this energy by bends protects the stream from excessive erosion and flooding and shelters benthic macroinvertebrates and fish.

A well-defined bend will usually have a point bar at the inside and a cut bank on the outside of the bend with flow directed toward the cut-bank side. Eddy currents are usually present in these bends. Moderately defined stream bends have somewhat less sinuosity, and the bends and point bars are not as well developed.

Poorly defined bends have almost no sinuosity or are straight as in channelized streams. In some situations stream-bend development can be evaluated from topographical maps.

The speed of water flow depends on several factors, including the angle of the bed slope, the roughness of the bed, the depth of the water, and the type of geologic materials the stream flows through. For example, streams flowing through soft soils tend to meander more and have less velocity than streams flowing through hard erosion-resistant rock. Generally, if the stream meanders a great deal, the stream's gradient is probably low.

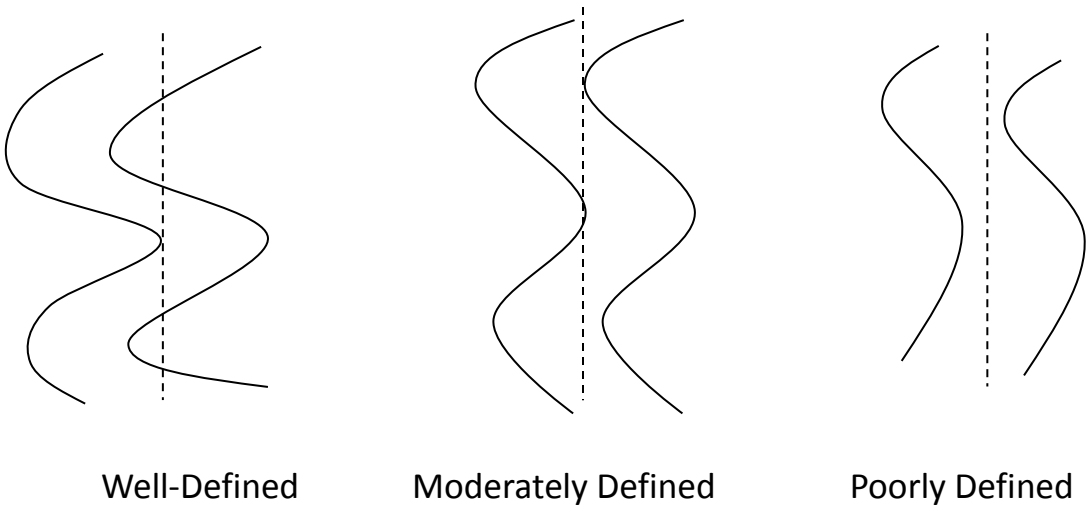


Figure 9.5. Stream bends.

Channel Obstructions or Modifications

Indicate observed channel obstructions such as fences, log jams, culverts, and low water bridges. Also indicate any channel modifications such as channelization, levees, concrete lining, or riprap within the reach; note whether these modifications are natural or artificial.

Water Level

The water level is the degree to which water covers the entire available channel substrate, from bank to bank. It is not to be confused with "flow severity" referred to in Volume 1 (RG-415) or measured discharge. Flow severity is a visual assessment of the amount of flowing water in the channel relative to base flow or normal flow conditions. Water level represents the percentage of substrate that is covered with water throughout the reach, or conversely, the percentage of substrate that is exposed in the channel. This attribute is a measure of how much of the potential habitat is available to aquatic organisms based on the amount of water in the channel at the time of assessment. When water does not cover much of the stream bed, the amount of substrate available for aquatic organisms is limited relative to times when water level is higher. For example, if the true channel is 20 m wide and water only fills 10 m of the channel, the channel flow status will be reported as "low," with water filling 25 to 75 percent of the available channel. This observation is especially useful for interpreting biological information under low-flow conditions.

Estimate the percentage of water in the available channel and the amount of substrate exposed as—

High: Water reaches the base of both banks. Very little (less than 5 percent), if any, of the channel substrate is exposed.

Moderate: Water fills more than 75 percent of the available channel, or less than 25 percent of channel substrate is exposed.

Low: Water fills 25 to 75 percent of the available channel and riffle substrates are mostly exposed.

Dry: Very little water in the channel—mostly present as standing pools—or the stream is dry.

Stream Width

Stream width is the horizontal distance along the transect line from water's edge to water's edge along the existing water surface. It is also referred to as the wetted width.

Measure the width of the water in the stream channel from water's edge to water's edge at a transect. The water's edge is the point where stream materials, such as rocks, are no longer surrounded by water. Record this width in meters.

Remember that stream width is only the wetted width, whereas channel flow status looks at the entire available channel, bank to bank.

Stream Depths at Points across the Transect

Stream depth is the vertical height of the water column from the existing water surface level to the channel bottom.

Measure the water depth in meters at 11 equally spaced points across each transect for wadable streams, beginning and ending with the depth at the water's edge. For streams less than 1.5 m wide or greater than 11 m wide, measure as many depths as will adequately profile the channel substrate. Also locate the *thalweg,* or deepest portion of the channel, and measure its depth. Indicate the thalweg depth as a separate depth measurement in the area labeled "Thalweg Depth" on Part I.

For non-wadable streams, measure the water depth at 11 or more equally spaced points across each transect. Locate and measure the depth of the thalweg as for wadable streams. In non-wadable streams, best professional judgment will be required to determine how many depth measurements to make; the number chosen must adequately profile the stream channel bottom at that transect. The number of depth measurements must increase as the stream bottom becomes more irregular.

Tertiary Attributes—The Riparian Environment

The riparian environment is defined as follows.

Riparian Zone

The *riparian zone* can be defined in many ways, but it is generally considered to be the area from the stream bank out onto the flood plain. The limit of the zone depends on many factors including plant community, soil moisture, and distance from the stream. It also depends on the limit of interaction between land and stream processes. The riparian zone is periodically inundated by floodwaters from the stream. Interaction with this terrestrial zone is vital for the health of the stream.

Natural Vegetative Buffer

Natural vegetative buffer refers to an area of either natural or native vegetation that buffers the water body from terrestrial runoff and human activities. In natural areas, it may be much wider

than the riparian zone. In human-altered settings, the natural vegetative buffer limit is at the point of human influence in the riparian zone, such as a road, parking lot, pasture, or crop field. It is the width of this buffer that the TCEQ is most interested in measuring for qualifying potential stream disturbances.

Aesthetics

Circle the descriptor that most adequately describes the reach as a whole. Make only one selection.

Wilderness (1): The surrounding landscape has outstanding natural beauty. Usually wooded or unpastured areas typical of what would be found in a wilderness area such as in a national forest or preserve. There is no evidence of human alterations to landscape. Water clarity **may** be exceptional.

Natural area (2): Trees or native vegetation (or both) are common. Some development or human alteration to the landscape may be evident, but is usually minimal. Could include fields, pastures, or rural dwellings.

Common setting (3): The landscape and stream are fairly altered by humans, but the alteration is not offensive. Could include an urban park setting.

Offensive (4): The stream does not enhance the aesthetics of the landscape. It is littered with trash, highly developed, or a dumping area. Water **may** be discolored or very turbid.

Riparian Vegetation (Percent)

Indicate the percentage of riparian vegetation types on each bank located in the riparian zone. If no plants exist in the riparian zone, indicate this by recording 100 percent in "other."

Bank Slope (Bank Angle)

Measure the slope of each bank at the transect with a clinometer and a survey rod or pole. Place one end of the survey rod at the water's edge and lay it on the ground perpendicular to the stream channel along the bank and pointed toward the top of the first main terrace. Lay the clinometer on top of the survey rod and record the angle reading. Refer to Figure 9.6 for bank-angle measurements. The clinometer can only measure angles less than 90°.

During low-flow conditions, the water's edge may recede from the true bank, revealing part of the stream bottom as the apparent bank. In these instances, measure the slope of this apparent bank from the water's edge.

A *vertical bank* has a bank angle of 90°. If the vertical portion of the bank is ≥ 0.3 m, record only the vertical measurement. If the vertical portion of the bank is < 0.3 m, measure the vertical portion of the bank as well as the angle at the top of the vertical section and average the readings. Record the average as the bank angle.

A *gently sloping bank* has a bank angle of < 90° and can be read directly off the clinometer.

For banks greater than 90° (*undercut banks*), place a survey rod flush against the roof of the undercut bank and in as far as possible. Turn the clinometer over, take the reading, and subtract it from 180°.

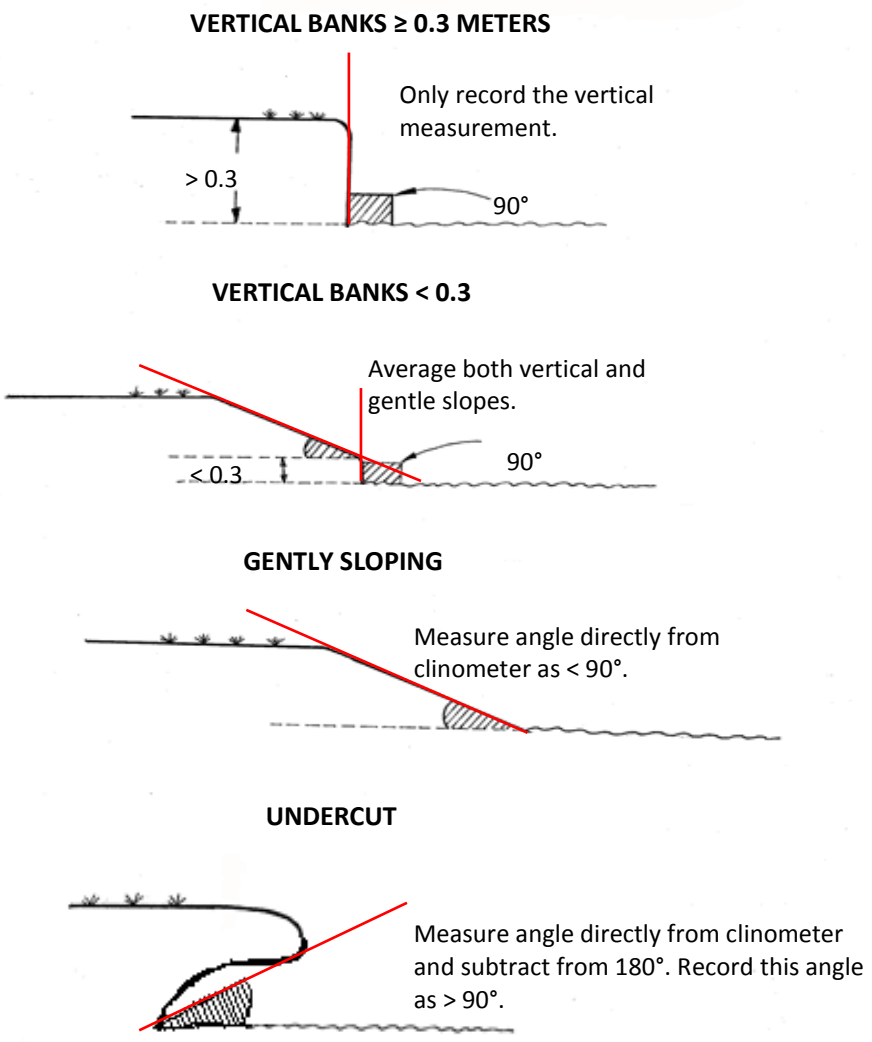


Figure 9.6. Bank-angle measurements.

If the bank is very irregular in shape or has many small, intermediate terraces, take several bank-angle readings at each elevation break and average the readings, or, if the irregularities are fairly small, lay the survey rod across the irregularities and take one average bank angle reading. Record the average as the bank angle.

Measure both left and right banks and record those angles separately.

Bank Erosion

Estimate the percentage of the areas of the stream bank that shows evidence of or potential for erosion. Assess each bank separately, up to the first terrace within the transect area—along and 3 m on either side of the transect. Record an estimate for each bank on the form. The range is as follows—

- 100 percent: totally bare, unconsolidated soil not stabilized by roots
- 0 percent: totally covered by thick vegetation or hard rock, such as a canyon wall

Tree Canopy

Tree canopy is the uppermost spreading, branching layer of stream-side trees that shades the water surface. Tree canopy is reported as percent cover and is measured with a densiometer. Tree canopy is an indicator measurement of stream corridor health and level of disturbance. The possible measurement range is from 0 percent (totally open canopy cover) to 100 percent (totally closed). See Figure 9.7. Measure the amount of tree canopy cover with a **convex** spherical densiometer along the transect line at mid-channel, once facing the left bank and once facing the right bank. Make two additional measurements along the transect line at the water's edge, once facing the left bank and once facing the right bank.

Use the following method for marking and reading a convex densiometer.

- With a black permanent fine-tipped marker, mark the densiometer, as shown in Figure 9.7, so that 17 grid intersections are located above the marked lines. Measure canopy cover by holding the densiometer level 0.3 m above the surface of the water.
- The observer's face must be kept from reflecting in the grids of the mirror. While concentrating on the 17 points of intersection, the observer then counts the number of intersections that are covered by reflected canopy cover. In this example, the densiometer reading would be 10.

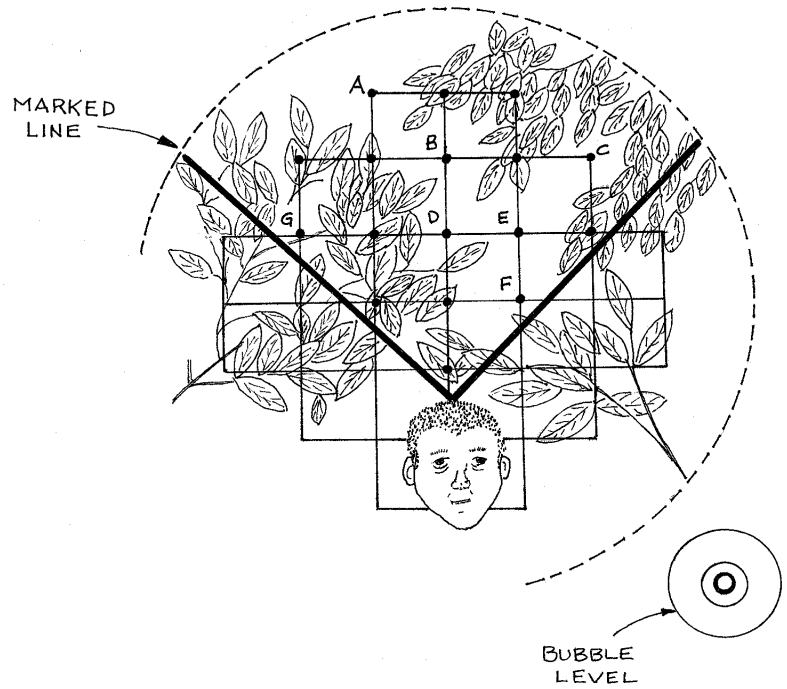


Figure 9.7. Convex spherical densiometer diagram. (From Mulvey et al., 1992.)

- Two densiometer readings are taken at midstream, one facing the left bank and one facing the right bank. Two additional readings are taken at the water's edge, one facing the left bank and one facing the right bank. The four readings are averaged and the percentage calculated. For example, if the average of the four densiometer readings is 9, the reported percent tree canopy would be:

  $9 \div 17 = 0.53$ or 53 percent

- Range: no trees, totally open = 0 percent
  large trees providing total shading = 100 percent

Dominant Types of Riparian Vegetation

Indicate the types of riparian vegetation observed within 3 m either side of the transect (oak trees, sunflowers, Bermuda grass) for each bank. Record this information for each bank separately. If a bank contains no riparian vegetation, indicate this by describing the conditions, such as a paved parking lot up to the edge of the stream bank.

Width of Natural Buffer Vegetation

Measure the width in meters of the natural vegetative buffer on each bank. This can be performed with a hip chain, a measuring tape, or an optical range finder. If the buffer is greater than 20 m, simply indicate "> 20 m" on the form.

General Observations

After finishing the transect measurements, complete the "general observation" portion of the worksheet. Count the number of riffles throughout the evaluated reach. Record the width and maximum depth, in meters, of the largest pool in the reach, if applicable. Also note the number and quality of bends in the reach.

At an appropriate location within the stream reach, measure streamflow. See Volume 1, Chapter 3, for details.

Photograph the stream reach from mid-channel, facing upstream and downstream. Ideally, take photographs at each transect from mid-channel facing the left bank, the right bank, upstream, and downstream.

Part II—Summary of Physical Characteristics of Water Body

Once the field worksheet (Part I) has been completed, summarize the measurements on the summary sheet (Part II) in preparation for calculating the habitat metrics. Use information from all transects and measurements in Part I, as well as from other sources, to complete this form. This summary is used primarily to calculate the habitat metrics but is also used in other areas of biological assessment, such as determining appropriate ALUs. The parameter codes for each habitat descriptor are listed in parentheses after each descriptor heading.

Streambed Slope

Using a USGS topographic map of the reach, measure the **change in elevation** between the first contour line crossing the stream upstream of the upstream reach boundary and the first contour line crossing the stream downstream of the downstream reach boundary. Convert to meters.

Divide this by the **length of the stream reach** in meters from Part I. Multiply by 1,000 to get m/km.

Example: 10 ft / 250 m = 3.048 m / 250 m × 1000 = 12.192 (1 ft = 0.3048 m)

For low-gradient streams or for short reach lengths, the reach may fall between two contour lines (see Figure 9.8). In these instances, determine the slope over the entire interval between the two contour lines that encompass the reach and assign that slope to the reach.

Drainage Area

Using GIS (or possibly either a USGS topographic map or a quarter-scale county highway map and a planimeter) determine the drainage area upstream of the furthest downstream transect. Record this area in square kilometers.

Stream Order

Using a USGS topographic map with a scale of 1 : 24,000, determine the stream-order classification. The smallest unbranched tributaries of a drainage basin (intermittent or perennial on the map) are designated *first-order streams*. Where two first-order streams join, a second-order stream is formed; where two second-order streams join, a third order stream is formed; and so on. Figure 9.9 depicts a typical stream-order pattern.

Length of Stream Evaluated

From Part I. Record it in meters.

Number of Lateral Transects Made

Record the number of transects measured in the stream reach. There will be anywhere from five to 11 transects, depending on the length of the reach.

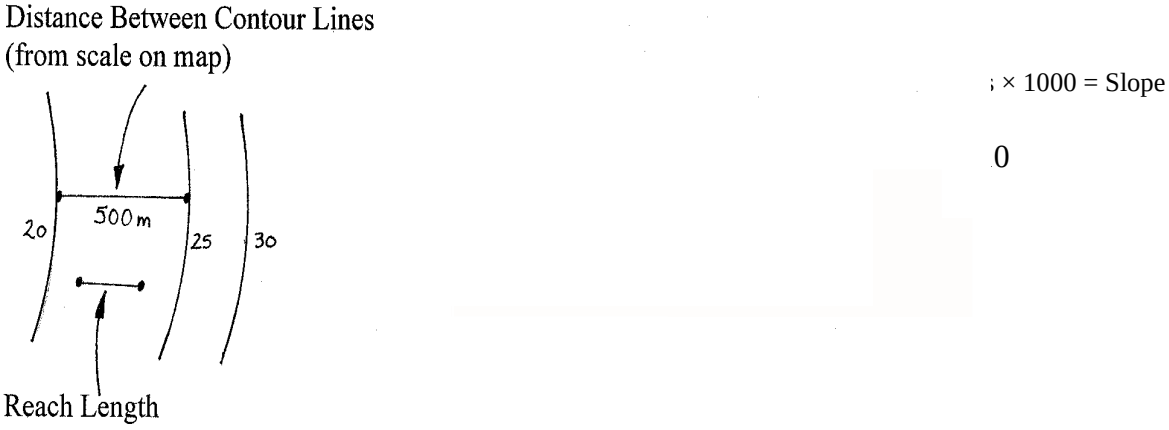


Figure 9.8. Streambed slope.

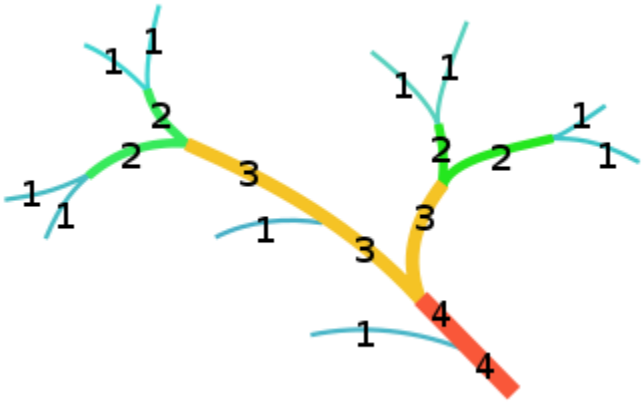


Figure 9.9. Stream order.

Average Stream Width

Average the stream width measurements from all transects. Record in meters.

Average Stream Depth

Average the individual stream depth measurements from all transects. For example, if there were five transects, with 10 depth measurements at each transect, calculate the average from all 50 individual depth measurements. Record it in meters.

Stream Discharge

Record the measured streamflow in the reach on the same day the transect measurements are made. It is preferable to measure flow in the field even if there is a USGS streamflow gauge nearby. Record in ft^3/sec.

Flow Measurement Method

Indicate the type of equipment used to measure flow.

Channel Flow Status

Record high, moderate, low, or no flow from Part I.

Maximum Pool Width

Record, in meters, the maximum width of the largest pool encountered in the reach. This is usually done when making general observations on the walk back after the last transect measurement.

Maximum Pool Depth

Record, in meters, the maximum depth of the largest pool encountered in the reach. This is usually done when making general observations on the walk back after the last transect measurement.

Total Number of Stream Bends

Record the sum of the following three sub-categories from Part I: well-defined, moderately defined, and poorly defined. These are usually tallied during the general observations. Additionally, record the number of bends in each bend category:

- well-defined bends
- moderately defined bends
- poorly defined bends

Total Number of Riffles

Record the number of riffles from Part I. These are usually tallied during general observations.

Dominant Substrate Type

Record the dominant substrate type from all transects in the reach. For example, if six transects were measured and four listed "sand" as the dominant substrate type and two listed "gravel" as the dominant substrate type, then "sand" would be recorded as the dominant type for the reach on Part II. If there is an even number of two types, use professional judgment to determine the most prevalent type.

Average Percent of Substrate Gravel-Sized or Larger

Average all percent gravel numbers recorded for each transect from Part I. Record as a percentage.

Average Percent Instream Cover

Average all percent instream cover numbers recorded for each transect from Part I. Record the average as a percentage.

Number of Instream Cover Types

Total the number of different types of instream cover such as macrophytes, gravel, snags, artificial, etc.

Average Percent Stream Bank Erosion Potential

Average the individual percent stream bank erosion determinations from all transects. For example, if five transects were made, and a left- and right-bank percent erosion was determined at each transect, the average is calculated from all 10 individual percent stream bank erosion numbers. Record the average as a percentage.

Average Stream-Bank Slope

Average the individual stream bank angle measurements from all transects. For example, if five transects were made, and a left and right bank-angle measurement was made at each transect, calculate the average from all 10 individual bank angle measurements. Record the average in degrees.

Average Width of Natural Buffer Vegetation

First, determine the minimum natural buffer vegetation width at each transect. Next, average the minimum widths for all transects in the reach.

Average Riparian Vegetation Percent Composition

Average the left and right bank determinations made in Part I for each category of vegetation type. For example, if the percent trees were 65 percent on the left bank and 40 percent on the right bank, record 52 percent for total percent trees. The total of all vegetation types equals 100 percent. Record average percent vegetation type as follows:

- average percent trees as riparian vegetation
- average percent shrubs as riparian vegetation
- average percent grasses as riparian vegetation
- average percent cultivated fields as riparian vegetation
- average percent other as riparian vegetation

Average Percent Tree Canopy Coverage

Average the individual percent tree canopy coverage measurements from all transects and record that value.

Overall Aesthetic Appraisal of the Stream

Record your assessment from Part I.

Part III—Habitat Quality Index

After completing the form summarizing physical characteristics of the water body (Part II), complete the HQI form (Part III) and calculate a total habitat score for the stream. Use the values from Part II and any field notes to score each metric. For example, if the average percent instream cover from Part II was 50 percent, the available instream cover metric would score a 3 as common. Once all metrics are scored individually, calculate the total score by adding all individual scores. The assigned habitat assessment category based on the HQI is as follows:

26–31	Exceptional
20–25	High
14–19	Intermediate
≤ 13	Limited

Assessing the Habitat of Non-Wadable Rivers and Streams

Streams are considered non-wadable if water depth in the stream channel prohibits wading and requires use of a flotation device (boat or tube) during normal flow conditions. Generally, these are streams of the fourth order or larger and are usually considered rivers. Riffle areas or low

flow may render the stream accessible to wading in certain places or at certain times; however, the stream will still be considered non-wadable when determining reach length.

Determine the stream reach using GIS tools before heading into the field or by boating the stream for several kilometers to locate the areas where biological collections will be made. Determine an average stream width during this initial reconnaissance.

The reach length of a non-wadable stream should include one full meander of the stream channel, if possible, and two examples each of at least two types of geomorphic channel units.

The minimum reach length for a non-wadable stream is 500 m and the maximum length is 1 km. On some rivers, one full meander may be longer than 1 km. In other rivers, the channel may be dominated by only one geomorphic unit, such as a glide. In these cases, limit the reach length to 1 km with as many different types of geomorphic units represented as possible.

Non-Wadable Streams

For reach lengths of 500 m to 1 km, place six to 11 evenly spaced transects over the reach length and include the reach boundaries as transects. Select an appropriate number of transects no more than 100 m apart.

Assessing the Habitats of Lakes and Reservoirs

At this time guidance is limited on assessing the physical habitats of lakes and reservoirs for regulatory purposes. The HQI is designed for freshwater streams. Some of the HQI are not applicable to lakes and reservoirs. As habitat assessments become an important part of assessing biological integrity, a uniform approach to assessing the habitat of lakes and reservoirs will need to be developed.

Preliminary work has begun to determine what habitat attributes are important for reservoirs. Some of the attributes being studied include aquatic macrophyte coverage, shoreline habitat, human disturbance, and volumetric surveys. The EPA has a field operation manual for environmental monitoring and assessment for lakes (U.S. EPA 1997) and a guide to lake and reservoir bioassessment and biocriteria (U.S. EPA 1998) that provides guidance on how to conduct habitat assessment for Texas reservoirs.

Assessing the Habitat of Tidal Streams and Estuaries

There are no standardized guidelines for evaluating habitat in Texas tidal streams and estuaries. A recommended resource for habitat evaluation in larger (non-wadable) tidal streams is Section 6 of the EPA's EMAP protocol for non-wadable streams (U.S. EPA 2000).

CHAPTER 10

BIOLOGICAL ASSESSMENT REPORTING REQUIREMENTS AND DATA MANAGEMENT

Biological Data Reporting

Whenever biological data are collected, field measurements and comments must be reported for that station on that day. This information is used to characterize the conditions in the water body at the time of collection.

Table 10.1 outlines the required and recommended data requirements for biological assessments. **The requirements include both data collection and data evaluation components.**

Table 10.1. Summary of data requirements.

Data Type	**ALM**	**ALA**	**RWA**	**ALUAA**
Field multiprobe parameters	X	X	X	X
Diel (24-hour) measurements	X	X		X
Routine water chemistry samples		X		X
Flow measurement (in non-tidal streams) and observations	X	X	X	X
Fish survey	X	X	X	X
Benthic macroinvertebrate survey	X	X	X	X
Survey of stream physical habitat	X	X	X	X
Field notes (copied pages of field data logbook)	X	X	X	X
Latitude and longitude coordinates	X	X	X	X
Forms	X	X	X	X
Color photographs	X	X	X	X
Biological Data Summary Packet (AAs must also include a report following the ALUAA report outline in Appendix C)	X	X	X	X

X = Required

Managing Biological Data

Data Handling

Transcription of data into electronic format creates a high possibility of error. Each phase of data generation and handling must have routine independent checks made on 10 percent of the data. Data from biological samples must be rechecked after data entry to ensure correct transcription. The TCEQ will supply a standard format for submission of data. This format will be outlined in the *SWQM DMRG.* Collectors must ensure that they submit all data necessary to calculate multi-metric indices.

Contracting and Institutional Standards

Data collectors will employ desktop QA methods to ensure identifications are correct. Those submitting biological data must check samples against known distributional information to determine if out-of-range organisms were identified. Primary sources include Hubbs et al. (1991) and Lee et al. (1980). The TCEQ will scrutinize all new citations and will require appropriate vouchers to ensure proper identification. If the out-of-range determinations prove incorrect upon review, then the TCEQ will review the collection for other similar species and those rechecked.

If a sample fails desktop QC checks and the error is uncorrectable, the data are invalid and the TCEQ will not accept them. Possible consequences of failing desktop QA include requiring resampling, more frequent QA visits, and the TCEQ withholding contractor payment.

Submitting Biological Data

Submit data associated with a biological sampling event (for fish, macroinvertebrates, habitat, water chemistry, or field data) electronically according to standard procedures described in this guide and in the *SWQM DMRG*. This includes the sample and results electronic flat files, as well as digital photos and PDFs of other records resulting from the biological monitoring (scanned logbooks, field-data worksheets, forms, and other records that can be converted to electronic images). Detailed information on reporting biological data is located in the *SWQM DMRG*. Additionally, hard copies of the data must be submitted as prescribed in the Biological Data Summary Packet in Appendix C.

CHAPTER 11

QUALITY ASSURANCE AND QUALITY CONTROL

Biological monitoring programs that contribute data to the TCEQ must conform to all QA measures outlined in this chapter and all QC measures outlined in the biological monitoring chapters of this document. These measures were developed with freshwater streams and rivers in mind, but may be modified to address other systems. Quality assurance of biological monitoring programs is accomplished through a number of measures, including a program's participation in technical systems audits (TSAs), in both the field and the laboratory, and by a TCEQ-approved QAPP.

All TCEQ regional biological-monitoring projects will require a separate QAP that details the unique aspects of the project. A QAP shell document appears in the SWQM QAPP. Types of biological-monitoring projects are detailed in Chapter 2.

Technical Systems Audits

The TCEQ conducts TSAs on agency regional SWQM personnel conducting biological monitoring and on contracted organizations collecting biological data as resources allow. If the TCEQ determines that a TSA is needed, it will conduct the TSA separately from a TSA on other monitoring activities, such as routine monitoring. If the TCEQ determines that a TSA is needed for a CRP partner or other cooperator, a TSA is performed during that organization's contract period and may or may not be separate from other TSAs. TSAs consist of both field and laboratory audits and include inspection of records kept on file at the offices of the organization submitting biological data.

Biological-Sample Records

Records that must be maintained and that must be kept available for inspection during a TSA include:

- Field notes containing the sampling station location and number, date and time of collection, details of collections including the area and duration of sampling, raw counts of specimens collected, and photographs of any large specimens released after identification.
- A sample-tracking logbook that details the event and sample information. Assign each sample a unique sample tracking number, such as *BM 020 14* for 'benthic macroinvertebrate number 020, year 2014.'
- Laboratory identification notes and bench sheets. Each sheet must contain the label information, unique sample tracking number from the logbook, the date of identification, the name of the identifier, the scientific name for each taxon, the number of individuals in each taxon, and other comments that may pertain to identification.
- Appropriately labeled sample and voucher specimen jars. The sample label must contain the information required in the appropriate chapter of this manual for a biological specimen type.

- Final counts of organisms reported on the basis of individuals per unit area, volume, or sampling effort.
- Raw data used to produce final counts that serve as evidence of the method of calculation. These records include:
    - sampling station location and number
    - date and time of day of collection
    - information on volume, area, effort and duration of the sampling
    - raw counts used in the calculation of reported values
    - verification that the data have been entered into a database or sent to the TCEQ SWQM Team

Training

Training in all aspects of biological monitoring takes place every few years and will be available to TCEQ personnel and other cooperators furnishing biological or habitat data to the TCEQ. These trainings are a significant part of the QA for the biological monitoring program and will be required regardless of level of expertise. Even experienced field biologists will be required to attend periodic trainings to ensure they are practicing current methodologies. In place of a major training event, employees or contractors may participate in a biological-monitoring event with experienced personnel.

Approval of Deviation from Methods

Biological collection methods for wadable streams are documented in this manual and any variation from those sampling protocols must be approved in advance by the TCEQ and detailed in a QAPP or QAP. It is imperative that monitoring initiatives on water bodies without prescribed protocols, such as reservoirs or tidal streams, be discussed at the beginning of study plan development with either the TCEQ SWQM Team or WQSG staff or with the TPWD. To ensure rigorous and skillful implementation of the procedures in this manual, the TCEQ (with assistance from the TPWD) will conduct TSAs of personnel involved in the collection of biological data.

Tracking Samples

Proper sample custody is a joint effort of the sampling crew, the sample transporter, and the laboratory staff (including sorters, pickers, and those performing taxa identification). The sampling crew places biological samples and the identifying labels in jars with screw-top lids. This label is the main sample documentation and is written in waterproof ink or pencil and placed in the jar. The laboratory staff is responsible for keeping this label with the sample and replacing it if damaged. The sample label includes the following information.

- county
- river basin
- stream name

- station ID or location of nearest landmark (for example, a road crossing)
- time and date of collection
- name of each collector
- collection methods
- type of preservative used

Sample-Tracking Log

Maintain a *sample-tracking logbook* that contains the following for each sample. Log this information immediately upon returning to the lab.

- unique sample tracking number
- name of person logging information
- name of each collector
- location of collection
- date of collection
- date entered in log
- date identification and enumeration began

After completing the log entries, inspect the sample label to ensure that it is in good condition and legible, and includes the following information.

- name of each collector
- station location
- station number, if applicable
- date and time of collection
- collection method
- preservative

Note: Replace the label if deterioration is obvious.

Laboratory Bench Sheet

When identification and sorting begins, handle the collections individually, working only on one sample at a time. Maintain a **laboratory bench sheet** for each sample that contains, at a minimum, the following information.

- sample number from tracking log
- name of identifier
- location of collection
- date of collection

- date entered in log
- date identification and enumeration began
- date identification and enumeration ended
- scientific name for each taxon in sample
- number of individuals in each taxon
- ID qualifiers (difficulties)

Voucher-Specimen Vials

Consistent with guidelines for voucher specimens found in previous chapters, maintain a **separate vial** for each taxon in the sample. Each vial may contain multiple specimens of the same taxon. Preserve specimens in 70 percent ethanol or isopropyl alcohol. Each vial must contain a label that includes the following information.

- name of each collector
- name of each identifier
- station location
- station number, if applicable
- date and time of collection
- collection method
- preservative
- scientific name of taxon contained in vial

When slide mounts of specimens (or parts of specimens) are needed to complete identification using a compound microscope, the slides must be labeled with the scientific name of the taxon, the initials of the identifier, and the sample tracking log number.

General Quality Assurance

To minimize misidentification of biological samples, the following steps are mandatory:

Vouchers

Retain voucher specimens of all species of fishes, benthic macroinvertebrates, algae samples, and permanent diatom slides for a minimum of five years or until the applicable regulatory decision is made (whichever is longer).

Voucher specimens serve as long-term physical proof that confirm 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.

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 experienced in the specific area of taxonomy required

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.

Confirmation Checks

Confirmation checks of species identification and distribution may be performed as an aspect of biological QA. If samples checked against known distributional information determine that the species in question was collected outside the known range where it would be expected to occur, then the collection should be reviewed and rechecked for other similar species. Where a species' identification is in question, the collecting organization may send specimens to agency experts at either the TCEQ or the TPWD for confirmation.

Fish

Identification of Fish-Assemblage Samples

Fish-assemblage samples must be identified and counted by personnel trained in taxonomy and familiar with appropriate keys and literature. The validity of identifications affects the quality of community analyses and, frequently, the ALU designated for a stream.

Appropriate equipment must be available for laboratory determinations of biological specimens, including a dissecting microscope, an assortment of probes, dividers, a ruler, forceps, and appropriate taxonomic references. For identifying Texas freshwater fishes, the primary reference is Hubbs et al. (1991), with supplemental sources as needed.

Retention and Preservation of Fishes

Large, easily identified fishes may be counted in the field after all collection activity at a sampling location has been completed. This will necessitate maintaining the fishes in some type of holding bucket or tank with adequate aeration. Retain small fishes for positive identification in the laboratory. The standard preservative is 10 percent formalin. Place specimens in this preservative while still alive; those that die before preservation normally do not retain distinctive markings. Do not crowd fishes into bottles, as the preservation will not be adequate. Slit larger specimens on the right side of the abdominal cavity to allow proper preservation. Each field container must include an internal label that includes the date, collection locality, the name of each collector, and the sampling method. This paper must be of high rag content and notations must be in pencil or waterproof ink.

Equipment Requirements

Before identification and counting, specimens are transferred to 70 percent ethanol or 45 percent isopropyl alcohol in the laboratory. See Chapters 3 and 4 for details of laboratory samples. Proper identification and counting of fish requires, at a minimum, the following equipment.

- stereo dissecting microscope, total magnification variable 7× to 30×; recommended 7× to 110×
- jeweler's forceps
- petri dishes
- preservative—70 percent ethanol or 45 percent isopropyl alcohol
- ruler

Taxonomic Keys

Required References

The following taxonomic references are required for identifying fish.

Freshwater

Hubbs, C., R.J. Edwards, and G.P. Garrett. 1991. An annotated checklist of the freshwater fishes of Texas, with keys to identification of species. *Tex. J. of Sci.* 43(4):1–56.

Saltwater

Hoese, H.D., and R.H. Moore. 1998. Fishes of the Gulf of Mexico—Texas, Louisiana, and Adjacent Waters. College Station: Texas A&M University Press.

Supplemental References

The following taxonomic references are recommended supplements.

Freshwater

Douglas, N.H. 1974. Freshwater Fishes of Louisiana. Baton Rouge, LA: Claitor's Publishing Division.

Hubbs, C., et al., eds. 1994. Freshwater and Marine Fishes of Texas and the Northwestern Gulf of Mexico. Austin: Texas System of Natural Laboratories.

Kuehne, R.A. and R.W. Barbour. 1983. The American Darters. University Press of Kentucky.

Lee, D.S., et al. 1980. Atlas of North American Fresh Water Fishes. North Carolina Biological Survey publication no. 1980-12. Raleigh: North Carolina State Museum of Natural History.

McGowan, N., R.J. Kemp, Jr. and R. McCune. 1971. Freshwater Fishes of Texas. Bulletin 5-A. Austin: Texas Parks and Wildlife Department.

Miller, R.J., and H.W. Robinson. 1973. The Fishes of Oklahoma. Stillwater: Oklahoma State University Press.

Nelson, J.S., et al. 2004. Common and Scientific Names of Fishes from the United States, Canada, and Mexico. Special Publication 29. Bethesda, MD: American Fisheries Society.

Page, L.M., and B.M. Burr. 1991. A Field Guide to Freshwater Fishes. Peterson Field Guide Series. Boston: Houghton Mifflin.

Pflieger, W.L. 1975. The Fishes of Missouri. Jefferson City: Missouri Department of Conservation.

Robison, H.W., and T.M. Buchanan. 1988. Fishes of Arkansas. Fayetteville: University of Arkansas Press.

Sublette, J.E., M.D. Hatch, and M. Sublette. 1990. The Fishes of New Mexico. Albuquerque: University of New Mexico Press.

Thomas, C., T.H. Bonner, and B.G. Whiteside, 2007. Freshwater Fishes of Texas. College Station: Texas A&M University Press.

Tomelleri, J.R., and M.E. Eberle. 1990. Fishes of the Central United States. Lawrence: University Press of Kansas.

Saltwater

Hubbs, C., et al. 1994. Freshwater and Marine Fishes of Texas and the Northwestern Gulf of Mexico. Austin: Texas System of Natural Laboratories.

Murdy, E.O. 1995. Saltwater Fishes of Texas. A Dichotomous Key. TAMU-SG-83-607. College Station: Texas A&M University Sea Grant College Program.

Shipp, R.L. 1999. Dr. Bob Shipp's Guide to the Fishes of the Gulf of Mexico. Mobile, AL: KME Seabooks.

Benthic Macroinvertebrates

Benthic macroinvertebrates must be identified and counted by persons with appropriate expertise, training, and knowledge of the literature.

Identifying and counting benthic macroinvertebrates must be consistent among samples. The taxonomic expertise of the identifier must, at a minimum, be adequate to allow identification of all specimens to the appropriate taxonomic level identified in Chapter 5.

Equipment Requirements

Proper identification and enumeration of benthic macroinvertebrates requires, at a minimum, the following equipment.

- stereo dissecting microscope, total magnification variable 7× to 30×; recommended 7× to 110×
- stereo compound microscope, total magnification 400×
- jeweler's forceps
- petri dishes

- preservative: 70 percent ethanol or 70 percent isopropyl alcohol
- microscope slides

Taxonomic Keys

Required References

The following taxonomic references are required for identifying benthic macroinvertebrates.

Freshwater

Merrit, R.W., and K.W. Cummins, eds. 2008. An Introduction to the Aquatic Insects of North America. 4th ed. Dubuque, IA: Kendall/Hunt.

Pennak, R.W. 1989. Freshwater Invertebrates of the United States: Protozoa to Mollusca. 3rd ed. New York: John Wiley and Sons.

Thorpe, J.H., and A.P. Covich, eds. 1991. Ecology and Classification of North American Freshwater Invertebrates. New York: Academic Press.

U.S. EPA. 1982. Freshwater Snails (Mollusca: Gastropoda) of North America. EPA-600/3/82/026. Washington: U.S. Environmental Protection Agency.

Saltwater

Andrews, J. 1977. Shells and Shores of Texas. Austin: University of Texas Press.

Fauchald, K. 1977. The Polychaete Worms: Definitions and Keys to the Orders, Families, and Genera. Science Series no. 28. Natural History Museum of Los Angeles.

Gosner, K.L. 1971. Guide to the Identification of Marine and Estuarine Invertebrates. New York: Wiley-Interscience.

Uebelaker, J.M., and P.G. Johnson, eds. 1984. Taxonomic Guide to the Polychaetes of the Northern Gulf of Mexico. 7 volumes. Metairie, LA: Mineral Management Services.

Williams, A.B. 1984. Shrimps, Lobsters and Crabs of the Atlantic Coast of the Eastern United States, Maine to Florida. Washington: Smithsonian Institution Press.

Supplemental References

The following taxonomic references are recommended as supplements.

Freshwater

Brigham, A.R., W.U. Brigham, and A. Gnilka. 1983. Aquatic Insects and Oligochaetes of North and South Carolina. Mahomet, IL: Midwest Aquatic Enterprises.

Edmondson, W.T., ed. 1959. Ward and Whipple's Fresh-Water Biology. 2nd ed. New York: John Wiley and Sons.

McCafferty, W.P. 1983. Aquatic Entomology. Boston: Jones and Bartlett.

Usinger, R.L., ed. 1968. Aquatic Insects of California. Berkeley and Los Angeles: University of California Press.

Saltwater

Abbott, R.T. 1974. American Seashells. 2nd ed. New York: Van Nostrand Reinhold.

Barnes, R.D. 1987. Invertebrate Zoology. 5th ed. New York: CBS College Publishing.

Farfante, I.P. 1988. Illustrated Key to Penaeoid Shrimps of Commerce in the Americas. NMFS 64. Springfield, VA: National Oceanic and Atmospheric Association Report.

Williams, Austin B. 1965. Marine Decapod Crustaceans of the Carolinas. *U.S. Fish and Wildlife Service Fishery Bulletin* 65(1): 1–298.

Wood, Carl E. 1974. Key to the Natantia (Crustacea, Decapoda) of the coastal waters on the Texas coast. *Contributions in Marine Science* 18: 35–56.

Benthic Algae and Plankton

Algae and plankton samples must be identified by persons with proper expertise, training, and knowledge of the literature. Identification must reach, at a minimum, the genus level for non-diatom algae and the species level for diatoms.

Equipment Requirements

Proper identification and enumeration of benthic algae or plankton requires, at a minimum, the following equipment.

- binocular compound microscope; 10× oculars with 10× to 100× (oil-immersion) objectives
- microscope slides and cover slips
- mounting media for permanent diatom slides
- hot plate for preparing permanent diatom slides
- diatom pencil for circling taxa on slides for vouchers

Taxonomic Keys

Required References

The following taxonomic references are required for identifying benthic algae and plankton.

Freshwater

Prescott, G.W. 1978. How to Know the Freshwater Algae. 3rd ed. Dubuque, IA: Wm. C. Brown.

Patrick, R., and C.W. Reimer. 1966, 1975. The Diatoms of the United States, exclusive of Alaska and Hawaii. Monograph no. 13, vols. 1 and 2. Academy of Natural Sciences of Philadelphia.

Saltwater

Tomas, C.R. 1997. Identifying Marine Phytoplankton. San Diego: Academic Press.

Supplemental References

The following taxonomic references are recommended as supplements.

Freshwater

Dillard, G.E. 1989–93. Freshwater Algae of the Southeastern United States. Parts 1–6. Bibliotheca Phycologica. Stuttgart, Germany: Cramer.

Krammer, K., and H. Lange-Bertalot. 1986–91. Susswasserflora von Mitteleuropa. Band 2. Parts 1–5. Bacillariophycea. Stuttgart, Germany: Gustav Fischer Verlag.

Prescott, G.W. 1962. The Algae of the Western Great Lakes Area. Dubuque, IA: Wm. C. Brown.

Wehr, J.D., and R.G. Sheath. 2002. Freshwater Algae of North America: Ecology and Classification. Waltham, MA: Academic Press.

Whitford, L.A, and G.J. Schumacher. 1973. A Manual of Fresh-Water Algae. Raleigh, NC: Sparks Press.

Aquatic Macrophytes

Taxonomic Keys

Required References

The following taxonomic references are required for identifying aquatic macrophytes.

Freshwater

Prescott, G.W. 1969. How to Know the Aquatic Plants. Dubuque, IA: Wm. C. Brown.

Riemer, D.N. 1984. Introduction to Freshwater Vegetation. New York: Van Nostrand Reinhold.

Tarver, D.P., et al. 1986. Aquatic and Wetland Plants of Florida. 3rd ed. Tallahassee: Bureau of Aquatic Plant Research and Control, Florida Department of Natural Resources.

Saltwater

Hotchkiss, N. 1972. Common Marsh Plants of the United States and Canada. New York: Dover Publications.

Stutzenbaker, C. D. 1999. Aquatic and Wetland Plants of the Western Gulf Coast. Austin: University of Texas Press.

Tarver, D.P., et al. 1986. Aquatic and Wetland Plants of Florida. 3rd ed. Tallahassee: Bureau of Aquatic Plant Research and Control, Florida Department of Natural Resources.

APPENDIX A

EQUIPMENT AND MATERIALS

Flow Measurement

Chapter 3, Volume 1 (RG-415)

- flow meter
- top-setting wading rod
- 100 ft tape measure (marked in tenths of a foot)
- hip and chest waders
- calculator
- forms for recording flow
- stakes or posts

Fish Sampling

Chapters 3 and 4

- TPWD Scientific Collection Permit
- boat-mounted electrofisher
- backpack electrofisher—Smith-Root Type VII or equivalent (for waters where conductivity allows use) and extra battery
- non-conductive dip nets (both medium and small mesh)
- battery charger
- seines (30' or 15' × 6' × ¼" mesh, 15' or 6' × 6' × $^{3}/_{16}$" mesh, and 6' × 6' × ⅛" mesh)
- seines 10', 15', or 30' long with $^{3}/_{16}$" mesh (height and length are based on site requirements)
- experimental gill nets (graduated mesh sizes)
- two holding buckets or tanks with aerators—one for use in boat or in stream while sampling, and one for maintaining fish for processing
- 5-gallon plastic buckets
- fish-measuring board
- 1 L plastic wide-mouth containers with screw-top lids
- preservative—10% formalin and 70% ethanol
- sample-labeling materials
- electrical-safety gloves

- chest waders for electrofishing
- personal flotation device
- fish-identification manuals for field and laboratory identification; see Chapter 11
- stereo dissecting microscope
- trawl
- scale
- data-recording form(s)

Freshwater Benthic-Macroinvertebrate Sampling

Chapters 5 and 6

- D-frame kicknet (mesh size 595 µm) or Surber sampler
- sorting trays and subsampling mechanism (for example, a Mason-jar lid)
- screen sieves—U.S. std. sieve no. 30, 595 µm
- jeweler's forceps
- magnifying glass
- petri dishes
- wide-mouth sample jars and 2-dram vials
- preservatives—70 percent ethanol and 10 percent formalin
- sample-labeling material
- hip and chest waders
- manuals for field and laboratory identification of freshwater benthic macroinvertebrates; see Chapter 11
- stereo dissecting microscope
- compound binocular microscope —10× and 15× eyepieces; 4×, 10×, 20×, and 45× objectives
- lopping shears for snag samples
- Surber sampler
- Ekman dredge
- data-recording form(s)

Marine Benthic-Macroinvertebrate Sampling

Chapter 6

- dredges—Ekman (soft sediment); Ponar or Van Deen (shell or sand)
- small bucket or pan (narcotizing sample)

- wide-mouth jars; 500 mL
- screen sieve bucket—U.S. std. sieve no. 30 (mesh size ≤ 595 µm) or no. 35 (mesh size = 500 µm)
- 0.5mm sieve
- preservatives—magnesium chloride; Rose Bengal; borax; 70 percent ethanol or isopropyl alcohol; 10 percent formalin
- compound binocular microscope —10× and 15× eyepieces; 4×, 10×, 20×, and 45× objectives
- stereo dissecting microscope
- manuals for field and laboratory identification of marine benthic macroinvertebrates; see Chapter 11
- forceps
- shallow white pan
- light magnifier (2×)
- small vials

Benthic-Algae Sampling

Chapter 7

- pocketknife or other scraping device
- pipettes
- sample-collection jars—60 mL, glass, snap-on caps
- preservative—glutaraldehyde or formalin
- compound microscope
- glass slides and coverslips
- hot plate
- mounting media
- data recording form(s)

Plankton Sampling

Chapter 8

- plankton net
- sample jars
- Lugol's solution
- compound binocular microscope—10× and 15× eyepieces; 4×, 10×, 20×, and 45× objectives
- glass slides and coverslips

- Sedgewick-Rafter counting chamber
- data-recording form(s)

Measurements of Physical Stream Habitat

Chapter 9

- 50 m tape measure, minimum
- clinometer with degrees and percent
- convex densiometer
- range finder
- metric survey rod
- hip and chest waders
- metric rangefinder or hip chain
- survey tape or flags
- flotation tube, small boat
- metric tag line or sturdy rope
- metal stakes or fence posts
- mallet
- data recording form(s)

Other Equipment

- field logbook and pencil
- GPS equipment (real-time correction mode capabilities preferred)
- 7.5-minute-series topographic maps for sampling area
- digital camera or camera with film
- Volume 1 of *SWQM Procedures* (RG-415) for water chemistry, flow measurement, field parameters (including 24-hour DO), and tissue sampling
- laboratory forms for submitting water-chemistry samples
- winch
- long rope

APPENDIX B

REFERENCE MATERIALS AND CRITERIA FOR BIOLOGICAL ASSESSMENTS

Table B.1. Estimated downstream distance of domestic-discharge impact on dissolved oxygen.

Permitted Effluent Flow (mgd)	Extent of Downstream Impact on Dissolved Oxygen (km)[a]
0–0.05	1.0
0.05–0.10	1.2
0.10–0.20	1.6
0.20–0.50	1.8
0.50–1.0	3.2
1.0–2.0	4.4
2.0–3.5	4.6
3.5–5.0	5.2
5.0–7.5	8.0
7.5–10.0	9.6
10.0–15.0	12.4
15.0–20.0	14.8
20.0–40.0	24.6

[a] Twice the estimated distance, based on a default QUAL-TX water quality simulation model with no site-specific information.

Table B.2. Dissolved-oxygen criteria, mg/L [30 TAC 307.7(b)(3)(A)(I)].

Aquatic-Life-Use Subcategory	**Freshwater Mean, Minimum**	**Springtime Freshwater Mean, Minimum**
Exceptional	6.0, 4.0	6.0, 5.0
High	5.0, 3.0	5.5, 4.5
Intermediate	4.0, 3.0	5.0, 4.0
Limited	3.0, 2.0	4.0, 3.0

- Apply dissolved-oxygen means as a minimum average over a 24-hour period.
- Daily minima are not to extend beyond 8 hours per 24-hour day. Lower dissolved-oxygen minima may apply at a specific site when natural daily fluctuations below the mean are greater than the difference between the mean and minima of the appropriate criteria.
- Apply springtime criteria to protect fish-spawning periods during that portion of the first half of the year, when water temperatures are 63.0°F to 73.0°F.
- Quantitative criteria to support aquatic-life attributes are described in *Procedures to Implement the Texas Surface Water Quality Standards*, RG-194, January 2003.
- Dissolved-oxygen analyses and computer models to establish effluent limits for permitted discharges are normally applied to mean criteria at steady-state, critical conditions.
- Determination of standards attainment for dissolved-oxygen criteria is specified in 30 TAC 307.9(e)(6).

Table B.3. Metrics for Ecoregion 24.

	Metric	Scoring Criteria		
1	**Total Number of Fish Species**	Use the graph in Figure B.1 to determine the score based on the basin size compared to the number of fish species collected (species richness)		
	Score_____	**5**	**3**	**1**
2	**Number of Native Cyprinid Species**	If there are > 4 species present	If there are 3–4 species present	If there are < 3 species present
	Score_____	**5**	**3**	**1**
3	**Number of Benthic Invertivore Species**	If there are > 1 species present	If there is 1 species present	If there are 0 species present
	Score_____	**5**	**3**	**1**
4	**Number of Sunfish Species**	If there are > 1 species present	If there is 1 species present	If there are 0 species present
	Score_____	**5**	**3**	**1**
5	**Number of Intolerant Species**	If there are > 1 species present	If there is 1 species present	If there are 0 species present
	Score_____	**5**	**3**	**1**
6	**% Individuals as Tolerant Species (excluding western mosquitofish)**	< 26 % tolerant species	26–50% tolerant species	> 50% tolerant species
	Score_____	**5**	**3**	**1**
7	**% of Individuals as Omnivores**	< 9 % omnivore species	9–16% omnivore species	> 16% omnivore species
	Score_____	**5**	**3**	**1**
8	**% of Individuals as Invertivores**	> 65 % invertivore species	33–65% invertivore species	< 33% invertivore species
	Score_____	**5**	**3**	**1**
9	**Number of Individuals in Sample**	**A. Number of individuals / seine haul**		
		> 160.4 individuals	80.2–160.4 individuals	< 80.2 individuals
		5	**3**	**1**
		B. Number of individuals / minutes of electrofishing		
	Score_____	> 26.5 individuals	13.3–26.5 individuals	< 13.32 individuals
		5	**3**	**1**
10	**% of Individuals as Non-Native Species**	< 1.4% non-native species	9–16% non-native species	> 16% non-native species
	Score_____	**5**	**3**	**1**
11	**% of Individuals with Disease or Other Anomaly**	< 9% diseased species	9–16% diseased species	> 16% diseased species
	Score_____	**5**	**3**	**1**

Aquatic-life use: ≥ 43, exceptional; 37–42, high; 35–36, intermediate; < 35 limited.

Figure B.1.

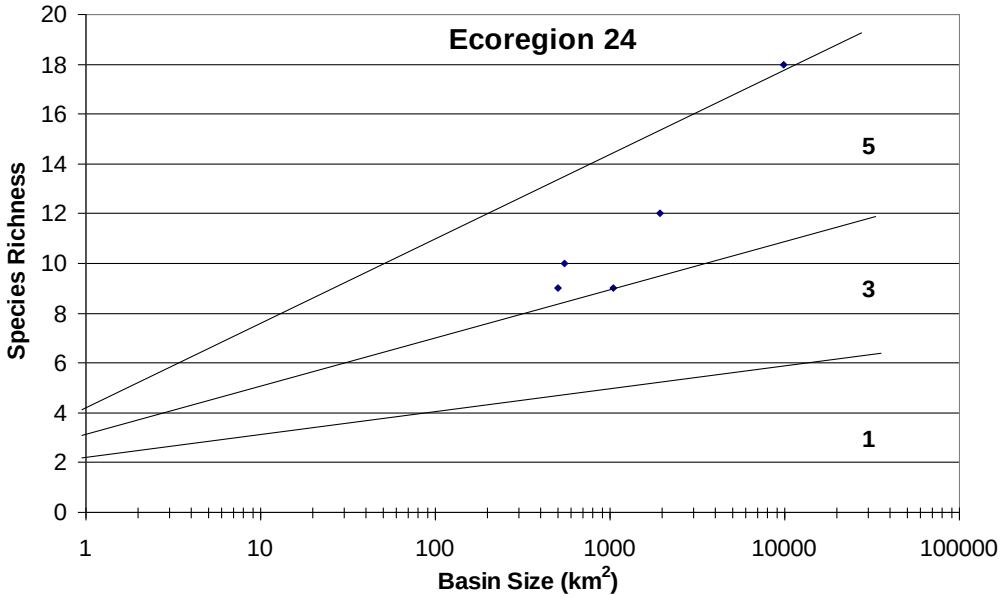

Ecoregion 24
Species Richness
Basin Size (km2)
5
3
1
0
2
4
6
8
10
12
14
16
18
20
1
10
100
1000
10000
100000

Table B.4. Metrics for Ecoregions 25 and 26.

Metric		**Scoring Criteria**		
		5	**3**	**1**
1	Total number of fish species	See Figure B.2		
2	Number of native cyprinid species	> 2	2	< 2
3	Number of sunfish species	> 1	1	0
4	% of individuals as omnivores	< 9	9–16	> 16
5	% of individuals as invertivores	> 65	33–65	< 33
6	Number of individuals/seine haul	> 41.7	20.9–41.7	< 20.9
7	% of individuals as non-native species	< 1.4	1.4–2.7	> 2.7
8	% of individuals with disease or other anomaly	< 0.6	0.6–1.0	> 1.0

Aquatic-life use: ≥ 36, exceptional; 34–35, high; 24–33, intermediate; < 24 limited.

Figure B.2.

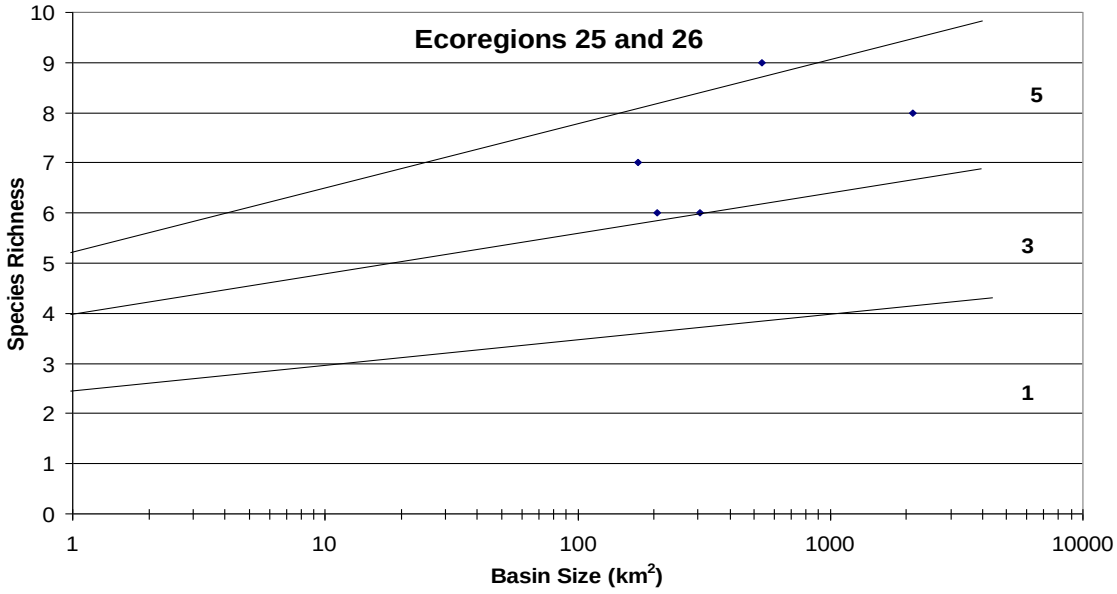

Ecoregions 25 and 26
Species Richness
Basin Size (km2)
5
3
1
0
1
2
3
4
5
6
7
8
9
10
1
10
100
1000
10000

Table B.5. Metrics for Ecoregions 27, 29, and 32.

Metric		**Scoring Criteria**		
		5	**3**	**1**
1	Total number of fish species	See Figure B.3		
2	Number of native cyprinid species	> 3	2–3	< 2
3	Number of benthic invertivore species	> 1	1	0
4	Number of sunfish species	> 3	2–3	< 2
5	% of individuals as tolerant species (excluding western mosquitofish)	< 26	26–50	> 50
6	% of individuals as omnivores	< 9	9–16	> 16
7	% of individuals as invertivores	> 65	33–65	< 33
8	% of individuals as piscivores	> 9	5–9	< 5
9	Number of individuals in sample			
	a. Number of individuals / seine haul	> 87	36–87	< 36
	b. Number of individuals / minute electrofishing	> 7.1	3.3–7.1	< 3.3
10	% of individuals as non-native species	< 1.4	1.4–2.7	> 2.7
11	% of individuals with disease or other anomaly	< 0.6	0.6–1.0	> 1.0

Aquatic-life use: ≥ 49, exceptional; 41–48, high; 35–40, intermediate; < 35, limited.

Figure B.3.

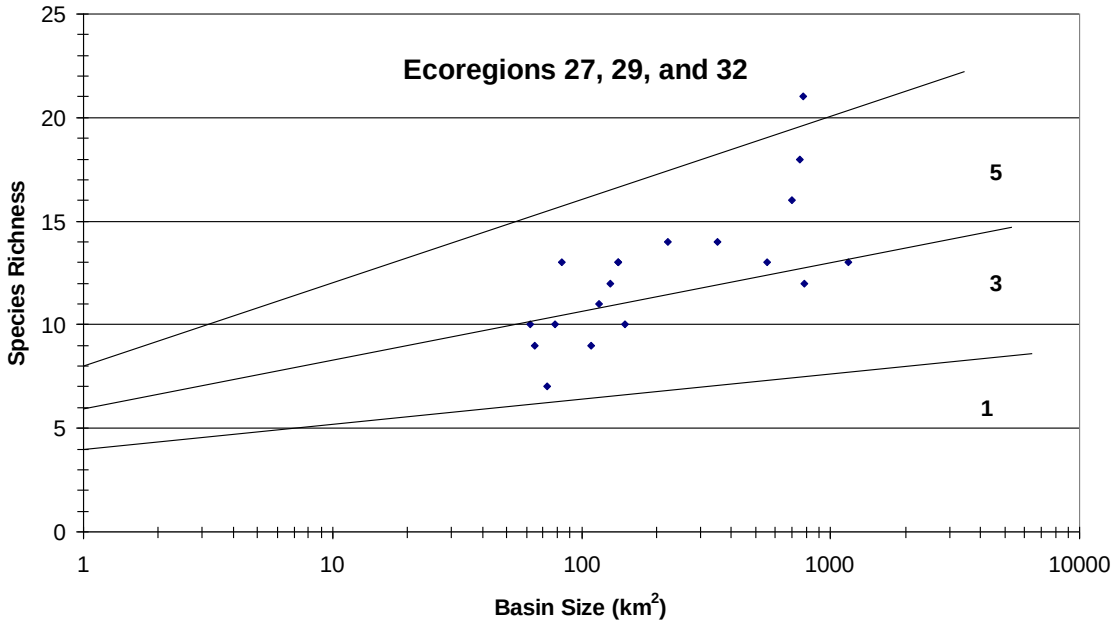

Ecoregions 27, 29, and 32
Species Richness
Basin Size (km2)
25
20
15
10
5
0
1
10
100
1000
10000
5
3
1

Table B.6. Metrics for Ecoregion 30.

Metric		**Scoring Criteria**		
		5	**3**	**1**
1	Total number of fish species	See Figure B.4		
2	Number of native cyprinid species	> 4	3–4	< 3
3	Number of benthic invertivore species	> 1	1	0
4	Number of sunfish species	> 3	2–3	< 2
5	Number of intolerant species	> 1	1	0
6	% of individuals as tolerant species (excluding western mosquitofish)	< 26	26–50	> 50
7	% of individuals as omnivores	< 9	9–16	> 16
8	% of individuals as invertivores	> 65	33–65	< 33
9	% of individuals as piscivores	> 8	3.9–8.0	< 3.9
10	Number of individuals in sample			
	a. Number of individuals / seine haul	> 48	37–48	< 37
	b. Number of individuals / minute electrofishing	> 5	2.5–5	< 2.5
11	% of individuals as non-native species	< 1.4	1.4–2.7	> 2.7
12	% of individuals with disease or other anomaly	< 0.6	0.6–1.0	> 1.0

Aquatic-life use: ≥ 52, exceptional; 42–51, high; 30–41, intermediate; < 30, limited.

Figure B.4.

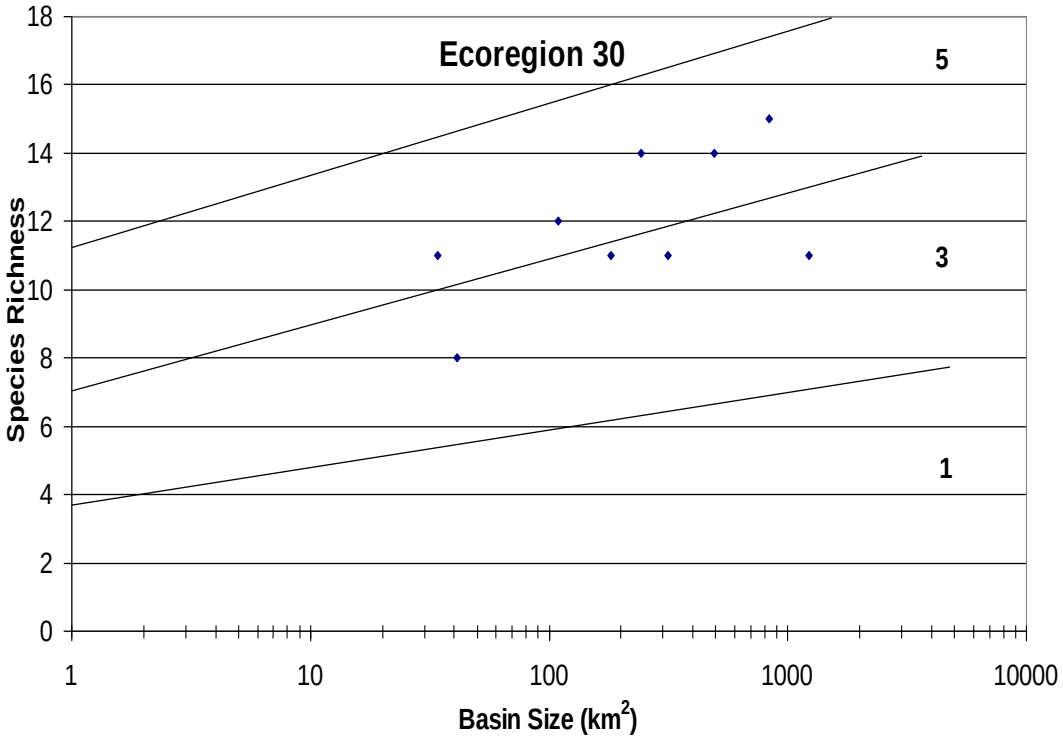

Ecoregion 30
5
3
1
Species Richness
18
16
14
12
10
8
6
4
2
0
1
10
100
1000
10000
Basin Size (km2)

Table B.7. Metrics for Ecoregion 31.

Metric		**Scoring Criteria**		
		5	**3**	**1**
1	Total number of fish species	See Figure B.5		
2	Number of native cyprinid species	> 5	3–5	< 3
3	Number of benthic species (catfish, suckers, and darters)	> 2	2	< 2
4	Number of sunfish species	> 4	3–4	< 3
5	% of individuals as tolerant species (excluding western mosquitofish)	< 26	26–50	> 50
6	% of individuals as omnivores	< 9	9–16	> 16
7	% of individuals as invertivores	> 65	33–65	< 33
8	% of individuals as piscivores	> 9	5–9	< 5
9	Number of individuals in sample			
	a. Number of individuals / seine haul	> 39.5	19.7–39.5	< 19.7
	b. Number of individuals / minute electrofishing	> 8.9	4.4–8.9	< 4.4
10	% of individuals as non-native species	< 1.4	1.4–2.7	> 2.7
11	% of individuals with disease or other anomaly	< 0.6	0.6–1.0	> 1.0

Aquatic-life use: ≥ 42, exceptional; 37–41, high; 25–36, intermediate; < 25, limited.

Figure B.5.

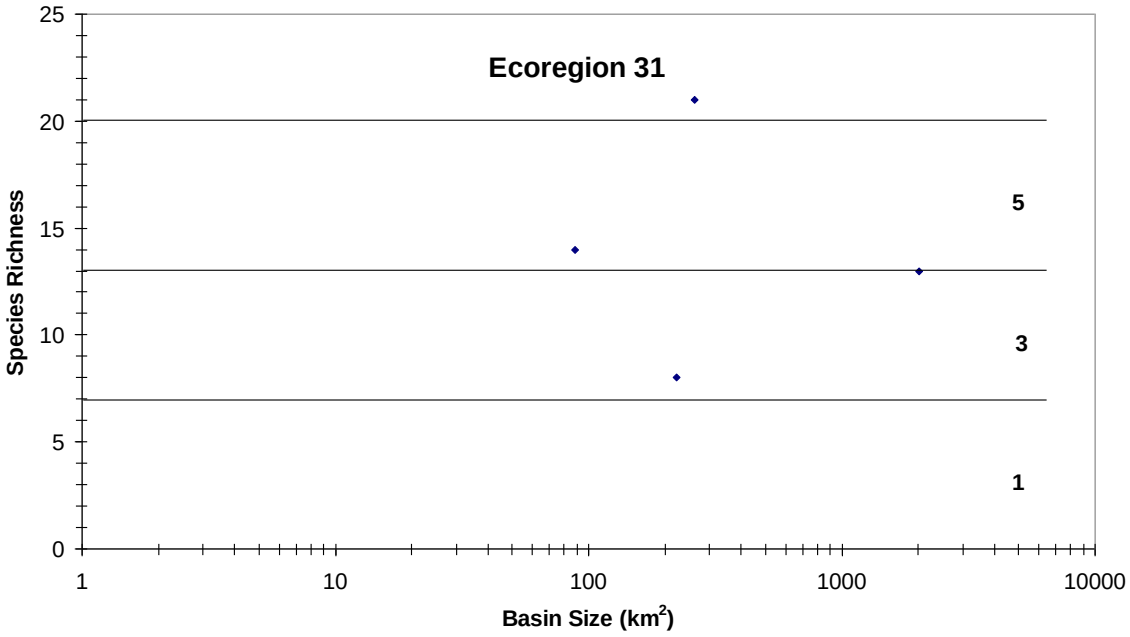

Ecoregion 31
Species Richness
25
20
15
10
5
0
5
3
1
1
10
100
1000
10000
Basin Size (km2)

Table B.8. Metrics for Ecoregions 33 and 35.

Metric		**Scoring Criteria**		
		5	**3**	**1**
1	Total number of fish species	See Figure B.6		
2	Number of native cyprinid species	> 4	2–4	< 2
3	Number of benthic invertivore species	> 4	3–4	< 3
4	Number of sunfish species	> 4	3–4	< 3
5	Number of intolerant species	> 3	2–3	< 2
6	% of individuals as tolerant species (excluding western mosquitofish)	< 26	26–50	> 50
7	% of individuals as omnivores	< 9	9–16	> 16
8	% of individuals as invertivores	> 65	33–65	< 33
9	% of individuals as piscivores	> 9	5–9	< 5
10	Number of individuals in sample			
	a. Number of individuals / seine haul	> 28	14–28	< 14
	b. Number of individuals / minute electrofishing	> 7.3	3.9–7.3	< 3.6
11	% of individuals as non-native species	< 1.4	1.4–2.7	> 2.7
12	% of individuals with disease or other anomaly	< 0.6	0.6–1.0	> 1.0

Aquatic-life use: ≥ 52, exceptional; 42–51, high; 36–41, intermediate; < 36, limited.

Figure B.6.

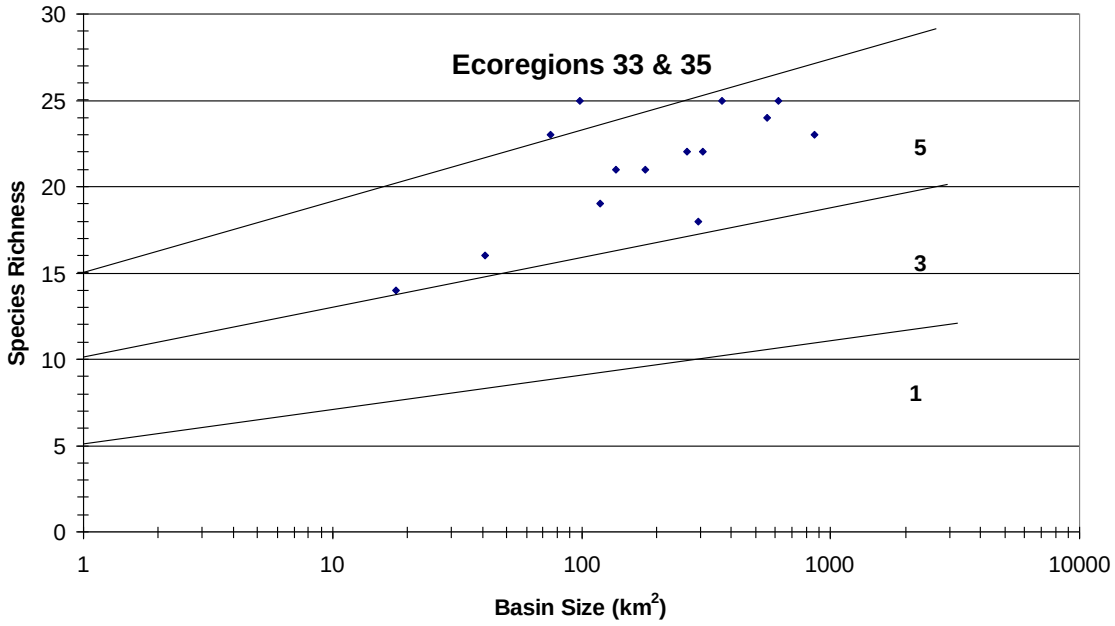

Ecoregions 33 & 35
Species Richness
Basin Size (km2)
30
25
20
15
10
5
0
1
10
100
1000
10000
5
3
1

Table B.9. Metrics for Ecoregion 34.

Metric		**Scoring Criteria**		
		5	**3**	**1**
1	Total number of fish species	See Figure B.7		
2	Number of native cyprinid species	> 2	2	< 2
3	Number of benthic invertivore species	> 1	1	0
4	Number of sunfish species	> 3	2–3	< 2
5	Number of intolerant species	≥ 1	—	0
6	% of individuals as tolerant species (excluding western mosquitofish)	< 26%	26–50%	> 50%
7	% of individuals as omnivores	< 9%	9–16%	> 16%
8	% of individuals as invertivores	> 65%	33–65%	< 33%
9	Number of individuals in sample			
	a. Number of individuals / seine haul	> 174.7	87.4–174.7	< 87.4
	b. Number of individuals / minute electrofishing	> 7.7	3.9–7.7	< 3.9
10	% of individuals as non-native species	< 1.4%	1.4–2.7%	> 2.7%
11	% of individuals with disease or other anomaly	< 0.6%	0.6–1.0%	> 1.0%

Aquatic-life use: ≥ 49, exceptional; 39–48, high; 31–38, intermediate; < 31, limited.

Figure B.7.

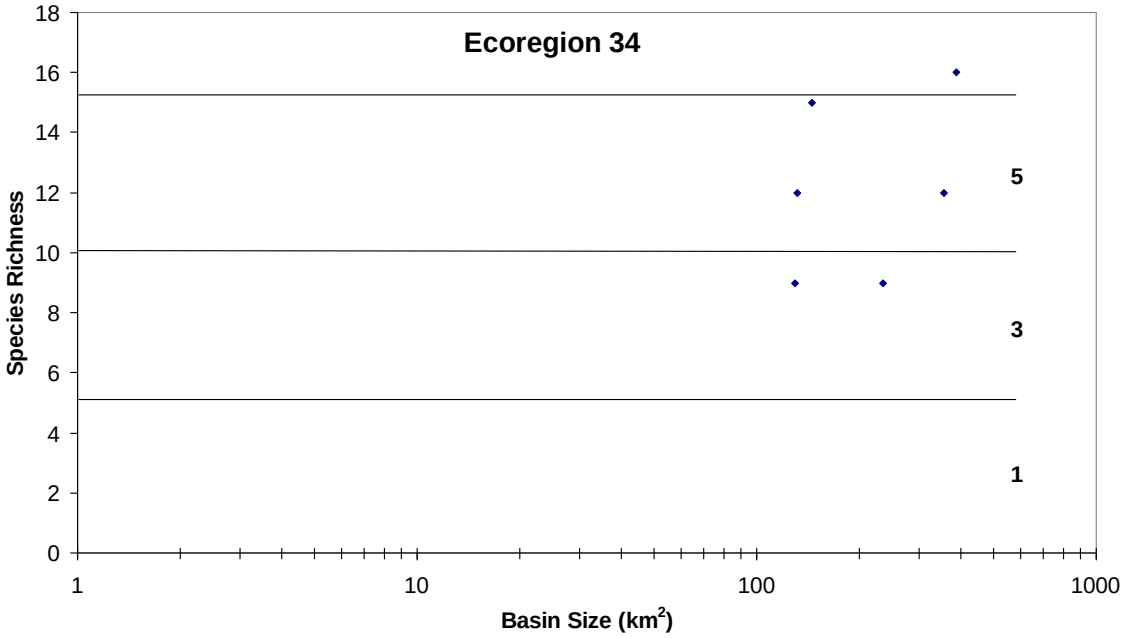

Ecoregion 34
Species Richness
Basin Size (km2)
18
16
14
12
10
8
6
4
2
0
1
10
100
1000
5
3
1

Figure B.8. Map of Texas Level IV ecoregions.

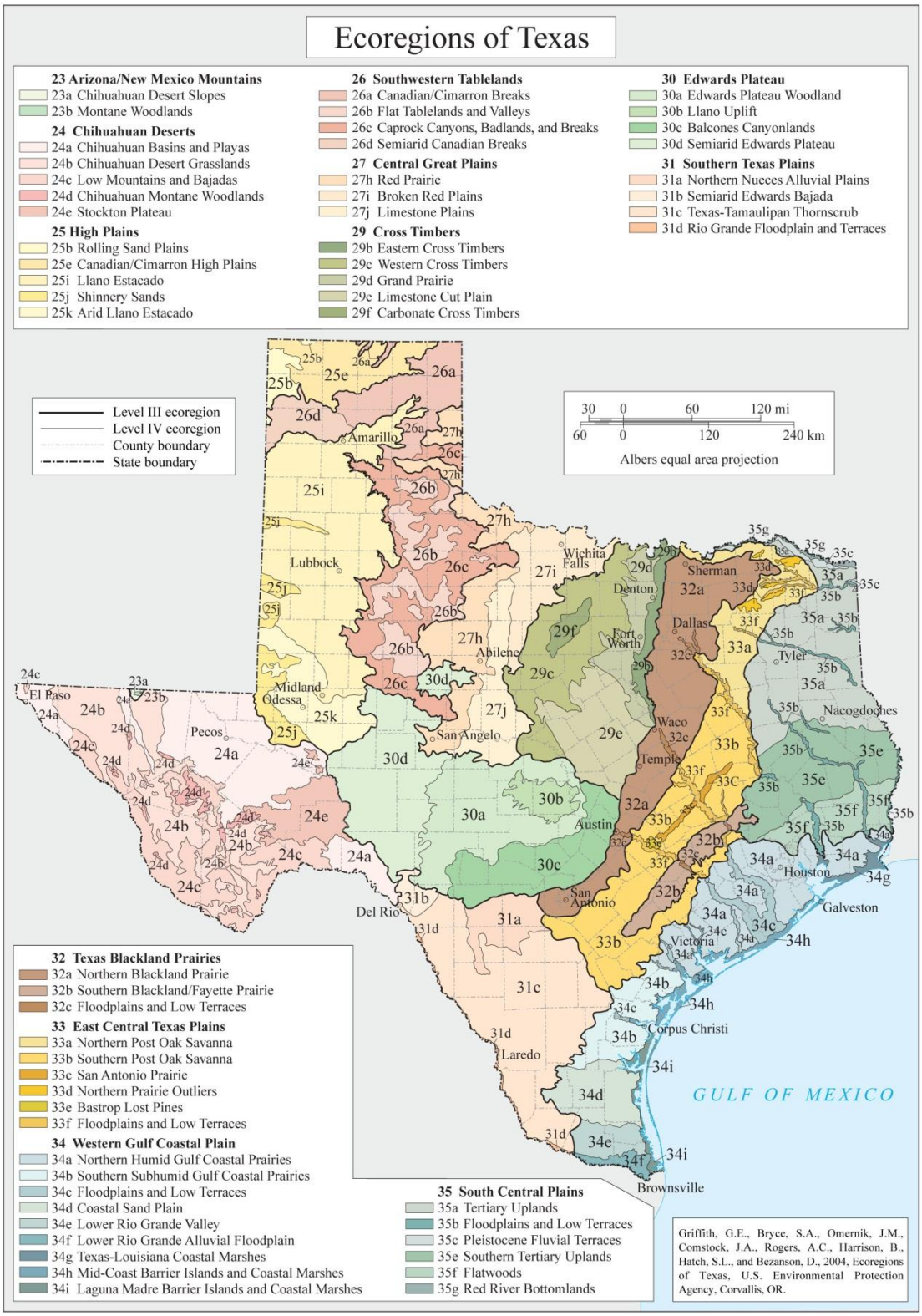


Table B.10. Classification of Texas freshwater fishes into trophic and tolerance groups. Adapted from G.W. Linam, and L.J. Kleinsasser (1998).

Trophic-group designations: IF—invertivore; P—piscivore; O—omnivore; and H—herbivore. Tolerance designations: T—tolerant; I—intolerant. Those species without a tolerance designation are considered intermediate.

Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
Paddlefishes	**Polyodontidae**			
Paddlefish	*Polyodon spathula*	98335	O	I
Gars	**Lepisosteidae**			
Spotted gar	*Lepisosteus oculatus*	98340	P	T
Longnose gar	*Lepisosteus osseus*	98341	P	T
Shortnose gar	*Lepisosteus platostomus*	98342	P	T
Alligator gar	*Atractosteus spatula*	98344	P	T
Bowfins	**Amiidae**			
Bowfin	*Amia calva*	98347	P	T
Freshwater eels	**Anguillidae**			
American eel	*Anguilla rostrata*	98361	P	
Snake eels	**Ophichthidae**			
Speckled worm eel	*Myrophis punctatus*	98388	P	
Lampreys	**Petromyzontidae**			
Chestnut lamprey	*Ichthyomyzon castaneus*	99297	P	I
Southern brook lamprey	*Ichthyomyzon gagei*	98013	None	I
Herrings	**Clupeidae**			
Skipjack herring	*Alosa chrysochloris*	98418	P	
Finescale menhaden	*Brevoortia gunteri*	98426	O	
Gizzard shad	*Dorosoma cepedianum*	98430	O	T
Threadfin shad	*Dorosoma petenense*	98429	O	
Scaled sardine	*Harengula jaguana*	98015	IF	
Minnows	**Cyprinidae**			
Central stoneroller	*Campostoma anomalum*	98502	H	
Mexican stoneroller	*Campostoma ornatum*	98503	H	
Goldfish	*Carassius auratus*	98439	O	T
Grass carp	*Ctenopharyngodon idella*	98528	H	T
Red shiner	*Cyprinella lutrensis*	98474	IF	T
Proserpine shiner	*Cyprinella proserpina*	98480	IF	
Blacktail shiner	*Cyprinella venusta*	98487	IF	
Common carp	*Cyprinus carpio*	98437	O	T
Manantial roundnose minnow	*Dionda argentosa*		O	I
Devils River minnow	*Dionda diaboli*	98490	IF	I
Roundnose minnow	*Dionda episcopa*	98491	O	I
Nueces roundnose minnow	*Dionda serena*		IF	I
Cypress minnow	*Hybognathus hayi*	98493	O	
Mississippi silvery minnow	*Hybognathus nuchalis*	98494	O	T
Plains minnow	*Hybognathus placitus*	98495	O	T
Striped shiner	*Luxilus chrysocephalus*	98432	IF	
Ribbon shiner	*Lythrurus fumeus*	98471	IF	
Redfin shiner	*Lythrurus umbratilis*	98486	IF	
Rio Grande chub	*Gila pandora*	98451	IF	I
Speckled chub	*Macrhybopsis aestivalis*	98449	IF	

Trophic-group designations: IF—invertivore; P—piscivore; O—omnivore; and H—herbivore. Tolerance designations: T—tolerant; I—intolerant. Those species without a tolerance designation are considered intermediate.

Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
Prairie chub	*Macrhybopsis australis*		IF	
Shoal chub	*Macrhybopsis hyostoma*		IF	
Burrhead chub	*Macrhybopsis marconis*		IF	
Peppered chub	*Macrhybopsis tetranema*		IF	
Silver chub	*Macrhybopsis storeriana*	98448	IF	
Golden shiner	*Notemigonus crysoleucas*	98441	IF	T
Texas shiner	*Notropis amabilis*	98459	IF	
Pallid shiner	*Hybopsis amnis*	98460	IF	
Emerald shiner	*Notropis atherinoides*	98461	IF	
Blackspot shiner	*Notropis atrocaudalis*	98462	IF	
Red River shiner	*Notropis bairdi*	98463	IF	
River shiner	*Notropis blennius*	98464	IF	
Tamaulipas shiner	*Notropis braytoni*	98465	IF	
Smalleye shiner	*Notropis buccula*	98466	IF	
Ghost shiner	*Notropis buchanani*	98467	IF	
Ironcolor shiner	*Notropis chalybaeus*	98468	IF	I
Chihuahua shiner	*Notropis chihuahua*	98469	IF	
Arkansas River shiner	*Notropis girardi*	98472	IF	
Bluehead shiner	*Pteronotropis hubbsi*	99136	IF	
Rio Grande shiner	*Notropis jemezanus*	98473	IF	
Taillight shiner	*Notropis maculatus*	98475	IF	
Sharpnose shiner	*Notropis oxyrhynchus*	98477	IF	
Chub shiner	*Notropis potteri*	98479	IF	
Sabine shiner	*Notropis sabinae*	98481	IF	
Silverband shiner	*Notropis shumardi*	98482	IF	
Sand shiner	*Notropis stramineus*	98484	IF	
Weed shiner	*Notropis texanus*	98485	IF	
Mimic shiner	*Notropis volucellus*	98488	IF	I
Pugnose minnow	*Opsopoeodus emiliae*	98452	IF	
Suckermouth minnow	*Phenacobius mirabilis*	98457	IF	
Fathead minnow	*Pimephales promelas*	98497	O	T
Bullhead minnow	*Pimephales vigilax*	98498	IF	
Flathead chub	*Platygobio gracilis*	98447	IF	
Longnose dace	*Rhinichthys cataractae*	98455	IF	
Rudd	*Scardinius erythrophthalmus*	98414	O	T
Creek chub	*Semotilus atromaculatus*	98443	P	
Suckers	**Catostomidae**			
River carpsucker	*Carpiodes carpio*	98511	O	T
Blue sucker	*Cycleptus elongatus*	98505	IF	I
Creek chub sucker	*Erimyzon oblongus*	98519	O	
Lake chubsucker	*Erimyzon sucetta*	98520	O	
Smallmouth buffalo	*Ictiobus bubalus*	98507	O	
Bigmouth buffalo	*Ictiobus cyprinellus*	98508	IF	T
Black buffalo	*Ictiobus niger*	98509	O	
Spotted sucker	*Minytrema melanops*	98517	IF	
Mexican redhorse	*Moxostoma austrinum*	98500	IF	

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Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
Gray redhorse	*Moxostoma congestum*	98513	IF	
Golden redhorse	*Moxostoma erythrurum*	98514	IF	
Blacktail redhorse	*Moxostoma poecilurum*	98515	IF	
Characins	**Characidae**			
Mexican tetra	*Astyanax mexicanus*	98435	IF	
Bullhead catfishes	**Ictaluridae**			
Black bullhead	*Ameiurus melas*	98563	O	T
Yellow bullhead	*Ameiurus natalis*	98564	O	
Blue catfish	*Ictalurus furcatus*	98562	P	
Headwater catfish	*Ictalurus lupus*	98554	O	
Channel catfish	*Ictalurus punctatus*	98561	O	T
Tadpole madtom	*Noturus gyrinus*	98574	IF	I
Freckled madtom	*Noturus nocturnus*	98575	IF	I
Flathead catfish	*Pylodictus olivaris*	98570	P	
Widemouth blindcat	*Satan eurystomus*	98572	IF	
Toothless blindcat	*Trogloglanis pattersoni*	98568	O	
Suckermouth catfishes	**Loricariidae**			
Suckermouth catfish	*Hypostomus plecostomus*	98553	H	T
Southern sailfin catfish	*Pterygoplichthys anisitsi*		H	T
Sea catfishes	**Ariidae**			
Gafftopsail catfish	*Bagre marinus*	98557	P	T
Hardhead catfish	*Ariopsis felis*	98559	IF	T
Thorny catfishes	**Doradidae**			
Southern striped Raphael	*Platydoras armatulus*			
Pikes	**Esocidae**			
Redfin pickerel	*Esox americanus*		P	
Northern pike	*Esox lucius*	98406	P	I
Chain pickerel	*Esox niger*	98405	P	
Salmons	**Salmonidae**			
Rainbow trout	*Oncorhynchus mykiss*	98527	IF-Lotic	I
		98527	P-Lentic	I
Pirate perch	**Aphredoderidae**			
Pirate perch	*Aphredoderus sayanus*	98773	IF	
Killifishes	**Cyprinodontidae**			
Leon Springs pupfish	*Cyprinodon bovinus*	98705	O	
Comanche Springs pupfish	*Cyprinodon elegans*	98706	O	
Conchos pupfish	*Cyprinodon eximius*	98707	O	
Pecos River pupfish	*Cyprinodon pecosensis*	98769	O	T
Red River pupfish	*Cyprinodon rubrofluviatilis*	98708	O	T
Sheepshead minnow	*Cyprinodon variegatus*	98709	O	T
Diamond killifish	*Adinia xenica*	98691	O	T
Western starhead topminnow	*Fundulus blairae*		IF	
Golden topminnow	*Fundulus chrysotus*	98694	IF	
Gulf killifish	*Fundulus grandis*	98695	O	
Saltmarsh topminnow	*Fundulus jenkinsi*	98696	IF	
Blackstripe topminnow	*Fundulus notatus*	98677	IF	

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Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
Blackspotted topminnow	*Fundulus olivaceus*	98678	IF	I
Bayou killifish	*Fundulus pulvereus*	98699	IF	
Longnose killifish	*Fundulus similis*	98700	O	I
Plains killifish	*Fundulus zebrinus*	98729	IF	T
Least killifish	*Heterandria formosa*	98832	IF	
Rainwater killifish	*Lucania parva*	98689	IF	
Livebearers	**Poeciliidae**			
Western mosquitofish	*Gambusia affinis*	98713	IF	T
Big Bend gambusia	*Gambusia gaigei*	98715	IF	
Largespring gambusia	*Gambusia geiseri*	98716	IF	
Clear Creek gambusia	*Gambusia heterochir*	98718	IF	
Pecos gambusia	*Gambusia nobilis*	98719	IF	
Tex-Mex gambusia	*Gambusia speciosa*		IF	
Amazon molly	*Poecilia formosa*	98725	O	
Sailfin molly	*Poecilia latipinna*	98724	O	T
Guppy	*Poecilia reticulata*	97770	IF	T
Silversides	**Atherinidae**			
Brook silverside	*Labidesthes sicculus*	98734	IF	I
Rough silverside	*Membras martinica*	98732	IF	
Inland silverside	*Menidia beryllina*	98728	IF	
Texas silverside	*Menidia clarkhubbsi*	98796	IF	
Tidewater silverside	*Menidia peninsulae*	98658	IF	
Temperate basses	**Percichthyidae**			
White bass	*Morone chrysops*	99163	P	
Yellow bass	*Morone mississippiensis*	99164	P	
Striped bass	*Morone saxatilis*	99165	P	
Sunfishes	**Centrarchidae**			
Rock bass	*Ambloplites rupestris*	99106	P	I
Flier	*Centrarchus macropterus*	99111	IF	
Banded pygmy sunfish	*Elassoma zonatum*	99113	IF	
Redbreast sunfish	*Lepomis auritus*	99093	IF	
Green sunfish	*Lepomis cyanellus*	99094	P	T
Warmouth	*Lepomis gulosus*	99095	P	T
Orangespotted sunfish	*Lepomis humilis*	99096	IF	
Bluegill	*Lepomis macrochirus*	99097	IF	T
Dollar sunfish	*Lepomis marginatus*	99098	IF	
Longear sunfish	*Lepomis megalotis*	99099	IF	
Redear sunfish	*Lepomis microlophus*	99100	IF	
Redspotted sunfish	*Lepomis miniatus*	99101	IF	
Bantam sunfish	*Lepomis symmetricus*	99102	IF	
Smallmouth bass	*Micropterus dolomieu*	99091	P	I
Spotted bass	*Micropterus punctulatus*	99089	P	
Largemouth bass	*Micropterus salmoides*	99090	P	
Guadalupe bass	*Micropterus treculii*	99086	P	I
White crappie	*Pomoxis annularis*	99108	P	
Black crappie	*Pomoxis nigromaculatus*	99109	P	

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Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
Percidae	Perches			
Western sand darter	*Ammocrypta clara*	99071	IF	
Scaly sand darter	*Ammocrypta vivax*	99072	IF	
Redspot darter	*Etheostoma artesiae*		IF	
Mud darter	*Etheostoma asprigene*	99074	IF	
Bluntnose darter	*Etheostoma chlorosoma*	99075	IF	
Fountain darter	*Etheostoma fonticola*	99076	IF	I
Swamp darter	*Etheostoma fusiforme*	99077	IF	
Slough darter	*Etheostoma gracile*	99078	IF	
Rio Grande darter	*Etheostoma grahami*	99079	IF	
Harlequin darter	*Etheostoma histrio*	99080	IF	
Greenthroat darter	*Etheostoma lepidum*	99081	IF	I
Goldstripe darter	*Etheostoma parvipinne*	99082	IF	I
Cypress darter	*Etheostoma proeliare*	99083	IF	I
Orangebelly darter	*Etheostoma radiosum*	99084	IF	I
Orangethroat darter	*Etheostoma spectabile*	99085	IF	
Yellow perch	*Perca flavescens*	99062	P	
Logperch	*Percina caprodes*	99068	IF	I
Texas logperch	*Percina carbonaria*	99060	IF	I
Bigscale logperch	*Percina macrolepida*	99069	IF	I
Blackside darter	*Percina maculata*	98540	IF	I
Dusky darter	*Percina sciera*	98541	IF	I
River darter	*Percina shumardi*	99168	IF	
Sauger	*Sander canadensis*	99057	P	I
Walleye	*Sander vitreus*	99058	P	
Drums	**Sciaenidae**			
Freshwater drum	*Aplodinotus grunniens*	98958	IF	T
Silver perch	*Bairdiella chrysoura*	98960	IF	
Sand seatrout	*Cynoscion arenarius*	98973	P	I
Spotted seatrout	*Cynoscion nebulosus*	98974	P	I
Spot	*Leiostomus xanthurus*	98964	O	
Atlantic croaker	*Micropogonias undulatus*	98968	IF	I
Black drum	*Pogonias cromis*	98970	IF	
Red drum	*Sciaenops ocellatus*	98962	P	
Cichlids	**Cichlidae**			
Rio Grande cichlid	*Cichlasoma cyanoguttatum*	98953	IF	
Blue tilapia	*Oreochromis aureus*	98583	O	T
Mozambique tilapia	*Oreochromis mossambicus*	98565	O	
Redbelly tilapia	*Tilapia zillii*	98584	O	
Sleepers	**Eleotridae**			
Fat sleeper	*Dormitator maculatus*		O	
Largescaled spinycheek sleeper	*Eleotris amblyopsis*		O	
Emerald sleeper	*Erotelis smaragdus*		IF	
Bigmouth sleeper	*Gobiomorus dormitor*		IF	
Mullets	**Mugilidae**			
Mountain mullet	*Agonostomus monticola*	98797	O	

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Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
Striped mullet	*Mugil cephalus*	98793	O	
White mullet	*Mugil curema*		O	
Requiem sharks	**Carcharhinidae**			
Fine tooth shark	*Carcharhinus isodon*	98014	P	
Bull shark	*Carcharhinus leucas*	98280	P	
Sawfishes	**Pristidae**			
Small tooth sawfish	*Pristis pectinata*	98299	P	
Stingrays	**Dasyatidae**			
Atlantic stingray	*Dasyatis sabina*	98318	IF	
Sturgeons	**Acipenseridae**			
Shovelnose sturgeon	*Scaphirynchus platorynchus*	98337	IF	
Goldeyes	**Hiodontidae**			
Goldeye	*Hiodon alosoides*	98408	IF	
Tarpons	**Elopidae**			
Ladyfish	*Elops saurus*	98352	P	
Tarpon	*Megalops atlanticus*	98356	P	T
Anchovies	**Engraulidae**			
Striped anchovy	*Anchoa hepsetus*	98410	IF	
Bay anchovy	*Anchoa mitchilli*	98412	IF	
Needlefishes	**Belonidae**			
Atlantic needlefish	*Strongylura marina*	98663	P	
Pipefishes	**Syngnathidae**			
Opposum pipefish	*Microphis brachyurus*	98857	IF	
Chain pipefish	*Syngnathus louisianae*	98757	IF	
Gulf pipefish	*Syngnathus scovelli*	98761	IF	
Snooks	**Centropomidae**			
Smallscale fat snook	*Centropomus parallelus*	98806	P	
Common snook	*Centropomus undecimalis*	98989	P	I
Jacks	**Carangidae**			
Crevalle jack	*Caranx hippos*	98900	P	I
Mojarras	**Gerreidae**			
Irish pompano	*Diapterus auratus*	99047	IF	
Spotfin mojarra	*Eucinostomus argenteus*	99044	IF	
Flagfin mojarra	*Eucinostomus melanopterus*	98578	IF	
Grunts	**Haemulidae**			
Barred grunt	*Conodon nobilis*	98993	IF	
Burro grunt	*Pomodasys crocro*		IF	
Porgies	**Sparidae**			
Sheepshead	*Archosargus probatocephalus*	99155	O	
Pinfish	*Lagodon rhomboides*	99153	O	
Threadfins	**Polynemidae**			
Atlantic threadfin	*Polydactylus octonemus*		IF	
Soles	**Soleidae**			
Hogchoker	*Trinectes maculatus*	99218	IF	
Lined sole	*Achirus lineatus*		IF	
Lefteye flounders	**Bothidae**			

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Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
Bay whiff	*Citharichthys spilopterus*		IF	
Southern flounder	*Paralichthys lethostigma*	99246	P	
Fringed flounder	*Etropus crossotus*		IF	
Gobies	**Gobiidae**			
River goby	*Awaous banana*		O	
Frillfin goby	*Bathygobius soporator*		IF	T
Darter goby	*Ctenogobius boleosoma*		O	
Mexican goby	*Ctenogobius claytonii*		O	
Freshwater goby	*Ctenogobius shufeldti*		IF	
Marked goby	*Ctenogobius stigmaticus*		O	
Lyre goby	*Evorthodus lyricus*		H	
Violet goby	*Gobioides broussonetii*		O	
Highfin goby	*Gobionellus oceanicus*		O	
Naked goby	*Gobiosoma bosc*		IF	T
Code goby	*Gobiosoma robustum*		IF	
Clown goby	*Microgobius gulosus*		IF	
Puffers	**Tetraodontidae**			
Least puffer	*Sphoeroides parvus*		IF	

Table B.11. Benthic Index of Biotic Integrity metrics and scoring criteria for kick samples, rapid bioassessment protocol—benthic macroinvertebrates (Harrison 1996).

METRIC	SCORING CRITERIA			
	4	**3**	**2**	**1**
Taxa richness	> 21	15–21	8–14	< 8
EPT taxa abundance	> 9	7–9	4–6	< 4
Biotic index (HBI)	< 3.77	3.77–4.52	4.53–5.27	>5.27
% Chironomidae	0.79–4.10	4.11–9.48	9.49–16.19	< 0.79 or > 16.19
% Dominant taxon	< 22.15	22.15–31.01	31.02–39.88	> 39.88
% Dominant FFG	< 36.50	36.50–45.30	45.31–54.12	> 54.12
% Predators	4.73–15.20	15.21–25.67	25.68–36.14	< 4.73 or >36.14
Ratio of intolerant : tolerant taxa	> 4.79	3.21–4.79	1.63–3.20	< 1.63
% of total Trichoptera as Hydropsychidae	< 25.50	25.51–50.50	50.51–75.50	> 75.50 or no Trichoptera
# of non-insect taxa	> 5	4–5	2–3	< 2
% Collector-gatherers	8.00–19.23	19.24–30.46	30.47–41.68	< 8.00 or > 41.68
% of total number as Elmidae	0.88–10.04	10.05–20.08	20.09–30.12	< 0.88 or > 30.12
Aquatic-life-use point-score ranges:	Exceptional: High: Intermediate: Limited:	> 36 29–36 22–28 < 22		

Table B.12. Metrics and scoring criteria for Surber samples—benthic macroinvertebrates (Davis, 1997).

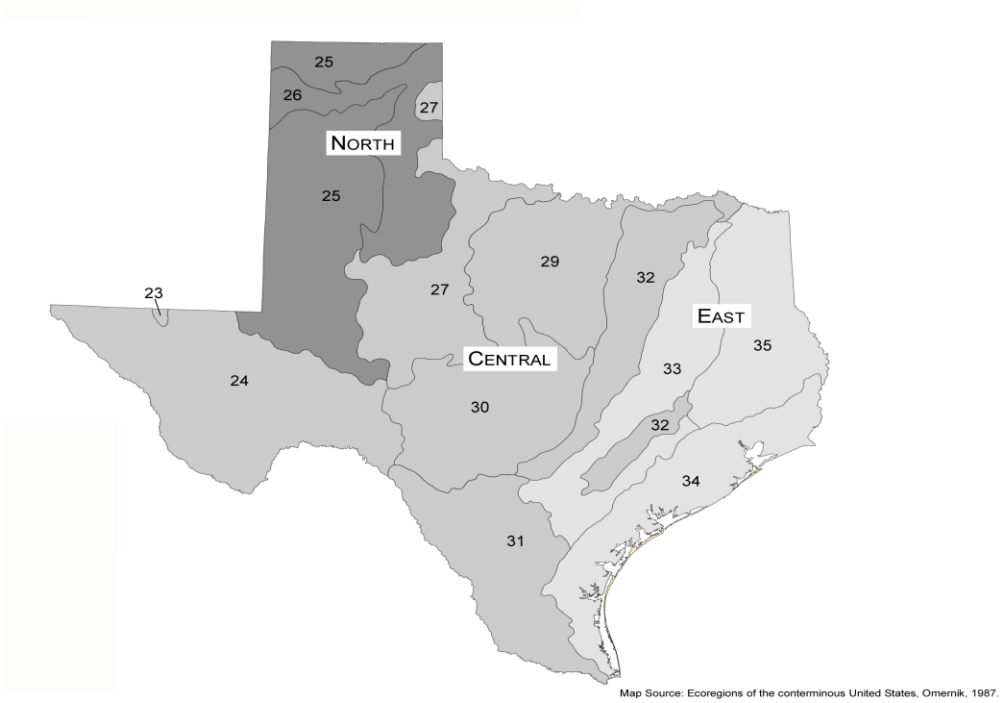
		SCORING CRITERIA		
	METRIC	**5**	**3**	**1**
Central bioregion	Total taxa	> 32	32–18	< 18
	Diptera taxa	> 7	7–4	< 4
	Ephemeroptera taxa	> 4	4–2	< 2
(Ecoregions: 23, 24, 27, 29, 30, 31, and 32)	Intolerant taxa	> 8	8–4	< 4
	% EPT taxa	> 30	30.0–17.4	< 17.4
	% Chironomidae	(*a*)	< 22.3	≥ 22.3
	% Tolerant taxa	(*a*)	< 10.0	≥ 10.0
	% Grazers	> 14.9	14.9–8.7	< 8.7
	% Gatherers	> 15.2	15.2–8.8	< 8.8
	% Filterers	(*a*)	> 11.9	≤ 11.9
	% Dominance (3 taxa)	< 54.6	54.6–67.8	> 67.8
East bioregion	Total taxa	> 30	30–17	< 17
	Diptera taxa	> 10	10–6	< 6
(Ecoregions: 33, 34, and 35)	Ephemeroptera taxa	(*b*)	> 3	≤ 3
	Intolerant taxa	> 4	4–2	< 2
	% EPT taxa	> 18.9	18.9–10.8	< 10.8
	% Chironomidae	(*a*)	< 40.2	≥ 40.2
	% Tolerant taxa	< 16.0	16.0–24.3	> 24.3
	% Grazers	> 9.0	9.0–5.2	< 5.2
	% Gatherers	> 12.5	12.5–7.3	< 7.3
	% Filterers	(*a*)	> 16.3	≤ 16.3
	% Dominance (3 taxa)	< 57.7	57.7–71.6	> 71.6

	METRIC	SCORING CRITERIA		
		5	**3**	**1**
North bioregion (Ecoregions 25 and 26)	Total taxa	> 33	33–19	< 19
	Diptera taxa	> 14	14–8	< 8
	Ephemeroptera taxa	(*b*)	> 2	≤ 2
	Intolerant taxa	> 3	3–2	< 2
	% EPT taxa	> 14.4	14.4–8.2	< 8.2
	% Chironomidae	< 36.9	36.9–56.2	> 56.2
	% Tolerant taxa	< 14.1	14.1–21.5	> 21.5
	% Grazers	(*b*)	> 5.4	≤ 5.4
	% Gatherers	(*a*)	> 14.9	≤ 14.9
	% Filterers	> 12.2	12.2–7.1	< 7.1
	% Dominance (3 taxa)	< 68.1	68.1–84.5	> 84.5

(*a*) The discriminatory power was less than optimal for this bioregion, so the metric was assigned only two scoring categories.

(*b*) The median value for this bioregion was less than the metric-selection criterion (< 5.5 for taxa richness metrics; < 12 for percentage metrics expected to decrease with disturbance), so the metric was assigned only two categories.

Figure B.9. Macrobenthic bioregions (North, Central, East) and ecoregions of Texas for use in Surber metric calculations using Table B.12. (Davis 1997.)



Ecoregions of Texas

23 Arizona–New Mexico Mountains
24 Southern Deserts
25 Western High Plains
26 Southwestern Tablelands
27 Central Great Plains
29 Central Oklahoma–Texas Plains
30 Central Texas Plateau
31 Southern Texas Plains
32 Texas Blackland Prairies
33 East Central Texas Plains
34 Western Gulf Coastal Plain
35 South Central Plains

Table B.13. Tolerance values and functional group classification for benthic macroinvertebrates.

Aquatic macroinvertebrates commonly collected in Texas streams. Shaded cells indicate tolerance values or functional classification taken from higher taxonomic levels (or both).

Functional groups: SCR = scraper; CG = collector gatherer; FC = filtering collector; P = predator; SHR = shredder. For different feeding habits for larvae and adult: L = larvae; A = Adult

Parameter Code	Genus or Species	Tolerance Value	Functional Group	Order	Family
91645	*Acentrella* sp.	4	SCR/CG	Ephemeroptera	Baetidae
91632	*Acerpenna* sp.	4	SCR/CG	Ephemeroptera	Baetidae
91646	*Baetis* sp.	4	SCR/CG	Ephemeroptera	Baetidae
91642	*Baetodes* sp.	4	SCR	Ephemeroptera	Baetidae
91650	*Callibaetis* sp.	4	CG	Ephemeroptera	Baetidae
91644	*Centroptilum* sp.	2	SCR/CG	Ephemeroptera	Baetidae
91648	*Cloeon* sp.	4	SCR/CG	Ephemeroptera	Baetidae
91649	*Dactylobaetis* sp.	4	SCR/CG	Ephemeroptera	Baetidae
91651	*Fallceon quilleri*	4	SCR/CG	Ephemeroptera	Baetidae
91579	*Labiobaetis* sp.	4	SCR/CG	Ephemeroptera	Baetidae
91656	*Paracloeodes* sp.	9	SCR/CG	Ephemeroptera	Baetidae
91654	*Pseudocloeon* sp.	4	SCR/CG	Ephemeroptera	Baetidae
91598	*Brachycercus* sp.	3	CG	Ephemeroptera	Caenidae
91600	*Caenis* sp.	7	SCR/CG	Ephemeroptera	Caenidae
91570	*Hexagenia* sp.	6	CG	Ephemeroptera	Ephemeridae
91590	*Isonychia* sp.	3	FC	Ephemeroptera	Oligoneuriidae
91619	*Stenacron* sp.	4	SCR/CG	Ephemeroptera	Heptageniidae
91620	*Stenonema* sp.	4	SCR/CG	Ephemeroptera	Heptageniidae
91596	*Leptohyphes* sp.	2	CG	Ephemeroptera	Tricorythidae
91594	*Tricorythodes* sp.	5	CG	Ephemeroptera	Tricorythidae
91549	*Leptophlebiidae*	2	CG/SCR	Ephemeroptera	Leptophlebiidae
91554	*Choroterpes* sp.	2	CG/SCR	Ephemeroptera	Leptophlebiidae
91661	*Farrodes texanus*	2	CG/SCR	Ephemeroptera	Leptophlebiidae
91550	*Paraleptophlebia* sp.	2	CG/SHR	Ephemeroptera	Leptophlebiidae
91562	*Thraulodes* sp.	2	CG/SCR	Ephemeroptera	Leptophlebiidae
91552	*Traverella* sp.	2	FC	Ephemeroptera	Leptophlebiidae
91628	*Eurylophella* sp.	4	CG	Ephemeroptera	Ephemerellidae
91896	*Isoperla* sp.	2	P	Plecoptera	Perlodidae
91861	*Allocapnia* sp.	2	SHR	Plecoptera	Capniidae
91879	*Anacroneuria* sp.	1	P	Plecoptera	Perlidae
91891	*Paragnetina* sp.	3.5	P	Plecoptera	Perlidae
91881	*Neoperla* sp.	1	P	Plecoptera	Perlidae
91883	*Perlesta* sp.	0	P	Plecoptera	Perlidae
91887	*Perlinella* sp.	2	P	Plecoptera	Perlidae
91871	*Taeniopteryx* sp.	2	SHR/CG	Plecoptera	Taeniopterygidae
91859	*Zealeuctra* sp.	0	FC	Plecoptera	Leuctridae
92292	*Cheumatopsyche* sp.	6	FC	Trichoptera	Hydropsychidae
92294	*Diplectrona* sp.	2	FC	Trichoptera	Hydropsychidae
92296	*Hydropsyche* sp.	5	FC	Trichoptera	Hydropsychidae
92302	*Macrostemum* sp. = *Macrostema*	4	C	Trichoptera	Hydropsychidae
92305	*Potamyia* sp.	4	FC	Trichoptera	Hydropsychidae
92308	*Smicridea* sp.	4	FC	Trichoptera	Hydropsychidae
92376	*Helicopsyche* sp.	2	SCR	Trichoptera	Helicopsychidae
92371	*Pycnopsyche* sp.	2	SHR	Trichoptera	Limnophilidae
92268	*Chimarra* sp.	2	FC	Trichoptera	Philopotamidae

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92334	*Dolophilodes* sp.	3	FC	Trichoptera	Philopotamidae
92324	*Hydroptila* sp.	2	SCR	Trichoptera	Hydroptilidae
92326	*Ithytrichia* sp.	4	SCR	Trichoptera	Hydroptilidae
92327	*Leucotrichia* sp.	3	CG/SCR	Trichoptera	Hydroptilidae
92329	*Mayatrichia* sp.	4	SCR	Trichoptera	Hydroptilidae
92332	*Ochrotrichia* sp.	4	CG	Trichoptera	Hydroptilidae
92335	*Oxyethira* sp.	2	CG/SCR	Trichoptera	Hydroptilidae
92337	*Stactobiella* sp.	3	SHR	Trichoptera	Hydroptilidae
92304	*Nectopsyche* sp.	3	SHR/CG/P	Trichoptera	Leptoceridae
92391	*Oecetis* sp.	5	P/SHR	Trichoptera	Leptoceridae
92365	*Setodes* sp.	2	CG/P	Trichoptera	Leptoceridae
92395	*Trianodes* sp.	3	P	Trichoptera	Leptoceridae
92274	*Cernotina* sp.	6	P	Trichoptera	Polycentropodidae
92278	*Neureclipsis* sp.	4	FC/SHR/P	Trichoptera	Polycentropodidae
92279	*Nyctiophylax* sp.	1	FC/P	Trichoptera	Polycentropodidae
92284	*Phylocentropus* sp.	5	FC	Trichoptera	Polycentropodidae
92281	*Polycentropus* sp.	3	FC/P	Trichoptera	Polycentropodidae
92539	*Polyplectropus* sp.	6	FC/P	Trichoptera	Polycentropodidae
92378	*Marilia* sp.	0	SHR	Trichoptera	Odontoceridae
92293	*Brachycentrus* sp.	1	FC/SCR	Trichoptera	Brachycentridae
92319	*Protoptila* sp.	1	SCR	Trichoptera	Glossosomatidae
92311	*Atopsyche* sp.	0	P	Trichoptera	Hydrobiosidae
92313	*Rhyacophila* sp.	0	P	Trichoptera	Rhyacophilidae
92076	*Corydalus cornutus*	6	P	Megaloptera	Corydalidae
92072	*Chauliodes* sp.	4	P	Megaloptera	Corydalidae
92069	*Sialis* sp.	4	P	Megaloptera	Sialidae
92731	*Acentria* sp.	1	SHR	Lepidoptera	Pyralidae
92726	*Crambus* sp.	5	SHR	Lepidoptera	Pyralidae
92659	*Paraponyx* sp.	5	SHR	Lepidoptera	Pyralidae
92686	*Petrophila* sp.	5	SCR	Lepidoptera	Pyralidae
92226	*Ancyronyx* sp.	2	SCR/CG	Coleoptera	Elmidae
92230	*Dubiraphia* sp.	5	SCR/CG	Coleoptera	Elmidae
92232	*Elsianus* sp.	2	SCR/CG	Coleoptera	Elmidae
92233	*Heterelmis* sp.	4	SCR/CG	Coleoptera	Elmidae
92235	*Hexacylloepus* sp.	2	SCR/CG	Coleoptera	Elmidae
92232	*Macrelmis* sp.	4	SCR/CG	Coleoptera	Elmidae
92240	*Macronychus* sp.	2	SCR/CG	Coleoptera	Elmidae
92243	*Microcylloepus* sp.	2	SCR/CG	Coleoptera	Elmidae
92244	*Narpus* sp.	2	SCR/CG	Coleoptera	Elmidae
92246	*Neoelmis* sp.	2	SCR/CG	Coleoptera	Elmidae
92253	*Stenelmis* sp.	7	SCR/CG	Coleoptera	Elmidae
92217	*Helichus* sp.	4	SCR/CG	Coleoptera	Dryopidae
92209	*Eubrianax* sp.	4	SCR	Coleoptera	Psephenidae
92211	*Psephenus* sp.	4	SCR	Coleoptera	Psephenidae
92090	*Dineutus* sp.	5	P	Coleoptera	Gyrinidae
92092	*Gyretes* sp.	6	P	Coleoptera	Gyrinidae

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92093	*Gyrinus* sp.	6	P	Coleoptera	Gyrinidae
92153	Hydrophilidae	5	L = P; A = CG	Coleoptera	Hydrophilidae
92154	*Berosus* sp.	9	CG	Coleoptera	Hydrophilidae
92161	*Enochrus* sp.	8	CG	Coleoptera	Hydrophilidae
92166	*Helochares* sp.	5	CG	Coleoptera	Hydrophilidae
92168	*Helophorus* sp.	8	SHR	Coleoptera	Hydrophilidae
92147	*Hydrobiomorpha sp*		CG	Coleoptera	Hydrophilidae
92142	*Hydrocanthus* sp.	7	L = P/CG; A = P	Coleoptera	Noteridae
92165	*Hydrochus* sp.		SHR	Coleoptera	Hydrochidae
92173	*Lacobius* sp.	8	L = P; A = CG	Coleoptera	Hydrophilidae
92143	*Sperchopsis* sp.	5	L = P; A = CG	Coleoptera	Hydrophilidae
92180	*Tropisternus* sp.	10	L = P; A = CG	Coleoptera	Hydrophilidae
92223	*Lutrochus* sp.		SHR/CG	Coleoptera	Lutrochidae
92108	*Agabus* sp.	5	P	Coleoptera	Dytiscidae
92086	*Bidessonotus* sp.	5	P	Coleoptera	Dytiscidae
92085	*Brachyvatus* sp.	5	P	Coleoptera	Dytiscidae
92111	*Celina* sp.	5	P	Coleoptera	Dytiscidae
92114	*Copelatus* sp.	9	P	Coleoptera	Dytiscidae
92119	*Deronectes* sp.	5	P	Coleoptera	Dytiscidae
92118	*Derovatellus* sp.	5	P	Coleoptera	Dytiscidae
92126	*Hydaticus* sp.	5	P	Coleoptera	Dytiscidae
92128	*Hydroporus* sp.	9	P	Coleoptera	Dytiscidae
92130	*Hydrovatus* sp.	5	P	Coleoptera	Dytiscidae
92083	*Laccophilus*	10	P	Coleoptera	Dytiscidae
92136	*Laccodytes* sp.	5	P	Coleoptera	Dytiscidae
92112	*Liodessus* sp.	5	P	Coleoptera	Dytiscidae
92129	*Oreodytes* sp.	5	P	Coleoptera	Dytiscidae
92127	*Uvarus* sp.	5	P	Coleoptera	Dytiscidae
92729	Scirtidae		SCR/CG/SHR	Coleoptera	Scirtidae
92198	*Cyphon* sp.	7	SCR/CG/SHR	Coleoptera	Scirtidae
92206	*Scirtes* sp.		SHR	Coleoptera	Scirtidae
92182	Curculionidae		SHR	Coleoptera	Curculionidae
92199	*Listronotus* sp.		SHR	Coleoptera	Curculionidae
92141	*Lixus* sp.		SHR	Coleoptera	Curculionidae
92095	Haliplidae	7	SHR/P	Coleoptera	Haliplidae
92098	*Haliplus* sp.	7	SHR/P		
92100	*Peltodytes* sp.	8	SHR/P	Coleoptera	Haliplidae
92193	Staphylinidae		P	Coleoptera	Staphylinidae
92196	*Stenus* sp.		P	Coleoptera	Staphylinidae
92146	*Suphisellus* sp.		P	Coleoptera	
91683	*Argia* sp.	6	P	Odonata	Coenagrionidae
91685	*Chromagrion* sp.	9	P	Odonata	Coenagrionidae
91687	*Enallagma* sp.	6	P	Odonata	Coenagrionidae
91695	*Ischnura* sp.	9	P	Odonata	Coenagrionidae
91667	*Calopteryx* sp.	5	P	Odonata	Calopterygidae

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91669	*Hetaerina* sp.	6	P	Odonata	Calopterygidae
91769	*Macromia* sp.	3	P	Odonata	Corduliidae
91741	*Aeshna* sp.	4	P	Odonata	Aeschnidae
91745	*Basiaeschna* sp.	2	P	Odonata	Aeschnidae
91747	*Boyeria* sp.	3	P	Odonata	Aeschnidae
91793	*Epiaeschna* sp.	1	P	Odonata	Aeschnidae
91757	*Nasiaeschna pentacantha*	8	P	Odonata	Aeschnidae
91764	*Cordulegaster* sp.	2	P	Odonata	Cordulegasteridae
91791	*Epitheca* sp.	4	P	Odonata	Corduliidae
91786	*Dorocordulia* sp.	5	P	Odonata	Corduliidae
91817	*Neurocordulia* sp.	3	P	Odonata	Corduliidae
91837	*Somatochlora* sp.	1	P	Odonata	Corduliidae
91843	*Tetragoneuria* sp.	8.5	P	Odonata	Libellulidae
91772	*Belonia* sp.	9	P	Odonata	Libellulidae
91776	*Brechmorhoga* sp.	6	P	Odonata	Libellulidae
91792	*Erythemis* sp.	5	P	Odonata	Libellulidae
91794	*Erythrodiplax* sp.	5	P	Odonata	Libellulidae
91806	*Libellula* sp.	8	P	Odonata	Libellulidae
91813	*Miathyria* sp.	9	P	Odonata	Libellulidae
91811	*Macrothemis* sp.	9	P	Odonata	Libellulidae
91819	*Orthemis* sp.	9	P	Odonata	Libellulidae
91822	*Pachydiplax longipennis*	10	P	Odonata	Libellulidae
91827	*Perithemis* sp.	4	P	Odonata	Libellulidae
91838	*Sympetrum* sp.	7	P	Odonata	Libellulidae
91706	Gomphidae	1	P	Odonata	Gomphidae
91709	*Arigomphus* sp.	1	P	Odonata	Gomphidae
91711	*Dromogomphus* sp.	4		Odonata	Gomphidae
91713	*Erpetogomphus* sp.	1	P	Odonata	Gomphidae
91715	*Gomphoides* sp.	1	P	Odonata	Gomphidae
91718	*Gomphus* sp.	7	P	Odonata	Gomphidae
91721	*Hagenius* sp.	3	P	Odonata	Gomphidae
91728	*Ophiogomphus* sp.	6	P	Odonata	Gomphidae
91696	*Phyllogomphoides* sp.	1	P	Odonata	Gomphidae
91730	*Progomphus* sp.	5	P	Odonata	Gomphidae
92016	Corixidae	9	P/CG	Hemiptera	Corixidae
92009	*Palmacorixa* sp.	9	P/CG	Hemiptera	Corixidae
92044	*Trichocorixa* sp.	5	P/CG	Hemiptera	Corixidae
92053	Naucoridae	5	P	Hemiptera	Naucoridae
92054	*Ambrysus* sp.	5	P	Hemiptera	Naucoridae
92057	*Cryphocricos* sp.	5	P	Hemiptera	Naucoridae
92060	*Limnocoris* sp.	5	P	Hemiptera	Naucoridae
92059	*Pelocoris* sp.	5	P	Hemiptera	Naucoridae
91953	*Mesovelia* sp.		P	Hemiptera	Mesoveliidae
91919	*Microvelia* sp.		P	Hemiptera	Veliidae
91923	*Rhagovelia* sp.		P	Hemiptera	Veliidae
91951	*Aquarius* sp.	5	P	Hemiptera	Gerridae

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91944	*Rheumatobates* sp.	5	P	Hemiptera	Gerridae
91946	*Trepobates* sp.	5	P	Hemiptera	Gerridae
91986	*Abedus* sp.		P	Hemiptera	Belostomatidae
91988	*Belostoma* sp.	10	P	Hemiptera	Belostomatidae
91994	*Lethocerus* sp.		P	Hemiptera	Belostomatidae
92002	*Ranatra* sp.	7	P	Hemiptera	Nepidae
91955	Hebridae		P	Hemiptera	Hebridae
91957	*Lipogomphus* sp.		P	Hemiptera	Hebridae
92051	*Notonecta* sp.		P	Hemiptera	Notonectidae
91913	*Hydrometra* sp.		P	Hemiptera	Hydrometridae
92008	*Neoplea* sp.		P	Hemiptera	Pleidae
92491	Chironomidae	6	P/CG/FC	Diptera	Chironomidae
Chironominae: Chironomini					
92507	Chironominae	6	CG/FC/P	Diptera	Chironomidae
92508	*Chironomus* sp.	10	CG/SHR	Diptera	Chironomidae
92522	*Cryptochironomus* sp.	8	P	Diptera	Chironomidae
92516	*Dicrotendipes* sp.	7	CG/FC	Diptera	Chironomidae
92512	*Einfeldia* sp.	10	CG	Diptera	Chironomidae
92520	*Endochironomus* sp.	6	SHR/CG/FC	Diptera	Chironomidae
92531	*Glyptotendipes* sp.	8	SHR/FC/CG	Diptera	Chironomidae
92525	*Goeldichironomus* sp.	8	CG	Diptera	Chironomidae
92524	*Harnischia* sp.	8	CG	Diptera	Chironomidae
92514	*Kiefferulus* sp.	10	CG	Diptera	Chironomidae
92535	*Lauterborniella* sp.	8	CG	Diptera	Chironomidae
91497	*Microchironomus* sp.	8	CG	Diptera	Chironomidae
92542	*Microtendipes* sp.	6	CG/FC	Diptera	Chironomidae
92544	*Paratendipes* sp.	5	CG	Diptera	Chironomidae
92526	*Parachironomus* sp.	9	P/CG	Diptera	Chironomidae
92528	*Paracladopelma* sp.	6	CG	Diptera	Chironomidae
92537	*Phaenopsectra* sp.	8	SCR/CG	Diptera	Chironomidae
92534	*Polypedilum* sp.	6	SHR/CG/P	Diptera	Chironomidae
91007	*Robackia* sp.	6	CG	Diptera	Chironomidae
92469	*Saetheria* sp.	8	CG	Diptera	Chironomidae
92547	*Stictochironomus* sp.	8	CG/SHR	Diptera	Chironomidae
91495	*Sergentia* sp.	6	SCR/CG	Diptera	Chironomidae
91901	*Stelechomyia* sp.	6	CG	Diptera	Chironomidae
92540	*Stenochironomus* sp.	6	CG/SHR	Diptera	Chironomidae
92511	*Tribelos* sp.	5	CG	Diptera	Chironomidae
Chironominae: Pseudochironomini					
92538	*Pseudochironomus* sp.	5	CG	Diptera	Chironomidae
Chironominae: Tanytarsini					
90996	Tanytarsini	6	CG/FC	Diptera	Chironomidae
92551	*Micropsectra* sp.	2	CG	Diptera	Chironomidae
92552	*Cladotanytarsus* sp.	7	CG/FC	Diptera	Chironomidae
91899	*Nimbocera* sp.	6	CG/FC	Diptera	Chironomidae
92441	*Paratanytarsus* sp.	8	CG/FC	Diptera	Chironomidae

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92555	*Rheotanytarsus* sp.	6	FC	Diptera	Chironomidae
92554	*Tanytarsus* sp.	7	CG/FC	Diptera	Chironomidae
92429	*Virgatanytarsus* sp.	6	CG/FC	Diptera	Chironomidae
Orthocladiinae: Corynoneurini					
92569	Orthocladiinae	6	CG	Diptera	Chironomidae
92573	*Corynoneura* sp.	6	CG	Diptera	Chironomidae
92588	*Thienemanniella* sp.	2	CG	Diptera	Chironomidae
Orthocladiinae: Orthocladiini					
91897	*Acricotopus* sp.	6	CG	Diptera	Chironomidae
92570	*Brillia* sp.	5	SHR/CG	Diptera	Chironomidae
91892	*Chaetocladius* sp.	6	CG	Diptera	Chironomidae
92575	*Cricotopus* sp.	8	CG	Diptera	Chironomidae
92579	*Eukiefferiella* sp.	4	CG/SCR/P	Diptera	Chironomidae
92614	*Hydrobaenus* sp.	10	SCR/CG	Diptera	Chironomidae
92444	*Lopescladius* sp.	2	CG	Diptera	Chironomidae
92581	*Metriocnemus* sp.	6	CG/P	Diptera	Chironomidae
91686	*Nanocladius* sp.	7	CG	Diptera	Chironomidae
92584	*Orthocladius* sp.	4	CG	Diptera	Chironomidae
91890	*Parakiefferiella* sp.	6	CG	Diptera	Chironomidae
92583	*Parametriocnemus* sp.	4	CG	Diptera	Chironomidae
91885	*Pseudosmittia* sp.	6	CG	Diptera	Chironomidae
91920	*Rheocricotopus* sp.	6	CG/SHR/P	Diptera	Chironomidae
91869	*Thienemannia* sp.	6	CG	Diptera	Chironomidae
Tanypodinae: Coelotanypodini					
90984	Tanypodinae	6	P	Diptera	Chironomidae
92374	*Alotanypus* sp.		P	Diptera	Chironomidae
92498	*Clinotanypus* sp.	6	P	Diptera	Chironomidae
92500	*Coelotanypus* sp.	6	P	Diptera	Chironomidae
Tanypodinae: Macropelopiini					
91866	*Fittkauimyia* sp.	6	P	Diptera	Chironomidae
92505	*Psectrotanypus* sp.	8	P	Diptera	Chironomidae
Tanypodinae: Procladiin					
91864	*Djalmabatista* sp.	6	P	Diptera	Chironomidae
92495	*Procladius* sp.	9	CG/P	Diptera	Chironomidae
Tanypodinae: Natarsini					
91862	*Natarsia* sp.	10	P	Diptera	Chironomidae
Tanypodinae: Pentaneurini					
92503	*Ablabesmyia* sp.	6	P/CG	Diptera	Chironomidae
92834	*Guttipelopia* sp.		P	Diptera	Chironomidae
92805	*Krenopelopia* sp.		P	Diptera	Chironomidae
91854	*Labrundinia* sp.	4	P	Diptera	Chironomidae
92678	*Larsia* sp.	6	P	Diptera	Chironomidae
92501	*Pentaneura* sp.	5	CG/P	Diptera	Chironomidae
92496	*Nilotanypus* sp.	4	P	Diptera	Chironomidae
92637	*Telopelopia* sp.	6	P	Diptera	Chironomidae
90976	*Thienemannimyia* sp.	6	P	Diptera	Chironomidae

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		Tanypodinae: Tanypodini			
92493	*Tanypus* sp.	10	P/CG	Diptera	Chironomidae
92474	Ceratopogonidae	5	P/CG	Diptera	Ceratopogonidae
91008	*Alluaudomyia* sp.	5	P	Diptera	Ceratopogonidae
92478	*Bezzia* sp.	7	P	Diptera	Ceratopogonidae
92480	*Culicoides* sp.	7	P/CG	Diptera	Ceratopogonidae
92369	*Forcipomyia* sp.	6	CG	Diptera	Ceratopogonidae
92367	*Sphaeromias* sp.	5	P/CG	Diptera	Ceratopogonidae
92481	*Dasyhelea* sp.	5	CG/SCR	Diptera	Ceratopogonidae
92840	*Serromyia* sp.		P	Diptera	Ceratopogonidae
92603	*Bittacomorpha* sp.	8	CG	Diptera	Ptychopteridae
91853	*Ptychoptera* sp.	8	CG/SHR	Diptera	Ptychopteridae
92445	*Anopheles* sp.	9	FC	Diptera	Culicidae
92442	Culicidae	8	FC/CG	Diptera	Culicidae
92447	*Chaoborus* sp.	4	P	Diptera	Chaoboridae
92564	*Cnephia* sp.	4	FC	Diptera	Simuliidae
92385	*Prosimulium* sp.	2	FC	Diptera	Simuliidae
92596	*Simulium* sp.	4	FC	Diptera	Simuliidae
92421	*Antocha* sp.	5	CG	Diptera	Tipulidae
92424	*Erioptera* sp.	3	CG	Diptera	Tipulidae
92425	*Helius* sp.	3	SHR/CG/P	Diptera	Tipulidae
92747	*Cryptolabis* sp.	3	SHR/CG	Diptera	Tipulidae
92427	*Hexatoma* sp.	4	P	Diptera	Tipulidae
92428	*Limnophila* sp.	4	P	Diptera	Tipulidae
91852	*Lipsothrix* sp.	3	SHR	Diptera	Tipulidae
92439	*Pseudolimnophila* sp.	7	SHR/P/CG	Diptera	Tipulidae
92440	*Tipula* sp.	8	SHR/CG	Diptera	Tipulidae
92625	*Atherix* sp.	4	P	Diptera	Athericidae
92722	*Chlorotabanus* sp.	7	P	Diptera	Tabanidae
92619	*Chrysops* sp.	7	P	Diptera	Tabanidae
92622	*Tabanus* sp.	7	P	Diptera	Tabanidae
91851	*Ochthera* sp.	8	P	Diptera	Ephydridae
92627	Empididae	8	P	Diptera	Empididae
92628	*Hemerodromia* sp.	6	P/CG	Diptera	Empididae
92470	*Pericoma* sp.	10	CG	Diptera	Psychodidae
92609	*Euparyphus* sp.		SCR/CG	Diptera	Stratiomyidae
92611	*Nemotelus* sp.		CG	Diptera	Stratiomyidae
92613	*Odontomyia* sp.	7	CG	Diptera	Stratiomyidae
91530	Collembola		CG	Collembola	
91839	*Ellipes minuta*		SHR	Orthoptera	Tridactylidae
91265	*Gammarus* sp.	3	CG/SHR	Amphipoda	Gammaridae
91267	*Gammarus lacustrus*		CG/SHR	Amphipoda	Gammaridae
91241	*Hyallela azteca*	8	CG/SHR	Amphipoda	Taltridae
91224	*Asellus* sp.	9	CG/SHR	Isopoda	Asellidae
91260	*Cragonyx*	8	CG/SHR	Isopoda	Asellidae
91227	*Lirceus* sp.	9	CG/SHR	Isopoda	Asellidae

Aquatic macroinvertebrates commonly collected in Texas streams. Shaded cells indicate tolerance values or functional classification taken from higher taxonomic levels (or both).

Functional groups: SCR = scraper; CG = collector gatherer; FC = filtering collector; P = predator; SHR = shredder. For different feeding habits for larvae and adult: L = larvae; A = Adult

Parameter Code	Genus or Species	Tolerance Value	Functional Group	Order	Family
91056	Ostracoda		CG/SCAV	Podocopa	
91397	*Palaemonetes* sp.	4	CG	Decapoda	Palaemonidae
91400	*Palaemonetes kadiakensis*	4	CG	Decapoda	Palaemonidae
91401	*Palaemonetes paludosis*	4	CG	Decapoda	Palaemonidae
91392	*Macrobrachium ohione*	4	CG	Decapoda	Palaemonidae
91409	Cambaridae	5	CG	Decapoda	Cambaridae
91419	*Cambarellus* sp.	5	CG	Decapoda	Cambaridae
91423	*Cambarus* sp.	8	CG	Decapoda	Cambaridae
91428	*Orconectes* sp.	3	CG	Decapoda	Cambaridae
91433	*Procambarus* sp.	9	CG	Decapoda	Cambaridae
93037	*Corbicula fluminea*	6	FC	Heterodonta	Corbiculidae
93026	*Eupera cubensis*		SCR	Heterodonta	Sphaeridae
93030	*Pisidium* sp.	7	FC	Heterodonta	Sphaeriidae
93032	*Sphaerium* sp.	5	FC	Heterodonta	Sphaeriidae
92900	*Ferrisia* sp.	7	SCR	Limnophila	Ancylidae
92905	*Ferrisia rivularis*	7	SCR	Limnophila	Ancylidae
92915	*Hebetancylus excentricus*		SCR	Limnophila	Ancylidae
92879	*Pseudosuccinea* sp.	7	SCR	Limnophila	Lymnaeidae
92894	*Pseudosuccinea columella*	7	SCR	Limnophila	Lymnaeidae
92920	*Stagnicola* sp.	7	SCR	Limnophila	Lymnaeidae
92885	*Gyraulus* sp.		SCR	Limnophila	Planorbidae
92887	*Helisoma* sp.	7	SCR	Limnophila	Planorbidae
92892	*Planorbella* sp.		SCR	Limnophila	Planorbidae
92891	*Planorbula* sp.	7	SCR	Limnophila	Planorbidae
92874	*Physella* sp.	9	SCR	Limnophila	Physidae
92783	Hydrobiidae	7	SCR	Mesogastropoda	Hydrobiidae
92763	*Amnicola* sp.	5	SCR	Mesogastropoda	Hydrobiidae
92779	*Somatogyrus* sp.	6	SCR	Mesogastropoda	Hydrobiidae
92780	*Elimia* sp.	2	SCR	Mesogastropoda	Pleuroceridae
92795	*Leptoxis* sp.	2	SCR	Mesogastropoda	Pleuroceridae
92898	*Melanoides tuberculata*		SCR	Mesogastropoda	Thiaridae
92760	*Valvata* sp.	2	SCR	Mesogastropoda	Valvatidae
92756	*Campeloma* sp.		SCR	Mesogastropoda	Viviparidae
92757	*Viviparus* sp.	1	SCR	Mesogastropoda	Viviparidae
91525	Hydracarina	6	P		
90913	Hirudinea	8	P		
90967	*Erpobdella* sp.	8	P	Erpobdelliformes	Erpobdellidae
93095	*Mooreobdella* sp.	7.8	P	Arhynchobdellida	Erpobdellidae
90931	*Placobdella* sp.	6	P	Rhynchobdellida	Glossiphoniidae
90382	Oligochaeta	8	CG		
90075	*Dugesia* sp.	7.5	P	Tricladida	Dugesiidae
90291	*Nematomorpha* sp.		P		
90196	Nematoda	5	P		

Site and Reach Selection for RWAs

Figure B.10. Example of **existing** 3.6 mgd discharge to intermittent and perennial stream. Extent of downstream impact on DO is 4.5 miles. Impact extends below second confluence.

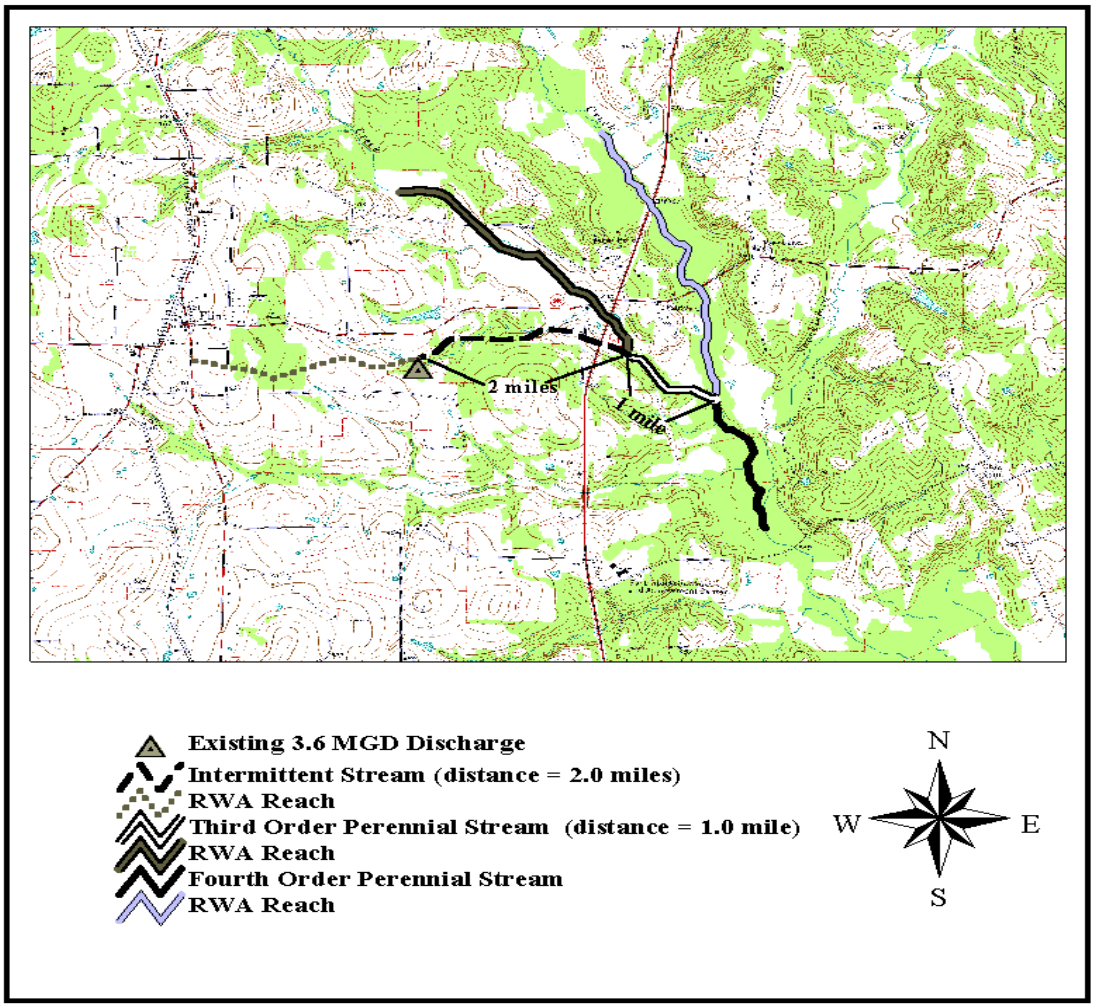
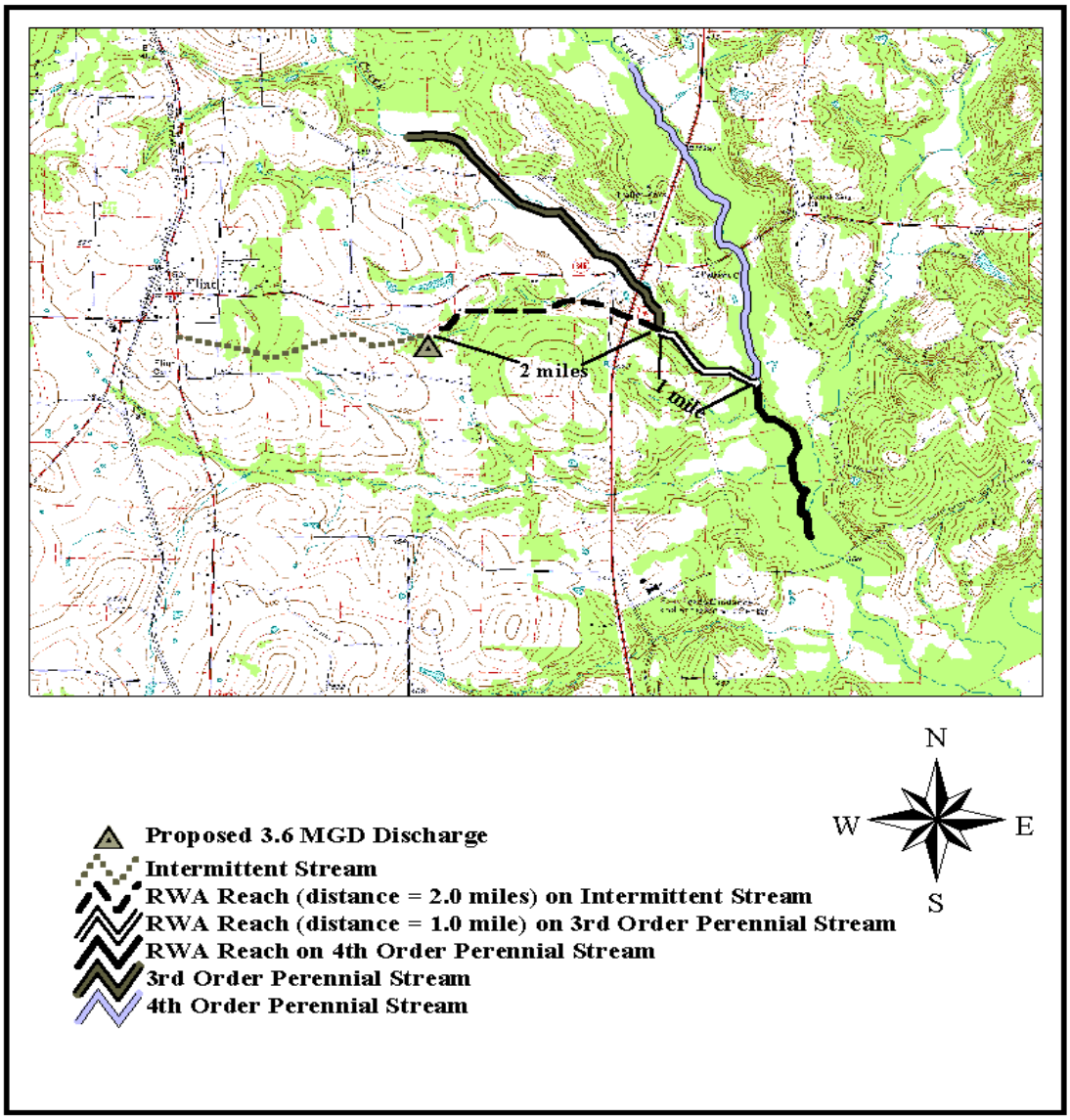


Figure B.11. Example of **proposed** 3.6 mgd discharge to intermittent and perennial stream. Extent of downstream impact on DO is 4.5 miles. Impact extends below second confluence.



APPENDIX C

FORMS FOR BIOLOGICAL-MONITORING PACKETS

Use the forms in this appendix when preparing a biological-monitoring packet to be submitted to the TCEQ. Some of the forms are to be used in every biological-monitoring packet and some will be specific to a particular purpose, such as an RWA.

Elements of the Biological-Data Summary Packet

This document provides guidance for submitting biological data that are collected for routine ALMs, ALUs, UAAs, and RWAs. For guidance in the **collection** of the biological data, consult the text of this manual in conjunction with the current approved version of the *2012 Guidance for Assessing and Reporting Surface Water Quality in Texas*, available online at <www.tceq.texas.gov/assets/public/waterquality/swqm/assess/12twqi/2012_guidance.pdf>.

Items 1 to 4 below are the minimum data which that should be submitted to the TCEQ, in a packet, as part of any biological assessment. If submitting the data as part of a UAA, please also use the UAA Report Outline to ensure the summary of the collection efforts is complete. The TCEQ regional staff should submit the packets to the SWQM Team. CRP Planning Agencies and other cooperating authorities should submit packets to the appropriate TCEQ CRP or appropriate project manager. Item 5 is optional.

1. Checklist for aquatic-life monitoring and habitat assessment.
2. Biological assessment
   - TCEQ Nekton Biological-Data Reporting Form or equivalent for seining.
   - TCEQ Nekton Biological-Data Reporting Form or equivalent for electrofishing.
   - TCEQ Benthic Macroinvertebrate Biological-Data Reporting Form or equivalent.
3. Habitat assessment
   - TCEQ Habitat Reporting Form or equivalent.
   - Part I—Stream physical characteristics worksheet.
   - Part II—Summary of physical characteristics of water body.
4. Field-Data Reporting Form or equivalent and Stream Flow (Discharge) Measurement Form or equivalent.
5. Metric sets for biological and habitat assessments
   Ecoregion scoring criteria for determining ALU—nekton
   Scoring criteria for benthic macroinvertebrate rapid bioassessment
   - Scoring criteria for benthic macroinvertebrate quantitative samples (Surber)
   - Part III—Habitat-Quality Index

Checklist: Aquatic-Life Monitoring and Habitat Assessment

Background Information

Name of water body: _____

Segment number: _____ Station ID: _____

On segment? Yes No

Permit number, if applicable: _____ Circle monitoring objective: ALM ALU UAA RWA

Historic stream characterization:

Intermittent	Intermittent with perennial pools sufficient to support significant aquatic life use	Perennial	Unknown

Basis for historic stream characterization (describe):

Current aquatic-life-use designation (if classified segment or site specific standard determined):

Exceptional High Intermediate Limited

Current assessment status on the (year)_____ water quality inventory, 305(b) report:

Supported Partially supported Not supported Concern Not assessed

Field data entry (FDE) information:

Date entered into FDE: _____ RTAG no.: _____
(TCEQ regional biologists only)

Field data (CRP partners only): Tag no.:

Objective for Aquatic-Life-Use Assessment

Is this water body supporting its designated uses? Yes No Reason:

Known or potential causes of aquatic life use concern or impairment:

Identify sources of pollution:

Point source? Yes No Identify:

Nonpoint source? Yes No Identify:

Ambient toxicity tests in water body? Yes No

Results:

	Sediment Chronic	Sediment Acute	Water Chronic	Water Acute
Significant effect				
No significant effect				

Monitoring Information

Biological monitoring conducted during index period (March 15–June 30 and Oct. 1–Oct. 15) and critical period (July 1–Sept. 30).

Stream characterization event 1, date:

Dry	Pools covering _____% of the _____ meters assessed	Flowing at cfs (measured)

Note: If the sampling event is for an RWA, characterize the receiving stream upstream of the existing discharge point or downstream of the proposed discharge point.

Stream characterization event 2, date:

Dry	Pools covering _____% of the _____ meters assessed	Flowing at cfs (measured)

Describe conditions that may have adversely affected the stream during each sampling event (for example, recent rains, drought, and construction):

Nekton sampling event 1

Minimum 15-minute (900 seconds) electrofishing? Yes No

Minimum 6 seine hauls (or equivalent effort to sample 60 meters)? Yes No

Fish sampling conducted in all available habitat types? Yes No

If no, please describe why:

Benthic-macroinvertebrate sampling event 1

Method(s) used:

Rapid bioassessment (5-minute kicknet or snags):

Quantitative (Surber, snags, or dredge):

Habitat-assessment event 1

TCEQ habitat protocols? Yes No

Streamflow-measurement event 1

Instantaneous measurement? Yes No

USGS gauge reading? Yes No

Nekton sampling event 2

Minimum 15-minute (900 seconds) electrofishing?	Yes	No
Minimum 6 seine hauls (or equivalent effort to sample 60 meters)?	Yes	No
Fish sampling conducted in all available habitat types?	Yes	No

If not, please describe why:

Benthic-macroinvertebrate sampling event 2

Method(s) used:

Rapid bioassessment (5-minute kicknet or snags):

Quantitative (Surber, snags or dredge):

Habitat-assessment event 2:

TCEQ habitat protocols?	Yes	No

If no, you must provide flow, wetted-channel width, photographs, description of bank conditions relative to first event, and description of canopy-cover conditions relative to the first event in this packet.

Streamflow-measurement event 2

Instantaneous measurement:	Yes	No
USGS gauge reading:	Yes	No

Assessment Results (Optional)

Fish-community index event 1:

Exceptional High Intermediate Limited

Fish community index event 2:

Exceptional High Intermediate Limited

Benthic-macroinvertebrate-community index event 1:

Exceptional High Intermediate Limited

Benthic-macroinvertebrate community index event 2:

Exceptional High Intermediate Limited

Habitat index event 1:

Exceptional High Intermediate Limited

Habitat index event 2:

Exceptional High Intermediate Limited

Outline for Use-Attainability-Analysis Report

Introduction

Problem statement
Objectives

Study Area

Description of water body and designated uses and criteria
Environmental features and population characteristics
Permitted discharges
Nonpoint sources
Summary of historical data

Methodologies

Station descriptions
Sampling methods
Survey descriptions

Results and Discussions

Physical evaluation
Hydrology
Habitat
Physicochemical evaluation
Biological evaluation
Fish
Benthic macroinvertebrates
Other

Conclusions

References

Appendixes

Packet for Reporting Biological-Monitoring Data

These forms are available online at <www.tceq.texas.gov/goto/biopacket>.

Nekton Data-Reporting Form

RTAG# | REGION | E-MAIL ID OF COLLECTOR

STATION ID | SEGMENT | SEQUENCE | DATA SOURCE

Station Description_____

Composite—coded as Space, Time, or Both

COMPOSITE SAMPLE

COMPOSITE CATEGORY: T = Time S = Space B = Both

M M D D Y Y Y Y — START DATE

H H M M — START TIME

START DEPTH (SHALLOWEST) . — M = meters F = feet

M M D D Y Y Y Y — END DATE

H H M M — END TIME

END DEPTH (DEEPEST) . — M = meters F = feet

PARAMETRIC DATA

Enter the codes and values appropriate for this sample. Enter "<" or ">" if necessary; otherwise, leave this column blank. Continue, if necessary, on additional worksheets. Codes to describe the habitat-sampling effort are on the back.

Code	< or >	Value	Description

Choose the most characteristic location and report data from this location as representative of the entire reach.

Nekton Parameter Codes

Note: Report all measurements in metric units.

Codes					
98005		Nekton, None Captured	98003		Total No. Fish Species (Richness)
89944		Electrofishing Effort, Duration of Shocking (sec.)	98008		Total No. of Sunfish Species (except bass)
89947		Seining Effort (No. of Seine Hauls)	98010		Total No. of Intolerant Fish Species
89948		Combined Length of Seine Hauls (meters)	98070		% of Individuals as Tolerant Species (Excluding Western Mosquitofish)
89949		Seining Effort, Duration (min.)	98017		Omnivore Individuals (% of community)
89930		Minimum Seine Mesh Size, net average bar (inches)	98021		Invertivore Individuals (% of community)
89931		Maximum Seine Mesh Size, net average bar (inches)	98022		Piscivore Individuals (% of community)
89941		Net Length (meters)	98039		Total No. of Individuals, Seining
89943		Electrofishing Method (1 = boat, 2 = backpack, 3 = tote barge)	98040		Total No. of Individuals, Electrofishing
89976		Area Seined (m^2)	98062		No. of individuals per seine haul
89961		Ecoregion (Texas Ecoregion Code)	98069		No. of individuals per minute electrofishing
98032		Total No. of Native Cyprinid Species	98052		Total No. of Benthic Invertivore Species
98033		Individuals as Nonnative Species (% of community)	98053		Total No. of Benthic Species (catfish, suckers, and darters)
98030		Individuals with Disease or Anomalies (% of community)			
Additional Parameters					
89942		Net or Hook-and-Line Effort, Duration in Water (hrs.)	89951		Cooling-Water Intake Screen (1 = revolving, 2 = static)
89945		Castnetting Effort (No. of casts)	89940		Intake-Screen Collection, Duration (min.)
89907		Trawl, Otter, Duration (min.)	89953		Trawl, Otter, Width (meters)

Benthic Macroinvertebrate Data-Reporting Form

RTAG# | REGION | E-MAIL ID OF COLLECTOR

STATION ID | SEGMENT | SEQUENCE | DATA SOURCE

Station Description_____

Composite—coded as Space, Time, or Both

COMPOSITE SAMPLE

COMPOSITE CATEGORY: T=Time S=Space B=Both

M M D D Y Y Y Y — START DATE

H H M M — START TIME

START DEPTH (SHALLOWEST) . — M = meters F = feet

M M D D Y Y Y Y — END DATE

H H M M — END TIME

END DEPTH (DEEPEST) . — M = meters

PARAMETRIC DATA

Enter the codes and values appropriate for this sample. Enter "<" or ">" if necessary; otherwise, leave this column blank. Continue, if necessary, on additional worksheets. Codes to describe the habitat-sampling effort are on the back.

Code	< or >	Value	Description

Choose the most characteristic location and report data from this location as representative of the entire reach.

Benthic-Macroinvertebrate Parameter Codes

Note: Report all measurements in metric units.
*Indicates parameter measured at sample point (for example, riffle from which benthic sample is collected)

Quantitative Benthic-Sample Descriptors					
89899		Biological-data reporting units (Values: 1= no. of individuals from subsample; 2 = no. of individuals/ft^2; 3 = no. of individuals/m^2; 4 = total no. in kicknet)	89946		Mesh size, any net or sieve (diagonal measurements) for benthic collection (cm)
89901		Surber-sampler effort, area sampled (m^2)	89961		Ecoregion (Texas Ecoregion Code)
89935		Ekman-sampler effort, area sampled (m^2)	84161		Stream order
89934		Petersen-sampler effort, area sampled ($m^{2)}$	90005		Benthos sampled—no organisms present
89933		Hester-Dendy duration (days)	90055		Total taxa (taxa richness), benthos no. taxa
89950		Benthic sampler (1 = Surber, 2 = Ekman, 3 = kicknet, 4 = Petersen, 5 = Hester-Dendy)	90056		Total no. of Diptera taxa
89975		Area of snag surface sampled (m^2)	90057		Total no. of Ephemeroptera taxa
*89921		Undercut bank at sample point (%)	90058		Total no. of intolerant taxa
*89922		Overhanging brush at sample point (%)	90060		EPT taxa (% of community)
*89923		Gravel substrate at sample point (%)	90062		Chironomidae (% of community)
*89924		Sand substrate at sample point (%)	90066		Tolerant taxa (% of community), benthos
*89925		Soft bottom at sample point (%)	90020		Benthic grazers (% of community)
*89926		Macrophyte bed at sample point (%)	90025		Benthic gatherers (% of community)
*89927		Snags and brush at sample point (%)	90030		Benthic filterers (% of community)
*89928		Bedrock at sample point (%)	90067		Dominance (3 taxa) (% of community)
RBAP Benthic Sample Descriptors					
89899		Biological-data reporting units (Values: 1 = no. of individuals from subsample; 2 = no. of individuals/ft^2; 3 = no. of individuals/m^2; 4 = total no. in kicknet)	89946		Mesh size, sieve (diagonal measurements) (cm)
89950		Benthic Sampler (1 = Surber, 2 = Ekman, 3 = kicknet, 4 = Petersen, 5 = Hester-Dendy)	89961		Texas Ecoregion Code
89902		Dip-net effort, area swept (m^2)	84161		Stream order
89903		Kicknet effort, area kicked (m^2)	90005		Benthos el
89904		Kicknet effort, minutes kicked (min.)	90055		Total taxa (taxa Richness), Benthos, no. taxa
89905		Snags-and-shoreline sampling effort, minutes picked	90008		EPT taxa abundance (no. taxa)
89906		Number of individuals in benthic RBA subsample (± 100)	90007		Biotic index (HBI)
89950		Benthic sampler (1= Surber, 2 = Ekman, 3 = kicknet, 4 = Petersen, 5 = Hester-Dendy)	90062		Chironomidae (% of community)
*89921		Undercut bank at sample point (%)	90042		Dominant taxon, benthos (% of community)
*89922		Overhanging brush at sample point (%)	90010		Dominant functional feeding group (% of community)
*89923		Gravel substrate at sample point (%)	90036		Benthic predators (% of community)
*89924		Sand substrate at sample point (%)	90050		Ratio of intolerant : tolerant taxa
*89925		Soft bottom at sample point (%)	90069		Total Trichoptera as Hydropsychidae (%)
*89926		Macrophyte bed at sample point (%)	90052		Total no. non-insect taxa
*89927		Snags and brush at sample point (%)	90025		Benthic collector-gatherers (% of community)
*89928		Bedrock at sample point (%)	90054		Total no. as Elmidae (% of community)

Habitat Data-Reporting Form

RTAG# | REGION | E-MAIL ID OF COLLECTOR

STATION ID | SEGMENT | SEQUENCE | DATA SOURCE

Station Description_____

Composite—code as Space, Time, or Both.

COMPOSITE SAMPLE

COMPOSITE CATEGORY: T=Time S=Space B=Both

M M D D Y Y Y Y — START DATE

H H M M — START TIME

START DEPTH (SHALLOWEST) . — M = meters F = feet

M M D D Y Y Y Y — END DATE

H H M M — END TIME

END DEPTH (DEEPEST) . — M = meters F = feet

PARAMETRIC DATA

Enter the codes and values appropriate for this sample. Enter "<" or ">" if necessary; otherwise, leave this column blank. Continue, if necessary, on additional worksheets. Codes to describe the habitat-sampling effort are on the back.

Code	< or >	Value	Description

Choose the most characteristic location and report data from this location as representative of the entire reach.

Habitat Parameter Codes

HABITAT DESCRIPTORS					
NOTE: All measurements reported in metric units (except for flow)					
72051		Streambed slope over evaluated reach (from USGS map; elevation change in meters / reach length in kilometers)	89844		Dominant substrate type (1 = clay, 2 = silt, 3 = sand, 4 = gravel, 5 = cobble, 6 = boulder, 7 = bedrock, 8 = other)
89859		Approximate drainage area above the most downstream transect from USGS map (km^2)	89845		Average substrate gravel > 2 mm or larger (%)
89860		Length of stream evaluated (km)	84159		Average instream cover (%)
89832		Number of lateral transects made	89929		Number of stream cover types
89861		Average stream width (m)	89846		Average stream-bank erosion (%)
89862		Average stream depth (m)	89847		Average stream-bank angle (degrees)
00061		Instantaneous stream flow (ft^3/sec)	89866		Average width of natural riparian vegetation (m)
89835		Flow measurement method (1=flow-gage station, 2= electronic, 3=mechanical, 4=weir or flume)	89849		Average trees as riparian vegetation (%)
			89850		Average shrubs as riparian vegetation (%)
89848		Channel flow (1 = none, 2 = low, 3 = moderate, 4 = high)	89851		Average grasses and forbs as riparian vegetation (%)
89864		Maximum pool width at time of study (m)	89852		Average cultivated fields as riparian vegetation (%)
89865		Maximum pool depth in study area (m)	89853		Average other as riparian vegetation (%)
89839		Total number of stream bends	89854		Average tree-canopy coverage (%)
89840		Number of well-defined stream bends	89867		Aesthetics (1 = wilderness, 2 = natural, 3 = common, 4 = offensive)
89841		Number of moderately defined stream bends	84161		Stream order
89842		Number of poorly defined stream bends	89961		Texas Ecoregion Code
89843		Total number of riffles	89962		Land-development impact (1 = none, 2 = low, 3 = moderate, 4 = high)

Specific to No Flow with Isolated Pools

Largest pool (m)	89910		Max. depth
	89908		Max. width
	89909		Max. length
Smallest pool (m)	89911		Max. depth
	89912		Max. width
	89913		Max. length
No. perennial pools evaluated	89914		

Quantitative Biological Scoring for Evaluating Aquatic-Life-Use Subcategories Regional-Criteria Worksheets for Fish

Ecoregion 24					
Stream name:			**Location:**	**Date:**	
Collector:			**County:**		
No. seine hauls:			**Electrofishing effort (min.):**		
Metric Category	**Intermediate Totals for Metrics**		**Metric Name**	**Raw Value**	**IBI Score**
	Drainage basin size (km^2)				
Species richness and composition	Number of fish species		Number of fish species		
	Number of native cyprinid species		Number of native cyprinid species		
	Number of benthic invertivore species		Number of benthic invertivore species		
	Number of sunfish species		Number of sunfish species		
	Number of intolerant species		Number of intolerant species		
	Number of individuals as tolerant species[a]		% of individuals as tolerant species[a]		
Trophic composition	Number of individuals as omnivores		% of individuals as omnivores		
	Number of individuals as invertivores		% of individuals as invertivores		
Fish abundance and condition	Number of individuals (seine)		Number of individuals in sample		
	Number of individuals (electrofishing)		Number of individuals / seine haul		
	Number of individuals in sample		Number of individuals / min. electrofishing		
	Number of individuals as nonnative species		% of individuals as nonnative species		
	Number of individuals with disease or anomaly		% of individuals with disease or anomaly		
			Index of Biotic Integrity numeric score:		
			Aquatic-life use:		

TCEQ-20155-A (Rev. 3-05-2014)

Note: These data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

[a] Excluding western mosquitofish.

Quantitative Biological Scoring for Evaluating
Aquatic-Life-Use Subcategories
Regional-Criteria Worksheets for Fish

Ecoregions 25 and 26					
Stream name:		**Location:**		**Date:**	
Collector:		**County:**			
No. seine hauls:		**Electrofishing effort (min.):**			
Metric Category	**Intermediate Totals for Metrics**		**Metric Name**	**Raw Value**	**IBI Score**
	Drainage basin size (km^2)				
Species richness and composition	Number of fish species		Number of fish species		
	Number of native cyprinid species		Number of native cyprinid species		
	Number of sunfish species		Number of sunfish species		
Trophic composition	Number of individuals as omnivores		% of individuals as omnivores		
	Number of individuals as invertivores		% of individuals as invertivores		
Fish abundance and condition	Number of individuals (seine)		Number of individuals in sample		
	Number of individuals in sample		Number of individuals / seine haul		
	Number of individuals as nonnative species		% of individuals as nonnative species		
	Number of individuals with disease or anomaly		% of individuals with disease or anomaly		
			Index of Biotic Integrity numeric score:		
			Aquatic-life use:		

TCEQ-20155-B (Rev. 3-05-2014)

Note: These data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

Quantitative Biological Scoring for Evaluating Aquatic-Life-Use Subcategories Regional-Criteria Worksheets for Fish

Ecoregions 27, 29, and 32

Stream name:	**Location:**	**Date:**
Collector:	**County:**	
No. seine hauls:	**Electrofishing effort (min.):**	

Metric Category	Intermediate Totals for Metrics		Metric Name	Raw Value	IBI Score
	Drainage basin size (km^2)				
Species richness and composition	Number of fish species		Number of fish species		
	Number of native cyprinid species		Number of native cyprinid species		
	Number of benthic invertivore species		Number of benthic invertivore species		
	Number of sunfish species		Number of sunfish species		
Trophic composition	Number of individuals as tolerant species[a]		% of individuals as tolerant species[a]		
	Number of individuals as omnivores		% of individuals as omnivores		
Fish abundance and condition	Number of individuals as invertivores		% of individuals as invertivores		
	Number of individuals as piscivores		% of individuals as piscivores		
	Number of individuals (seine)		Number of individuals in sample		
	Number of individuals (electrofishing)		Number of individuals / seine haul		
	Number of individuals in sample		Number of individuals / min. electrofishing		
	Number of individuals as nonnative species		% of individuals as nonnative species		
	Number of individuals with disease or anomaly		% of individuals with disease or anomaly		
			Index of Biotic Integrity numeric score:		
			Aquatic-life use:		

TCEQ-20155-C (Rev. 3-05-2014)

Note: These data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

[a] Excluding western mosquitofish.

Quantitative Biological Scoring for Evaluating Aquatic-Life-Use Subcategories Regional-Criteria Worksheets for Fish

Ecoregion 30					
Stream name:			**Location:**	**Date:**	
Collector:			**County:**		
No. seine hauls:			**Electrofishing effort (min):**		
Metric Category	**Intermediate Totals for Metrics**		**Metric Name**	**Raw Value**	**IBI Score**
	Drainage basin size (km^2)				
Species richness and composition	Number of fish species		Number of fish species		
	Number of native cyprinid species		Number of native cyprinid species		
	Number of benthic invertivore species		Number of benthic invertivore species		
	Number of sunfish species		Number of sunfish species		
	Number of intolerant species		Number of intolerant species		
Trophic composition	Number of individuals as tolerant species[a]		% of individuals as tolerant species[a]		
	Number of individuals as omnivores		% of individuals as omnivores		
	Number of individuals as invertivores		% of individuals as invertivores		
	Number of individuals as piscivores		% of individuals as piscivores		
Fish abundance and condition	Number of individuals (seine)		Number of individuals in sample		
	Number of individuals (electrofishing)		Number of individuals / seine haul		
	Number of individuals in sample		Number of individuals / min. electrofishing		
	Number of individuals as nonnative species		% of individuals as nonnative species		
	Number of individuals with disease or anomaly		% of individuals with disease or anomaly		
			Index of Biotic Integrity numeric score:		
			Aquatic-life use:		

TCEQ-20155-D (Rev. 3-05-2014)

Note: These data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

[a] Excluding western mosquitofish.

Quantitative Biological Scoring for Evaluating Aquatic-Life-Use Subcategories Regional-Criteria Worksheets for Fish

Ecoregion 31					
Stream name:			**Location:**	**Date:**	
Collector:			**County:**		
No. seine hauls:			**Electrofishing effort (min.):**		
Metric Category	**Intermediate Totals for Metrics**		**Metric Name**	**Raw Value**	**IBI Score**
	Drainage basin size (km^2)				
Species richness and composition	Number of fish species		Number of fish species		
	Number of native cyprinid species		Number of native cyprinid species		
	Number of benthic species		Number of benthic species		
	Number of sunfish species		Number of sunfish species		
	Number of individuals as tolerant species[a]		% of individuals as tolerant species[a]		
Trophic composition	Number of individuals as omnivores		% of individuals as omnivores		
	Number of individuals as invertivores		% of individuals as invertivores		
	Number of individuals as piscivores		% of individuals as piscivores		
Fish abundance and condition	Number of individuals (seine)		Number of individuals in sample		
	Number of individuals (electrofishing)		Number of individuals / seine haul		
	Number of individuals in sample		Number of individuals / min. electrofishing		
	Number of individuals as nonnative species		% of individuals as nonnative species		
	Number of individuals with disease or anomaly		% of individuals with disease or anomaly		
			Index of Biotic Integrity numeric score:		
			Aquatic-life use:		

TCEQ-20155-E (Rev. 3-05-2014)

Note: These data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

[a] Excluding western mosquitofish.

Quantitative Biological Scoring for Evaluating Aquatic-Life-Use Subcategories Regional-Criteria Worksheets for Fish

Ecoregions 33 and 35					
Stream name:			**Location:**	**Date:**	
Collector:			**County:**		
No. seine hauls:			**Electrofishing effort (min.):**		
Metric Category	**Intermediate Totals for Metrics**		**Metric Name**	**Raw Value**	**IBI Score**
	Drainage basin size (km^2)				
Species richness and composition	Number of fish species		Number of fish species		
	Number of native cyprinid species		Number of native cyprinid species		
	Number of benthic invertivore species		Number of benthic invertivore species		
	Number of sunfish species		Number of sunfish species		
	Number of intolerant species		Number of intolerant species		
	Number of individuals as tolerant species[a]		% of individuals as tolerant species[a]		
Trophic composition	Number of individuals as omnivores		% of individuals as omnivores		
	Number of individuals as invertivores		% of individuals as invertivores		
	Number of individuals as piscivores		% of individuals as piscivores		
Fish abundance and condition	Number of individuals (seine)		Number of individuals in sample		
	Number of individuals (electrofishing)		Number of individuals / seine haul		
	Number of individuals in sample		Number of individuals / min. electrofishing		
	Number of individuals as nonnative species		% of individuals as nonnative species		
	Number of individuals with disease or anomaly		% of individuals with disease or anomaly		
			Index of Biotic Integrity numeric score:		
			Aquatic-life use:		

TCEQ-20155-F (Rev. 3-05-2014)

Note: These data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

[a] Excluding western mosquitofish.

Quantitative Biological Scoring for Evaluating
Aquatic Life Use Subcategories
Regional Criteria Worksheets for Fish

Ecoregion 34					
Stream name:			**Location:**		**Date:**
Collector:			**County:**		
No. seine hauls:			**Electrofishing effort (min.):**		
Metric Category	**Intermediate Totals for Metrics**		**Metric Name**	**Raw Value**	**IBI Score**
	Drainage basin size (km^2)				
Species richness and composition	Number of fish species		Number of fish species		
	Number of native cyprinid species		Number of native cyprinid species		
	Number of benthic invertivore species		Number of benthic invertivore species		
	Number of sunfish species		Number of sunfish species		
	Number of intolerant species		Number of intolerant species		
	Number of individuals as tolerants[a]		% of individuals as tolerant species[a]		
Trophic composition	Number of individuals as omnivores		% of individuals as omnivores		
	Number of individuals as invertivores		% of individuals as invertivores		
Fish abundance and condition	Number of individuals (seine)		Number of individuals in sample		
	Number of individuals (electrofishing)		Number of individuals / seine haul		
	Number of individuals in sample		Number of individuals / min. electrofishing		
	Number of individuals as nonnative species		% of individuals as nonnative species		
	Number of individuals with disease or anomaly		% of individuals with disease or anomaly		
			Index of Biotic Integrity numeric score:		
			Aquatic-life use:		

TCEQ-20155-G (Rev. 3-05-2014)

Note: These data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

[a] Excluding western mosquitofish.

BIBI Metrics and Scoring for Kick Samples, Rapid Bioassessment Protocol—Benthic Macroinvertebrates

Stream name:		
Date:	**Collectors:**	
Location:		
County:	**Ecoregion No.:**	
Type of assessment: UAA ALA ALM RWA		

Metric	**Value**	**Score**
1. Taxa richness		
2. EPT taxa abundance		
3. Biotic index (HBI)		
4. % Chironomidae		
5. % Dominant taxon		
6. % Dominant FFG		
7. % Predators		
8. Ratio of intolerant : tolerant taxa		
9. % total Trichoptera as Hydropsychidae		
10. No. of non-insect taxa		
11. % Collector-gatherers		
12. % of total number as Elmidae		
Aquatic-life-use point-score ranges:	Exceptional: High: Intermediate: Limited:	> 36 29–36 22–28 < 22
Total score:		
Aquatic-life use:		

The scoring form for Surber samples—previously included in the Biological Monitoring Packet—entitled *Metrics and Scoring for Surber Samples for Benthic Macroinvertebrates by Bioregion: Central, East, or North*—now appears in Appendix F, "Surber-Sampler Protocols."

Streamflow (Discharge) Measurement Form

Streamflow (Discharge) Measurement Form

Stream:____Date:______________

Station ____

Description:____

Time Began:___________ Time Ended: ___________Meter Type:_____________

Observers: _____________Total Stream Width: ________Section Width (W):_______

Observations:____

Section Midpoint (ft)	Section Depth (ft) (D)	Sensor Depth (ft)	Velocity (V)		Flow (Q) (ft^3/s) Q = (W)(D)(V)
			At Point (ft/s)	Average (ft/s)	
$m^3/s \times 35.3 = ft^3/s$			**Total Flow (Discharge)**		

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Page 1 of ___	**Part I—Stream Physical-Characteristics Worksheet**	

Observers:	Date:	Time:
Weather conditions:		
Stream:	Stream segment no.	
Location of site:	Length of reach:	
Observed stream uses:		
Stream type (circle one): **perennial** or **intermittent with perennial pools**		

Stream bends:	No. well defined		No. moderately defined		No. poorly defined	

Aesthetics (circle one): **(1) wilderness (2) natural (3) common (4) offensive**

Channel obstructions or modifications:	No. of riffles	

Channel flow status (circle one): **high moderate low no flow**

Riparian vegetation (%):	**Left Bank**	**Right Bank**	Maximum pool depth:
Trees			Maximum pool width:
Shrubs			**Notes:**
Grasses or forbs			
Cultivated fields			
Other			

Site map:

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Part I—Stream Physical-Characteristics Worksheet (continued)

Date: **Stream Name:**

Location of transect	Stream width (m)	Left-bank slope (°)	Left-bank erosion potential (%)	Stream depths (m) at points across transect Thalweg Depth:	Right-bank slope (°)	Right-bank erosion potential (%)	Tree canopy (%)
							Total
	Habitat type (circle one) **Riffle** **Run** **Glide** **Pool**		Dominant substrate type	Dominant types riparian vegetation Left bank: Right bank:		% Gravel or larger	CL
							CR
Macrophytes (circle one) **Abundant** **Common** **Rare** **Absent**	Algae (circle one) **Abundant** **Common** **Rare** **Absent**		Width of natural buffer vegetation (m) LB:	Instream cover types RB:		% Instream cover	LB
							RB

Location of Transect	Stream width (m)	Left-bank slope (°)	Left-bank erosion potential (%)	Stream depths (m) at points across transect Thalweg depth:	Right-bank slope (°)	Right-bank erosion potential (%)	Tree canopy (%)
							Total
	Habitat type (circle one) **Riffle** **Run** **Glide** **Pool**		Dominant substrate type	Dominant types riparian vegetation Left bank: Right bank:		% Gravel or larger	CL
							CR
Macrophytes (circle one) **Abundant** **Common** **Rare** **Absent**	Algae (circle one) **Abundant** **Common** **Rare** **Absent**		Width of natural buffer vegetation (m) LB:	Instream cover types RB:		% Instream cover	LB
							RB

Location of transect	Stream width (m)	Left-bank slope (°)	Left-bank erosion potential (%)	Stream depths (m) at points across transect Thalweg depth:	Right-bank slope (°)	Right-bank erosion potential (%)	Tree canopy (%)
							Total
	Habitat type (circle one) **Riffle** **Run** **Glide** **Pool**		Dominant substrate type	Dominant types riparian vegetation Left bank: Right bank:		% Gravel or larger	CL
							CR
Macrophytes (circle one) **Abundant** **Common** **Rare** **Absent**	Algae (circle one) **Abundant** **Common** **Rare** **Absent**		Width of natural buffer vegetation (m) LB:	Instream cover types RB:		% Instream cover	LB
							RB

Part I—Stream Physical-Characteristics Worksheet (continued)

Date: **Stream Name:**

Location of transect	Stream width (m)	Left-bank slope (°)	Left-bank erosion potential (%)	Stream depths (m) at points across transect Thalweg Depth:	Right-bank slope (°)	Right-bank erosion potential (%)	Tree canopy (%)
							Total
	Habitat type (circle one) **Riffle** **Run** **Glide** **Pool**		Dominant substrate type	Dominant types riparian vegetation Left bank: Right bank:		% Gravel or larger	CL
							CR
Macrophytes (circle one) **Abundant** **Common** **Rare** **Absent**	Algae (circle one) **Abundant** **Common** **Rare** **Absent**		Width of natural buffer vegetation (m) LB:	RB:	Instream cover types	% Instream cover	LB
							RB

Location of Transect	Stream width (m)	Left-bank slope (°)	Left-bank erosion potential (%)	Stream depths (m) at points across transect Thalweg depth:	Right-bank slope (°)	Right-bank erosion potential (%)	Tree canopy (%)
							Total
	Habitat type (circle one) **Riffle** **Run** **Glide** **Pool**		Dominant substrate type	Dominant types riparian vegetation Left bank: Right bank:		% Gravel or larger	CL
							CR
Macrophytes (circle one) **Abundant** **Common** **Rare** **Absent**	Algae (circle one) **Abundant** **Common** **Rare** **Absent**		Width of natural buffer vegetation (m) LB:	RB:	Instream cover types	% Instream cover	LB
							RB

Location of transect	Stream width (m)	Left-bank slope (°)	Left-bank erosion potential (%)	Stream depths (m) at points across transect Thalweg depth:	Right-bank slope (°)	Right-bank erosion potential (%)	Tree canopy (%)
							Total
	Habitat type (circle one) **Riffle** **Run** **Glide** **Pool**		Dominant substrate type	Dominant types riparian vegetation Left bank: Right bank:		% Gravel or larger	CL
							CR
Macrophytes (circle one) **Abundant** **Common** **Rare** **Absent**	Algae (circle one) **Abundant** **Common** **Rare** **Absent**		Width of natural buffer vegetation (m) LB:	RB:	Instream cover types	% Instream cover	LB
							RB

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Part II—Summary of Physical Characteristics of Water Body

Using information from all of the transects and measurements in Part I and other sources, report the following general characteristics or averages for the entire reach:

Stream Name:	**Date:**
Physical Characteristics	**Value**
Stream bed slope over evaluated reach (from USGS map; elevation change in meters / reach length in kilometers)	
Approximate drainage area above the transect furthest downstream (from USGS or county highway map in km^2)	
Stream order	
Length of stream evaluated (meters or kilometers)	
Number of lateral transects made	
Average stream width (meters)	
Average stream depth (meters)	
Stream discharge (ft^3/sec)	
Flow measurement method	
Channel flow status (high, moderate, low, or no flow)	
Maximum pool width (meters)	
Maximum pool depth (meters)	
Total number of stream bends	
Number of well-defined bends	
Number of moderately defined bends	
Number of poorly defined bends	
Total number of riffles	
Dominant substrate type	
Average percent of substrate gravel-sized or larger	
Average percent instream cover	
Number of stream cover types	
Average percent stream-bank erosion potential	
Average stream-bank slope (degrees)	
Average width of natural buffer vegetation (meters)	
Average percent composition of riparian vegetation by: (total to equal 100%)	
Trees	
Shrubs	
Grasses and forbs	
Cultivated fields	
Other	
Average percent of tree-canopy coverage	
Overall aesthetic appraisal of the stream	

Part III—Habitat-Quality Index

Habitat Parameter		Scoring Category		
Available Instream Cover	**Abundant** > 50% of substrate favorable for colonization and fish cover; good mix of several stable (not new fall or transient) cover types such as snags, cobble, undercut banks, macrophytes	**Common** 30–50% of substrate supports stable habitat; adequate habitat for maintenance of populations; may be limited in the number of different habitat types	**Rare** 10–29.9% of substrate supports stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed	**Absent** < 10% of substrate supports stable habitat; lack of habitat is obvious; substrate unstable or lacking
Score_____	4	3	2	1
Bottom Substrate Stability	**Stable** > 50% gravel or larger substrate; gravel, cobble, boulders; dominant substrate type is gravel or larger	**Moderately Stable** 30–50% gravel or larger substrate; dominant substrate type is mix of gravel with some finer sediments	**Moderately Unstable** 10–29.9% gravel or larger substrate; dominant substrate type is finer than gravel, but may still be a mix of sizes	**Unstable** < 10% gravel or larger substrate; substrate is uniform sand, silt, clay, or bedrock
Score_____	4	3	2	1
Number of Riffles To be counted, riffles must extend >50% the width of the channel and be at least as long as the channel width	**Abundant** ≥ 5 riffles	**Common** 2–4 riffles	**Rare** 1 riffle	**Absent** No riffles
Score_____	4	3	2	1
Dimensions of Largest Pool	**Large** Pool covers more than 50% of the channel width; maximum depth is > 1 meter	**Moderate** Pool covers approximately 50% or slightly less of the channel width; maximum depth is 0.5–1 meter	**Small** Pool covers approximately 25% of the channel width; maximum depth is < 0.5 meter	**Absent** No existing pools, only shallow auxiliary pockets
Score_____	4	3	2	1
Water Level	**High** Water reaches the base of both lower banks; < 5% of channel substrate is exposed	**Moderate** Water fills >75% of the channel; or < 25% of channel substrate is exposed	**Low** Water fills 25–75% of the available channel or riffle substrates are mostly exposed	**No Flow** Very little water in the channel and mostly present in standing pools, or stream is dry
Score_____	3	2	1	0

Part III—Habitat-Quality Index (continued)

Habitat Parameter	Scoring Category			
Bank Stability	**Stable** Little evidence (< 10%) of erosion or bank failure; bank angles average < 30º	**Moderately Stable** Some evidence (10–29.9%) of erosion or bank failure; small areas of erosion mostly healed over; bank angles average 30–39.9°	**Moderately Unstable** Evidence of erosion or bank failure is common (30–50%); high potential of erosion during flooding; bank angles average 40–60°	**Unstable** Large and frequent evidence (> 50%) of erosion or bank failure; raw areas frequent along steep banks; bank angles average > 60°
Score_____	3	2	1	0
Channel Sinuosity	**High** ≥ 2 well-defined bends with deep outside areas (cut banks) and shallow inside areas (point bars) present	**Moderate** 1 well-defined bend **or** ≥ 3 moderately-defined bends present	**Low** < 3 moderately-defined bends **or** only poorly-defined bends present	**None** Straight channel; may be channelized
Score_____	3	2	1	0
Riparian Buffer Vegetation	**Extensive** Width of natural buffer is > 20 meters	**Wide** Width of natural buffer is 10.1–20 meters	**Moderate** Width of natural buffer is 5–10 meters	**Narrow** Width of natural buffer is < 5 meters
Score_____	3	2	1	0
Aesthetics of Reach	**Wilderness** Outstanding natural beauty; usually wooded or unpastured area; no obvious indications of human activity	**Natural Area** Trees or native vegetation is common; some development evident (from fields, pastures, rural dwellings) little evidence of human activity	**Common Setting** Not offensive; area is developed, but uncluttered such as in an urban park	**Offensive** Stream does not enhance the aesthetics of the area; cluttered; highly developed; may be a dumping area
Score_____	3	2	1	0
Total Score_____				

Habitat-Quality Index

26– 31	**Exceptional**
20–25	**High**
14– 19	**Intermediate**
≤ 13	**Limited**

APPENDIX D

BIOLOGICAL FACT SHEETS

Use the information and forms in this appendix to support the biological-monitoring program in addition to the forms included in the biological-monitoring packets.

Biological-Monitoring Fact Sheets

Aquatic-Life Monitoring

ALM events are typically scheduled as part of the cooperative monitoring schedule and are conducted to derive baseline data on environmental conditions and to determine if criteria for aquatic-life uses and dissolved oxygen are being attained. ALM samples can contribute to the establishment of an appropriate aquatic-life use, if the optional diel event is included in the data gathering. An ALM is appropriate for routine monitoring sites, and should be representative of the water body being assessed. Data are gathered over a year of sampling period with at least one month between each monitoring event.

Biological Events	Number of Index-Period Events	Number of Critical-Period Events
Characterization of the fish assemblage	1	1
Characterization of the benthic macroinvertebrate community		
Assessment of the stream's physical habitat		
Instantaneous field measurements		
Measurement of flow discharge		
24-hour DO monitoring		
Conventional water-chemistry sample*		

***Conventional water chemistry is optional, but strongly recommended for the evaluation of the biological event.**

Two biological events are required over **one year**. One event is to be conducted during the critical period (July 1–September 30) and the other event during the non-critical portion of the index period (March 15–June 30 or October 1–15) with at least one month between events.

When the ALM is conducted and the samples indicate that the presumed use is supported, this will be adequate information to confirm the aquatic-life use. However, if the ALM is conducted on a water body not listed in either Appendix A or D of the Texas Surface Water Quality Standards and the samples indicate that the presumed use is not supported, an ALA or UAA (detailed in the following sections) may be necessary to determine the appropriate aquatic-life use and the **optional diel events** (detailed below) must be included in the data gathering. The WQSG must be notified and consulted with to determine the appropriateness of an ALA or UAA.

When the ALM is conducted on a water body listed in either Appendix A or D of the TSWQS and the samples indicate that the adopted use is not supported, the water body will be placed on the 303(d) List. A UAA may be necessary to determine the appropriate aquatic-life use and the **optional diel events** (detailed below) must be included in the data gathering. You must notify and consult with the WQSG to determine the appropriateness of a UAA.

Optional Diel Events

Biological Events	Number of Index-Period Events	Number of Critical-Period Events
24-hour DO monitoring	1	2
Measurement of flow discharge		

Besides the two monitoring events described above, a minimum of **three additional diel events coupled with flow discharge measurements** must be conducted. Two events must be conducted during the critical period and one event must be conducted during the non-critical portion of the index period.

Aquatic-Life Assessment

An ALA is conducted on an unclassified water body not already included in Appendix D of the TSWQS that has previously been assessed and determined not to attain the presumed aquatic-life use or the associated dissolved-oxygen criterion (i.e., listed in Category 5c). The purpose is to determine the appropriate aquatic-life use and the associated dissolved-oxygen criterion.

Biological Events	Number of Index-Period Events	Number of Critical-Period Events
Characterization of the fish assemblage	1st year 1; 2nd year 1	1st year 1; 2nd year 1
Characterization of the benthic macroinvertebrate community		
Assessment of the stream's physical habitat assessment		
Instantaneous field measurements		
Measurement of flow discharge		
24-hour DO monitoring		
Conventional water-chemistry sample		

Four biological events are required over **two years**. For each year, one event is to be conducted during the critical period (July 1–September 30) and the other event during the non-critical portion of the index period (March 15–June 30 or October 1–15) with at least one month between monitoring events.

Site and reach selection must ensure that adequate data are generated to accurately characterize biotic integrity through the entire study area. This may involve more than one site, depending on the size of the water body. **Sites and reaches must be selected in consultation with the WQSG.**

Exceptions to the number of biological events required as determined by the WQSG. If an ALA was required based on the results of ALM and the first year's samples from the ALA *agree* with the results of the ALM, then the second year's biological events for the ALA are not required. If an ALA was required based on the results of ALM and the first year's samples from the ALA *do not agree* with the results of the ALM, then the second year's biological events for the ALA are required. The aquatic-life use indicated by the combined results of the ALA and ALM will be considered for Appendix D in the next TSWQS revision.

Additional Diel Events

Biological Events	Number of Index-Period Events	Number of Critical-Period Events
24-hour DO monitoring	1st year 1; 2nd year 1	1st year 2; 2nd year 2
Measurement of flow discharge		

Besides the four monitoring events described above, a minimum of **six additional diel events coupled with flow-discharge measurements** must be conducted. Four of the events should be conducted during the critical period with two during year 1, and two during year 2. The remaining two events should be conducted during the index period with one during year 1, and one during year 2.

Try to collect all samples when flows are at or above critical low flow. Discuss any deviations from the above procedure with the WQSG.

Exceptions to the number of additional diel events required as determined by the WQSG. If an ALA was required based on the results of ALM and the first year's samples from the ALA *agree* with the results of the ALM, then the second year's additional diel events for the ALA are not required. If an ALA was required based on the results of ALM and the first year's samples from the ALA *do not agree* with the results of the ALM, then the second year's additional diel events for the ALA are required.

Use-Attainability Analysis

A UAA is conducted to establish or change an assigned aquatic-life use or dissolved-oxygen criteria. The purpose is to determine the appropriate aquatic-life use and the associated dissolved-oxygen criteria. All activities should be coordinated through the WQSG to determine the appropriateness of the UAA study plan.

Biological Events	Number of Index-Period Events	Number of Critical-Period Events
Characterization of the fish assemblage	1	1st year 1; 2nd year 1
Characterization of the benthic macroinvertebrate community		
Assessment of the stream's physical habitat		
Instantaneous field measurements		
Measurement of flow discharge		
24-hour DO monitoring		
Conventional water-chemistry sample		

Three biological events are required over **two years**. Two of the events are to be conducted during the critical period (July 1–September 30) with one during year 1 and the second during year 2. The third event should be conducted during the non-critical portion of the index period (March 15–June 30 or October 1–15) in either year 1 or year 2. There should be at least one month between monitoring events.

Site and reach selection must ensure that adequate data are generated to accurately characterize biotic integrity through the entire study area. To accomplish this, sampling of multiple sites or reaches will be required for most water bodies. **Sites and reaches should be selected in consultation with the WQSG.**

Additional Diel Events

Biological Events	Number of Index-Period Events	Number of Critical-Period Events
24-hour DO monitoring	3	1st year 2; 2nd year 2
Measurement of flow discharge		

Besides the three monitoring events described above, a minimum of **seven additional diel events coupled with flow-discharge measurements** must be conducted. Three of the events should be conducted during the critical period with two during year 1, and two during year 2. The remaining three events should be conducted during the index period with no more than six events from both the critical and index periods in any one year.

Try to collect all samples when flows are at or above critical low flow. Discuss any deviations from the above procedure with the WQSG.

Receiving-Water Assessment

An RWA is conducted on unclassified water bodies that are the subject of a permitted activity involving wastewater. The purpose is to generate physical, chemical, and biological data to be used in identifying the appropriate aquatic-life use and the associated dissolved-oxygen criteria.

Biological Events	Number of Index-Period Events	Number of Critical-Period Events
Characterization of the fish assemblage		1
Characterization of the benthic macroinvertebrate community		
Assessment of the stream physical habitat		
Instantaneous field measurements		
Measurement of flow discharge		
24-hour DO monitoring*		
Conventional water-chemistry sample*		

One biological event is required, but two are strongly recommended for determining the appropriate aquatic-life use. Try to ensure that data are collected during the index period (March 15–October 15) and preferably within the critical period (July 1–Sept. 30).

***Conventional water-chemistry and 24-hour DO monitoring are optional, but strongly recommended, for the evaluation of the biological event.**

The RWA typically involves a single site upstream of an existing discharge or downstream of a proposed new discharge. Additional sites may be required depending on the size of the discharge. Study sites and reaches should be representative of the water bodies being evaluated and should be selected in consultation with the WQSIT.

The aquatic-life use indicated by the RWA will be considered for Appendix D in the next TSWQS revision.

APPENDIX E

GLOSSARY

Algae

Plants that lack true roots, stems, and leaves. For the physical assessment described herein, algae consist of nonvascular plants that attach to rocks and debris or are free floating in the water. Such plants may be green, blue-green, or olive, may be slimy to the touch, and usually have a coarse filamentous structure.

Aquatic-life assessment (ALA)

A category of biological monitoring conducted on unclassified water bodies not included in Appendix D of the Texas Surface Water Quality Standards that have previously been assessed and found not to support the presumed aquatic-life use.

Aquatic-life use (ALU)

A beneficial-use designation (in state water quality standards) in which the water body provides suitable habitat for survival and reproduction of desirable fish, benthic macroinvertebrates, shellfish, and other aquatic organisms.

Aquatic macrophytes

Vascular plants that usually are arranged in zones corresponding closely to successively greater depths in shallow water. The characteristic plant forms that dominate these gradients (in order of decreasing depth) are: (1) submerged rooted aquatics, (2) rooted aquatics with floating leaves, (3) emergent rooted aquatics, and (4) marginal mats. Some vascular plants (like duckweed) may live unattached in the water and may occur anywhere on the water surface.

Aquatic-life monitoring (ALM)

A category of biological monitoring that is routine and conducted to provide baseline data on environmental conditions or to determine if criteria for aquatic-life use or dissolved-oxygen are being attained. This category also includes reference-condition or ecoregion monitoring.

Bank

The portion of the channel that tends to restrict lateral movement of water. It often has a slope less than 90º and exhibits a distinct break in slope from the stream bottom. Also, a distinct change in the substrate materials or vegetation may delineate the bank.

Bankfull

The elevation on a stream bank where flooding begins. It is associated with the flow that fills the channel to its top and just begins to spill out onto the floodplain. In incised channels, this

elevation is determined by using a series of common stage indicators that may be situated along the boundary of the bankfull channel. Bankfull condition, recurs, on average, every 1.5 years.

Benthic organisms

Aquatic, bottom-dwelling organisms that include worms, leeches, snails, flatworms, burrowing mayflies, clams, and various insects.

Biological diversity

The variety and variability among living organisms and the ecological complexes in which they occur. Diversity can be defined as the number of different items and their relative frequencies. For biological diversity, these items are organized at many levels, ranging from complete ecosystems to the biochemical structures that are the molecular basis of heredity. Thus, the term encompasses different ecosystems, species, and genes.

Biological integrity

The ability of an aquatic ecosystem to support and maintain a balanced, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats within a region.

Bloom

The accelerated growth of algae or higher aquatic plants in a body of water. This is often related to pollutants that increase the rate of growth.

$CBOD_5$

The quantity of oxygen used after five days in the biochemical oxidation of organic matter present in wastewater as measured by procedures described in *Standard Methods*.

Channel

That portion of the landscape that contains the bank and the stream bottom. It is distinct from the surrounding area due to breaks in the general slope of the land, lack of terrestrial vegetation, and changes in the composition of substrate materials.

Channelization

Straightening and deepening streams so water will move faster, a method of flood control that disturbs fish and wildlife habitats and can interfere with a water body's ability to assimilate waste.

Classified water body

Also "designated water body." A water body that is protected by site-specific criteria. The classified segments are listed and described in Appendixes A and C of Chapter 307.10 of the Texas Surface Water Quality Standards. Classified waters include most rivers and their major tributaries, major reservoirs, and estuaries.

Criteria

Water quality conditions that are to be met in order to support and protect desired uses.

Cubic foot per second (ft^3/s or cfs)

A commonly used measure of the rate of flow where a 1-cubic-foot volume of water travels 1 foot in 1 second.

Cut bank

The outside (concave) bank of a stream-channel bend characterized by high erosion. Streamflow usually increases along the cut-bank side of the channel.

Detritus

Decaying organic material.

Dissolved oxygen

The oxygen freely available in water. Dissolved oxygen is vital to fish and other aquatic life and for the prevention of odors. Traditionally, the level of dissolved oxygen has been accepted as the single most important indicator of a water body's ability to support desirable aquatic life.

Ecological impact

The effect that a human or natural activity has on living organisms and their abiotic (non-living) environment.

Eddy current

A circular water movement formed on the side of a main current. Eddies may be formed where the main stream passes obstructions (logs, rocks).

Effluent

Wastewater (treated or untreated) that flows out of a treatment plant or industrial outfall (point source) before entering a water body.

Emergent vegetation

Aquatic macrophytes (plants), such as cattails, that are rooted in the sediment, near shore or in marshes, with nearly all of the leaves above the water surface.

Euphotic zone

The depth of water in a lake or ocean exposed to enough sunlight for photosynthesis to occur.

Family

A group of related plants or animals forming a category ranking above a genus and below an order and usually comprising several to many genera.

Floating vegetation

Rooted plants (some free floating) with leaves floating on the surface (for example, water lily, water shield, duckweed, and water hyacinths).

Floodplain

A level land area adjacent to rivers and streams that is subject to recurring inundation. Formed by the deposition of sediment during periodic floods. Floodplains contain such features as levees, back swamps, delta plains, and oxbow lakes.

Fork length

Fish—Greatest distance in a straight line from the tip of the snout to the center of the fork in the caudal fin.

Genus

A category of biological classification ranking between family and species, comprising structurally or phylogenetically (evolutionarily) related species and designated by a Latin or Latinized capitalized singular noun.

Glide

Portion of the water column in which the flow is characterized by slow moving laminar flow, similar to that which would be found in a shallow canal. Water-surface gradient over a glide is nearly zero, so velocity is slow, but flow is from shore to shore without eddy development. A glide is too shallow to be a pool but has too little water velocity to be a run.

Habitat

The area in which an organism lives.

Index of biotic integrity (IBI)

A composite index of the overall condition of a fish or benthic community based on the cumulative score of separate metrics.

Indicator organism

An organism, species, or community that indicates the presence of a certain environmental condition or conditions.

Intermittent stream

A stream that has a period of zero flow for at least one week during most years. Where flow records are available, a stream with a 7Q2 flow of less than 0.1 cfs is considered intermittent. The critical low flow (7Q2) is the lowest flow that occurs for seven consecutive days during a two-year period as statistically determined from historical data.

Intermittent stream with perennial pools

A stream that may have periods of zero flow or a 7Q2 flow of less than 0.1 cfs, but maintains pools that create significant aquatic-life uses.

Intolerant organism

An organism that is sensitive to degradation in water quality and habitat. Sensitive organisms are usually driven from an area or killed as the result of some contaminant, especially organic pollution (for example, sewage, feedlot runoff, food waste).

Invertebrate

Animal lacking a backbone.

Lotic

Of, relating to, or living in moving fresh water.

Macrophyte

Any large vascular plant that can be seen without the aid of a microscope or magnifying device (cattails, rushes, arrowhead, water lily, and other aquatic species).

Natural vegetative buffer

An area of either natural or native vegetation that buffers the water body from terrestrial runoff and the activities of man. In natural areas, it may be much greater than the riparian zone width. In human-altered settings, the natural vegetative buffer limit is at the point of human influence in the riparian zone such as a road, parking lot, pasture, or crop field. It is the width of this buffer that we are most interested in measuring for quantifying potential stream impairments.

Nekton

Free swimming organisms (for example, fish, insects).

Nonpoint sources

Pollution sources that are diffuse and do not have a single point of origin or are not introduced into a receiving stream from a specific outfall. The pollutants are generally carried off the land by stormwater runoff. The commonly used categories for nonpoint sources are agriculture, silviculture, urban, mining, construction, dams and channels, land disposal, and saltwater intrusion.

Nutrient

Any substance used by living things to promote growth. The term is generally applied to nitrogen and phosphorus in water and wastewater, but is also applied to other essential and trace elements.

Outfall

A designated point of effluent discharge.

Overhanging vegetation

Vegetation that overhangs the water column and provides food or cover for fish and benthic macroinvertebrates or shades the water from solar radiation.

Periphyton

Organisms that cling to rocks, plants, logs, tires, and other instream debris.

Perennial stream

A stream that does not have a period of zero flow for more than one week or where the 7Q2 flow is greater than 0.1 cfs.

pH

The hydrogen-ion activity of water caused by the breakdown of water molecules and presence of dissolved acids and bases.

Photosynthesis

The manufacture by plants of carbohydrates and oxygen from carbon dioxide and water in the presence of chlorophyll using sunlight as an energy source.

Point bar

The inside (convex) bank of a stream channel bend characterized by high deposition of sand, gravel, or cobble. The top of the point bar defines the floodplain. Point bars are built up during periods of flooding and are usually devoid of woody vegetation.

Point source

A specific location from which pollutants are discharged. It can also be defined as a single identifiable source of pollution (for example, a pipe or a ship).

Pool

A portion of a stream where water velocity is low and the depth is greater than the riffle, run, or glide. Pools often contain large eddies with widely varying directions of flow compared to riffles and runs, where flow is nearly exclusively downstream. The water-surface gradient of pools is very close to zero and their channel profile is usually concave.

Rapid bioassessment protocols (RBPs)

A set of protocols to evaluate the biological conditions of a water body that uses biological surveys of the resident plants, animals, and other living organisms that depend upon the aquatic resource.

Receiving water

A river, stream, lake, or other body of surface water into which wastewater or treated effluent is discharged.

Receiving-water assessment (RWA)

A category of biological monitoring designed as a single study conducted on a stream (usually with existing or proposed wastewater discharges) to assess its physical, chemical, and biological characteristics.

Riffle

A shallow portion of the stream extending across a stream bed characterized by relatively fast moving turbulent water. The water column in a riffle is usually constricted and water velocity is fast due to a change in surface gradient. The channel profile in a riffle is usually straight to convex.

Riparian zone

Generally includes the area of the stream bank and out onto the floodplain that is periodically inundated by floodwaters from the stream. The limit of the zone depends on many factors including the makeup of the native plant community, soil moisture levels, and distance from the stream (or the limit of interaction between land and stream processes). Interaction between this terrestrial zone and the stream is vital for the health of the stream.

Run

A relatively shallow portion of a stream characterized by relatively fast moving non-turbulent flow. A run is usually too deep to be considered a riffle and too shallow to be considered a pool. The channel profile under a run is usually a uniform flat plane.

Segment

Waters designated by the TCEQ in the TSWQS, which include most rivers and their major tributaries, major reservoirs, lakes, and marine waters. Segmented waters have designated physical boundaries, specific uses, and numerical physicochemical criteria (e.g., DO, temperature, fecal coliform, chloride, sulfate) in the state's water quality standards.

Seven-day, two-year low-flow (7Q2)

The seven-day, two-year low flow, or the lowest average streamflow for seven consecutive days with a recurrence interval of two years, as statistically determined from historical data.

Species

A category of biological classification ranking immediately below genus, comprising related organisms potentially capable of interbreeding. A species is identified by a two-part name—the name of the genus followed by a Latin or Latinized uncapitalized noun agreeing grammatically with the genus name.

Specific conductance

A measure of the electrical current carrying capacity, in µS/cm, of 1 cm^3 of water at 25ºC. Dissolved substances in water dissociate into ions with the ability to conduct electrical current. Conductivity is a measure of how salty the water is; salty water has high conductivity.

Standard length

Fish—The greatest distance in a straight line from the tip of the snout to the base of the caudal peduncle.

Stream bend

The curved part of a stream. A well-defined bend has a deep outside area (cut bank) and a shallow inside area accentuated by point-bar development. Due to sharp bending, streamflow is forced to the cut-bank side, and eddies develop on the inside of the bend. A moderately developed bend forces some flow to the outside and has only a slight change in depth across the channel. A poorly defined bend has no noticeable change in water depth across the channel, and streamflow is generally not forced to one side.

Stream order

A scheme for classifying stream sizes in which the smallest, unbranched tributaries in a watershed are designated first-order streams. Where two first-order streams join, a second-order stream is formed; where two second order streams join, a third-order stream is formed, and so on.

Stream terrace

A relatively level bench or step-like surface breaking the continuity of a slope. These occur due to erosion by a river on its floodplain. A terrace that is above the current level of a river is the location of the river at an earlier time. The river has continued to incise itself, leaving the terraces as remnants of its earlier elevation.

Submerged vegetation

Rooted plants with almost all leaves below the water surface (for example, alligator weed, hydrilla or elodea).

Surface water quality standards

The designation of water bodies for desirable uses and the narrative and numerical criteria deemed necessary to protect those uses.

Tolerant organism

An organism that has the capacity to grow and thrive when subjected to unfavorable environmental factors.

Total length

Fish—Tip of snout (mouth closed) to the tip of longest caudal ray (caudal fin compressed).

Shrimp—Tip of rostrum to tip of telson.

Crab—Lateral spine tip to lateral spine tip or trident point of body if no lateral spine.

Skates and rays—Maximum wingspan.

Squid—Posterior mantle margin to top of pen.

Transect line

A straight line, perpendicular to the streamflow, between two points on opposite stream banks.

Tree canopy

The uppermost spreading branching layer of stream side trees that shades the water surface. Reported as percent cover and measured with a canopy densiometer. Possible measurement range is from 0 percent (totally open canopy cover) to 100 percent (totally closed).

Tributary

A stream or river that flows into a larger stream or river.

Unclassified water body

A smaller water body that does not have site-specific water quality standards assigned to it (not included in Appendix D of the Texas Surface Water Quality Standards), but instead is protected by general standards that apply to all surface waters in the state.

Use-attainability analysis (UAA)

A category of biological monitoring to assess the physical, chemical, biological, and economic characteristics of a water body. It is used to establish site-specific standards for classified water bodies.

Watershed

The area of land from which precipitation drains to a single point. Sometimes referred to as a drainage basin, drainage area, or catchment basin.

APPENDIX F

SURBER-SAMPLER PROTOCOLS

Sample Collection

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

Records

In addition to other sample-labeling requirements specified in this appendix, 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, the location of the sample site (station ID), the name of each collector, the method of collection, the number and type of samples collected, the number of sample containers, and the preservative used.

Sample-Tracking Logbook

Maintain a sample-tracking logbook that contains the information described in Chapter 11. This logbook documents when samples arrive at the laboratory or headquarters facility, when each sample enters each processing step, and who has custody or responsibility for it.

Laboratory Bench Sheets

Maintain laboratory bench sheets where specimen identification and enumeration occur. These sheets document the raw counts of individuals for each taxon and contain notes relevant to identification and enumeration.

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 according to the following priorities:

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 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 one or more riffles, spending most of the sampling time in these microhabitats.

Consider alternate 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 RBP snag or RBP kicknet samples. See Chapter 5 for details.

Collecting a Surber Sample

To collect a Surber sample, firmly push the 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. Collect and preserve a total of three individual replicate Surber samples by following these procedures for each replicate.

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 midstream about halfway up the riffle; and collect the third 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.

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, it must be done in the laboratory. Inspect the complete sample under magnification to ensure that all individuals are counted and identified.

Transfer the entire sample from the net to one or more sample containers and preserve it 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, it may be preserved in 95 percent ethanol.

Use enough preservative to cover the sample. To ensure adequate preservation of benthic macroinvertebrate collections, do not fill sample containers more than half full of sample. The amount of preservative should at least equal the volume of organic material, including detritus. 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 safety data sheet for alcohol and formalin solutions for proper handling requirements.

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 each collector

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.

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, an instance of numbering may look like *BM 040 13*, 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 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 number to the outside of the container. Wrap the label with clear tape to ensure that the label will not come off. Do not put the label on the container lid.

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 for evaluation of the variability between replicates.

Rinsing a Sample

Thoroughly rinse the sample using a No. 30 or smaller (≤ 595 µm) sieve to remove preservative and fine sediments. Place rinsed sample in a shallow white pan.

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 it to alcohol before sorting. Follow your organization's plan for collection and disposal of hazardous formalin and alcohol waste.

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 them 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 them using a dissecting scope. Repeat this process until you have inspected the entire replicate sample under magnification.

Labeling a Sample

Label the sample bottle or vial containing the benthic macroinvertebrates obtained using this 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 each collector
- name of person subsampling, 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. Make sure the container is dry, and wrap it with clear tape to ensure the label will not come off. Do not put the label on the container lid.

Quantitative Snag Samples

The method for collecting snag samples, 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 long enough 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 (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 material to fill two 1 qt mason jars.

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 that all organisms are removed from the snags. A soft-bristled brush may be appropriate for removing 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," above.

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

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

Use all appropriate references, a stereo dissecting microscope, and a compound phase-contrast microscope to identify organisms to the appropriate taxonomic level listed below.

- 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

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 gives complete required and recommended references for identifying freshwater macroinvertebrates.

Data Evaluation 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 F.1. The BIBI criteria were derived for three bioregions (central, east, and north) that overlap ecoregions as defined by Omernik and Gallant (1987). Figure F.1 illustrates the three bioregions, whose boundaries all coincide with ecoregion lines:

Central bioregion. The region composed 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 data on benthic macroinvertebrates 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 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—the optimal approach according to Karr et al. (1986) and 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 ≥ 8.5, and

intolerant taxa, values ≤ 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 (stone flies), and Trichoptera (caddis flies) 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 most 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 (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, domination of a community by relatively few taxa may indicate environmental stress, and a high-percentage contribution by a few taxa often represents an imbalance in community structure.

Pool, Reservoir, or Lake: Protocols for Sampling by Ekman, Ponar, Petersen, or Van Veen Dredge

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

Sample-Collection Procedures

The Ekman dredge is the preferred tool for collecting benthic macroinvertebrate samples from lentic or depositional habitats, such as pools or reservoirs whose bottom is primarily mud, silt, or fine sand. Use of the Ekman dredge is considered quantitative sampling and collection and processing should be similar to those for Surber samples. In pools or reservoirs with substrates composed of gravel, hard sand, or clay, a Ponar or Van Veen dredge may be necessary. Before using any of these devices, inspect it 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 dredge in ambient water. Once it 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 bucket with a No. 30 or smaller sieve (mesh size ≤ 595 µm) and open the jaws of the dredge to allow all of the material collected in it 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 it 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 it, taking care to minimize destruction of soft-bodied organisms.

Repeat this process three more times to produce a total of four separate replicate benthic macroinvertebrate samples.

Preserving the Sample

Empty the washed contents of the sieve bucket into a clean, wide-mouthed bottle. Transfer the entire sample from the sieve bucket to one or more sample containers and preserve it in

10 percent formalin. Alternatively, if the sample is to be sorted shortly after reaching the laboratory, preserve it in 95 percent ethanol.

Use enough preservative to cover the sample. To ensure adequate preservation of benthic macroinvertebrate collections, fill sample containers no more than 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)
- container replicate number (for example, *1 of 4* or *2 of 4*)
- preservative used
- name of each collector

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 "Tracking Requirements for Surber Samples."

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

Use all appropriate references, a stereo dissecting microscope, and a compound phase-contrast microscope to identify organisms to the appropriate taxonomic level listed below. Chapter 11 gives a complete list of required and recommended references on identifying freshwater macroinvertebrates.

- 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

Maintain a separate count of individuals and list of taxa for each replicate dredge sample to allow an evaluation of variability between replicates. Methodologies for assessing ALUs have not been developed for Texas freshwater depositional habitats, including pools and reservoirs. Before any biological monitoring 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.

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 any applicable regulatory decision (whichever is longer) to allow verification of identification if necessary. 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.

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 sample maintenance or archiving in a university or museum natural-history collection.

Table F.1. Metrics and scoring criteria for Surber samples—Benthic Index of Biotic Integrity. (Davis, 1997.) (Footnotes appear on following page.)

	Metric	Scoring Criteria		
		5	**3**	**1**
Central bioregion **(Ecoregions: 23, 24, 27, 29, 30, 31, and 32)**	1. Total taxa	> 32	32–18	< 18
	2. Diptera taxa	> 7	7–4	< 4
	3. Ephemeroptera taxa	> 4	4–2	< 2
	4. Intolerant taxa	> 8	8–4	< 4
	5. % EPT taxa	> 30	30.0–17.4	< 17.4
	6. % Chironomidae	[a]	< 22.3	≥ 22.3
	7. % Tolerant taxa	[a]	< 10.0	≥ 10.0
	8. % Grazers	> 14.9	14.9–8.7	< 8.7
	9. % Gatherers	> 15.2	15.2–8.8	< 8.8
	10. % Filterers	[a]	> 11.9	≤ 11.9
	11. % Dominance (3 taxa)	< 54.6	54.6–67.8	> 67.8
East bioregion **(Ecoregions: 33, 34, and 35)**	1. Total taxa	> 30	30–17	< 17
	2. Diptera taxa	> 10	10–6	< 6
	3. Ephemeroptera taxa	[b]	> 3	≤ 3
	4. Intolerant taxa	> 4	4–2	< 2
	5. % EPT taxa	> 18.9	18.9–10.8	< 10.8
	6. % Chironomidae	[a]	< 40.2	≥ 40.2
	7. % Tolerant taxa	< 16.0	16.0–24.3	> 24.3
	8. % Grazers	> 9.0	9.0–5.2	< 5.2
	9. % Gatherers	> 12.5	12.5–7.3	< 7.3
	10. % Filterers	[a]	> 16.3	≤ 16.3
	11. % Dominance (3 taxa)	< 57.7	57.7–71.6	> 71.6

	Metric	Scoring Criteria		
		5	**3**	**1**
North bioregion (Ecoregions: 25 and 26)	1. Total taxa	> 33	33–19	< 19
	2. Diptera taxa	> 14	14–8	< 8
	3. Ephemeroptera taxa	[b]	> 2	≤ 2
	4. Intolerant taxa	> 3	3–2	< 2
	5. % EPT taxa	> 14.4	14.4–8.2	< 8.2
	6. % Chironomidae	< 36.9	36.9–56.2	> 56.2
	7. % Tolerant taxa	< 14.1	14.1–21.5	> 21.5
	8. % Grazers	[b]	> 5.4	≤ 5.4
	9. % Gatherers	[a]	> 14.9	≤ 14.9
	10. % Filterers	> 12.2	12.2–7.1	< 7.1
	11. % Dominance (3 taxa)	< 68.1	68.1–84.5	> 84.5

[a] The discriminatory power was less than optimal for this bioregion, so the metric was assigned only two scoring categories.

[b] The median value for this bioregion was less than the metric-selection criterion (< 5.5 for taxa richness metrics; < 12 for percentage metrics expected to decrease with disturbance), so the metric was assigned only two categories.

Metrics and Scoring for Surber Samples for Benthic Macroinvertebrates by Bioregion: Central, East, or North

Stream Name:						
Date:		**Collectors:**				
Location:						
County:		**Ecoregion #:**				
Type of assessment:			UAA	ALA	ALM	RWA

Metric	**Value**	**Score**
1. Total taxa		
2. Diptera taxa		
3. Ephemeroptera taxa		
4. Intolerant taxa		
5. % EPT taxa		
6. % Chironomidae		
7. % Tolerant taxa		
8. % Grazers		
9. % Gatherers		
10. % Filterers		
11. % Dominance (3 taxa)		
Aquatic life use point score ranges:	Exceptional: High: Intermediate: Limited:	> 40 31–40 21–30 < 21
Total Score:		
Aquatic-Life Use:		

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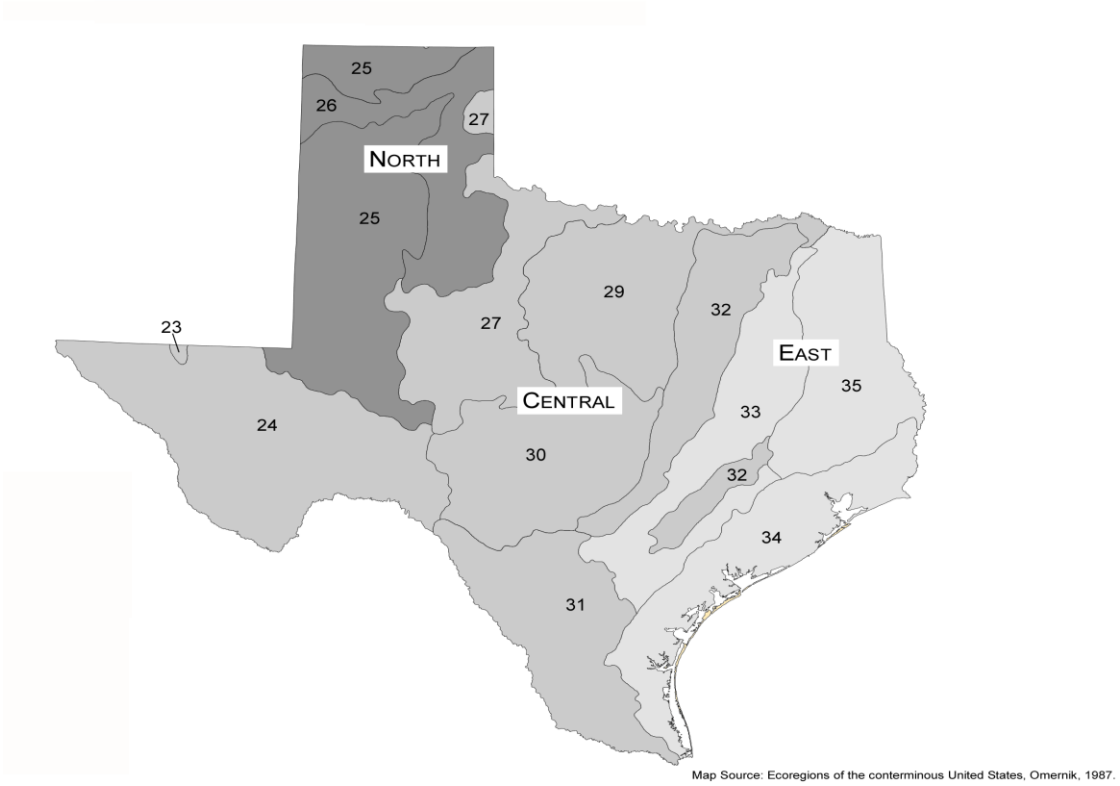
Note: This form should be used as part of the biological monitoring packet. If you chose to use another format, all information must be included.

Figure F.1. Macrobenthic bioregions (North, Central, East) and Level III ecoregions of Texas for use with Surber BIBI.



Level III Ecoregions of Texas

23 Arizona–New Mexico Mountains
24 Southern Deserts
25 Western High Plains
26 Southwestern Tablelands
27 Central Great Plains
29 Central Oklahoma–Texas Plains
30 Central Texas Plateau
31 Southern Texas Plains
32 Texas Blackland Prairies
33 East Central Texas Plains
34 Western Gulf Coastal Plain
35 South Central Plains

APPENDIX G

ABBREVIATIONS

Abbreviation	Description
µm	micrometer
µS/cm	microsiemens per centimeter
7Q2	seven-day, two-year low flow
AFMD	ash-free dry mass
AI	autotrophic index
ALA	aquatic-life assessment
ALM	aquatic-life monitoring
ALU	aquatic-life use
BIBI	benthic index of biotic integrity
$CBOD_5$	chemical biochemical oxygen demand, 5-day
cm	centimeter
CONUS	continental United States
cfs or ft^3/s	cubic feet per second
CPOM	course particulate organic matter
CRP	Clean Rivers Program
CWA	Clean Water Act
DMRG	Data Management Reference Guide
DO	dissolved oxygen
EPT	Ephemeroptera-Plecoptera-Trichoptera
FC	filterer-collector
FFG	functional feeding groups
FPOM	fine particulate organic matter
ft^3/s	cubic feet per second
CG	collector-gatherer
g	gram
GPS	global positioning system
HBI	Hilsenhoff biotic index
HQI	habitat quality index
Hz	hertz
IBI	index of biotic integrity
IR	integrated report
km	kilometer
m	meter
MGD	million gallons per day
mL	milliliter
$MgCl_2$	magnesium chloride
mm	millimeter
mm^2	square millimeters

Abbreviation	Description
ms	millisecond
QA	quality assurance
QC	quality control
QAPP	quality-assurance project plan
P	predator
PCSI	percent community similarity index
POM	particulate organic matter
PTI	pollution-tolerance index
RBP	rapid bioassessment protocol
RWA	Receiving-water assessment
SCP	Scientific Collection Permit
SCR	scraper
SIT	Standards Implementation Team
SLOC	station location
SHR	shredder
SWQM	surface water quality monitoring
SWQMIS	Surface Water Quality Monitoring Information System
TAC	Texas Administrative Code
TCEQ	Texas Commission on Environmental Quality
TMDL	total maximum daily load
TNRCC	Texas Natural Resource Conservation Commission
TPWD	Texas Parks and Wildlife Department
TPDES	Texas Pollution Discharge Elimination System
TSA	technical systems audit
TSWQS	Texas Surface Water Quality Standards
USEPA	United States Environmental Protection Agency
UAA	use-attainability analysis
USGS	United States Geological Survey
WQSG	Water Quality Standards Group
WQSIT	Water Quality Standards Implementation Team
WWTP	wastewater treatment plant

APPENDIX H

LABORATORY BENCH SHEETS

Example Laboratory Bench Sheet: Fish

Laboratory Bench Sheet: Fish			**Page ____ of _____**
Stream Name:		Location:	
Station No.:	Log No.:	River Basin:	County:
Date Collected:	Time:	Collector:	
Date Identified:		Identifier:	
Sample Type:		Rep No. _____ of _____ (if applicable)	
Tag ID:		Sample-Set ID:	
Collection Method: Electrofishing Seining Other			

Taxon	**No. of Individuals**	**Parameter Code**	**Trophic Group**	**Tolerance**

Example Laboratory Bench Sheet: Benthic Macroinvertebrates

Laboratory Bench Sheet: Benthic Macroinvertebrates			**Page ____ of _____**
Stream Name:		Location:	
Station No.:	Log No.:	River Basin:	County:
Date Collected:	Time:	Collector:	
Date Identified:		Identifier:	
Sample Type:			Rep No. _____ of _____ (if applicable)
Tag ID:			Sample-Set ID:
Collection Method: Kicknet Snag Sample Surber Multiplate Other			

Taxon	No. of Individuals	Parameter Code	Tolerance Value	Functional Group

Example Laboratory Bench Sheet: Algae

Laboratory Bench Sheet: Algae			Page ____ of _____
Stream Name:		Location:	
Station No.:	Log No.:	River Basin:	County:
Date Collected:	Time:	Collector:	
Date Identified:		Identifier:	
Sample Type:		Rep No. _____ of _____ (if applicable)	
Tag ID:		Sample Set ID:	
Substrate (List Type): Natural Artificial			

Taxon	**No. of Individuals**	**Parameter Code**	**Tolerance Value**

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