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Surface Water Quality Monitoring Procedures, Volume 2:

Methods for Collecting and Analyzing Biological Assemblage and Habitat Data

Monitoring Operations Division

TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

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Surface Water Quality Monitoring Procedures, Volume 2:

Methods for Collecting and Analyzing Biological Assemblage and Habitat Data

Prepared by
Surface Water Quality Monitoring Program
Monitoring Operations Division
Texas Commission on Environmental Quality

RG-416
June 2007

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LIST OF ABBREVIATIONS USED

AFDM	ash-free dry mass	mm^2	square millimeters
AI	autotrophic index	ms	millisecond
ALA	aquatic life assessment	QA	quality assurance
ALM	aquatic life monitoring	QC	quality control
ALU	aquatic life use	QAPP	quality assurance project plan
BIBI	benthic index of biotic integrity	P	predator
$CBOD_5$	chemical biochemical oxygen demand, 5-day	PCSI	percent community similarity index
cm	centimeter	POM	particulate organic matter
CONUS	continental United States	PTI	pollution tolerance index
cfs or ft^3/s	cubic feet per second	RBP	rapid bioassessment protocol
CPOM	coarse particulate organic matter	RWA	receiving water assessment
CRP	Clean Rivers Program	SCP	scientific collection permit
CWA	Clean Water Act	SCR	scraper
DMRG	Data Management Reference Guide	SLOC	station location
DO	dissolved oxygen	7Q2	seven-day, two-year low-flow
EPT	Ephemeroptera-Plecoptera-Trichoptera	SHR	shredder
FC	filterer-collector	SWQM	surface water quality monitoring
FFG	functional feeding groups	TAC	Texas Administrative Code
FPOM	fine particulate organic matter	TCEQ	Texas Commission on Environmental Quality
ft^3/s	cubic feet per second	TMDL	total maximum daily load
CG	collector-gatherer	TNRCC	Texas Natural Resource Conservation Commission
g	gram	TPWD	Texas Parks and Wildlife Department
GPS	global positioning system	TPDES	Texas Pollution Discharge Elimination System
HBI	Hilsenhoff biotic index	TSA	technical systems audit
HQI	habitat quality index	TSWQS	Texas Surface Water Quality Standards
Hz	hertz	USEPA	United States Environmental Protection Agency
IBI	index of biotic integrity	UAA	use attainability analysis
km	kilometer	USGS	United States Geological Survey
m	meter	WQST	Water Quality Standards Team
MGD	million gallons per day	WWTP	wastewater treatment plant
µm	micrometer		
µS/cm	microSiemens per centimeter		
mL	milliliter		
$MgCl_2$	magnesium chloride		
mm	millimeter		

CHAPTER 1
INTRODUCTION

Purpose

This publication, *Surface Water Quality Monitoring Procedures, Volume 2: Methods for Collecting and Analyzing Biological Assemblage and Habitat Data*, replaces the *Receiving Water Assessment Procedures Manual*, GI-253. It is intended to be used with a companion publication *Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue*, RG-415 (TCEQ 2003).

This publication provides a comprehensive source of 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 Texas Commission on Environmental Quality (TCEQ) generated these procedures in coordination with other water programs of the TCEQ and the Texas Parks and Wildlife Department (TPWD) through an established biological workgroup process.

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 monitoring programs such as the Total Maximum Daily Load (TMDL) Program and the Texas Clean Rivers Program (CRP). Monitoring entities, such as the CRP 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.

Use attainability analyses (UAAs). 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 2000).

Receiving water assessments (RWAs). 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). ALM is applicable for routine monitoring sites and is conducted to provide baseline data on environmental conditions and/or to determine if ALU/dissolved oxygen (DO) criteria are being attained. This category also includes reference condition, or ecoregion monitoring.

Aquatic life assessments (ALAs). 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 the *SWQM Procedures* are important because they document the quality assurance (QA) procedures that must be used to demonstrate that SWQM data collected by monitoring personnel are of known and comparable quality across the state.

The statewide SWQM Program is responsible for the collection of data that accurately describes 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 water quality standards compliance;
- Describe seasonal variation and frequency of occurrence of selected water quality constituents;
- Produce the *State of Texas Water Quality Inventory*, required by Section 305(b) of the Clean Water Act (CWA). This assessment enables the public, local governments, state agencies, the Texas Legislature, the United States Environmental Protection Agency (USEPA), and Congress to make water quality management decisions; and
- Establish water quality standards.

Legal Authority

Texas law requires all monitoring personnel, including partners and contractors, who collect and analyze biological samples for the TCEQ SWQM Program to follow procedures outlined in a TCEQ manual. The rule is in Title 30 of the Texas Administrative Code, Section 307.9.

Contact Information

For questions or comments about this manual or surface water quality monitoring, you can contact the SWQM Team 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 phone: 512/239-1716

By mail: SWQM Team, MC 165
TCEQ
P.O. Box 13087
Austin, TX 78711-3087

By fax: 512/239-1605

Online: Go to <www.tceq.state.tx.us/goto/swqm/links>, click on "Contacts," and then click on "TCEQ Surface Water Quality Monitoring Program Contacts."

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. You can also find the manuals on the TCEQ Web site at <www.tceq.state.tx.us/goto/publications>. Also check the Web page of the SWQM Program for other publications and resources, including those that are referenced later in this publication.

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 and habitat assessment of freshwater benthic macroinvertebrates and fish. Assessment methods for saltwater nekton (Chapter 4), saltwater benthic macroinvertebrates (Chapter 6), benthic algae and macrophytes (Chapter 7), and plankton (Chapter 8) are also addressed; however, assessment methods for salt waters, 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 be added to later revisions of this manual.

Index Period

In order to determine aquatic life uses (ALUs) or to evaluate support of existing ALUs, the TCEQ has established an *index period* during which most bioassessments of aquatic assemblages in lotic systems should be conducted. The time frame which describes the index period was established to:

- Minimize year-to-year variability resulting from natural events.
- Maximize gear efficiency.
- Maximize accessibility of targeted assemblage(s).
- Allow adequate time for work to be done 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 TSWQS establishes the criteria (for example: DO) for water quality conditions required to support and protect desired uses (30 TAC 2000).

Collecting a portion of the samples during critical conditions ensures that, with few exceptions (for example: acute toxic criteria), criteria for desired use are met and maintained when stream flow is at or above critical low flow (for example: seven-day, two-year low-flow [7Q2] flow), and water temperature approximates critical summer values.

In order to make the most efficient use of available resources, bioassessment data should be collected during summertime critical conditions. **Note**: If the criteria are met during these conditions, it would be expected that the criteria would be met during other seasons when flow would be expected to be greater and water temperatures would be expected to be lower.

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

If only one sample is to be collected, schedule the single event during the critical period that runs from July 1 to September 30. If it is not possible to conduct the single bioassessment during the critical period, submit with the sample results a written justification explaining why this objective was not met.

If two samples are collected at the same site during the same year, both samples must be collected during the index period with one event in the non-critical period, and one event conducted during the critical period. If more than two samples are collected at the same sample site, the period of study must be at least two years long, with a minimum of two samples per year collected.

At least one-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. Sample events must be separated by at least one month.

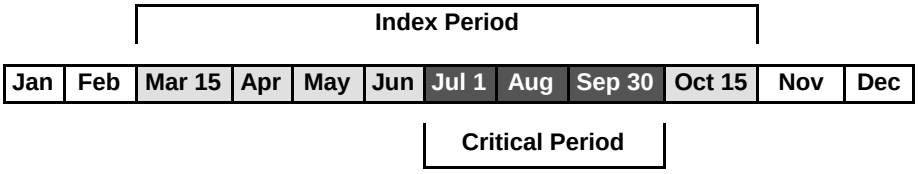


Figure 2-1. The Index Period

The objective for scheduling bioassessment events, when the intent is to use sample results to assess use support or to establish the appropriate use, should be to conform to the temporal guidelines provided above, which describe the timing of sample events within the index period and the critical and non-critical portions of the index period.

However, strict adherence to these temporal guidelines may not always be feasible as a result of normal and/or anomalous variability of local flow and temperature conditions. For example, during a year with abnormally high flows, critical low-flow/temperature conditions may not begin exactly on July 1 so that the non-critical period might extend into early July. In situations such as these, when conditions preclude meeting exact calendar guidelines, consult the TCEQ SWQM Team or Water Quality Standards Team (WQST) before adjusting sample regimes and provide written explanation for deviation from temporal guidelines along with results.

Site Representativeness

Select monitoring sites that best represent conditions (both biological and water quality) of an entire water body. 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 conditions 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 (depending on size of bridge and road crossing) upstream from any road or bridge 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. The 7Q2 of a stream is defined as the seven-day, two-year low flow, or the lowest average stream flow 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 a minimum of one month of normal flow before collecting biological samples. Use best professional judgment to determine the appropriate sampling condition, since the return of the stream to normal conditions may depend on recruitment sources.

Other Monitoring Requirements

Documentation and Field Notes

Use a bound field-data logbook to record biological information in the field. Record preliminary header information, field measurements, and other field observations. Preliminary header information includes station ID, location, sampling date, sampling time, sampling depth, and 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 event 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, make some note of this in the comments.

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

Latitude and Longitude Coordinates

Collect latitude and longitude coordinates using global positioning system (GPS) equipment or measured from 7.5-minute series topographic maps. Take the coordinates at the midpoint of each reach or, for RWAs, at the wastewater discharge point. If using GPS equipment, equipment must comply with TCEQ OPP 8.12 requirements. When collecting GPS data, real-time corrected mode data are preferable using an acceptable real-time correction source. If post-processing data, collect a minimum of 120 data points at each GPS site, average, and correct using a suitable base station.

Collect coordinates in degrees, minutes, and seconds. To ensure accuracy, data integrity, and enable global information system (GIS) mapping, export all GPS data into a ".dbf" file as lat-long in decimal degrees, NAD-1983 (CONUS). The person collecting the latitude and longitude coordinates must have completed the TCEQ GPS certification training and be certified. Questions about GPS certification training are directed to the TCEQ CRP for monitoring entities outside of TCEQ. All entities must complete a station location (SLOC) form for each new site. The SWQM Data Management Reference Guide (DMRG) contains detailed instructions and information necessary to complete a SLOC form. The SWQM DMRG can be found at <www.tceq.state.tx.us/goto/swqm/dmrg>.

Nonbiological Parameters

The following parameters may or may not be required as part of a biological monitoring event:

- Flow
- 24-hour DO
- Water chemistry

Refer to the monitoring categories below for specific requirements. Methods for these parameters may be found in *Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue*, RG-415 (TCEQ 2003).

Monitoring Categories for Wadeable Freshwater Streams

There are four categories for biological monitoring in freshwater. These categories are used primarily for the monitoring of wadeable streams. Each is designed to serve a specific regulatory purpose. They are:

- Aquatic Life Monitoring (ALM)
- Aquatic Life Assessment (ALA)
- Receiving Water Assessment (RWA)
- Use Attainability Analysis (UAA)

The data collection for each category is 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 Tables B-14 through B-17 in Appendix B.

Aquatic Life Monitoring

ALM, a SWQM Program and CRP activity, is for individual, 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 to begin 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 Water Quality Inventory or CWA Section 305(b) assessment. The details of the required biological events and number of events for an ALM are outlined in Appendix E, Table E-l.

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 (Omernik 1987).

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 that meet the following criteria:

- Watershed contains no, or very little, urban development.
- Watershed contains no significant point sources of pollution.
- Watershed contains no channelization.
- Watershed contains no atypical nonpoint sources of pollution.
- Watershed is characterized by perennial flow or perennial pools.

Once candidate reference streams are identified, each watershed is mapped to form part of a "desktop" evaluation of the upstream geomorphologic characteristics and to provide the opportunity to use GIS data on watershed land-use patterns. As a part of this step, the proportion of the watershed within a single Level III ecoregion (Omernik 1987) is determined. Ideally, the watershed for a particular sample site must be contained entirely within a single Level III ecoregion (Omernik 1987).

Mapping the watershed also allows determination of areal coverage and provides the necessary information 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). It has been demonstrated that 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).

Ground truthing for candidate sites is conducted, 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 that appropriate habitat for sampling the target group (for example: benthic macroinvertebrates and fish) is available. 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 Assessment

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. Unclassified, perennial water bodies, not listed in Appendix D, have a presumed high ALU. The purpose of an ALA is to confirm if indications of nonsupport are appropriate, and to identify the appropriate ALU and DO criterion. The details of the required biological events and number of events for an ALA are outlined in Appendix E, Table E-2.

Site and reach selection must ensure that adequate data are generated to accurately characterize biotic integrity through the entire study area. This will require a minimum of 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 WQST. Sampling of multiple sites and reaches may be necessary for most water bodies. Data collected as part of an ALA are used by the WQST to determine if the water body is meeting its presumed high ALU designation. The result of this type of monitoring may lead to a proposed site-specific ALU designation and corresponding DO criterion.

Receiving Water Assessments

RWAs are conducted on unclassified water bodies with existing or proposed wastewater discharges during a single study event, on a specific reach of a stream, to assess their physical, chemical, and biological characteristics. 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. RWAs are requested by the WQST 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. The data collected during an RWA are used to determine the appropriate ALU and DO criterion. RWAs are conducted on freshwater streams only. The details of the required biological events and number of events for an RWA are outlined in Appendix E, Table E-3.

RWA data are used primarily by the WQST for two TCEQ objectives: reviewing wastewater permit applications and establishing site-specific standards. As part of the Texas Pollutant Discharge Elimination System (TPDES) Program, wastewater permit applications are reviewed for potential water quality impacts to surface waters of the state. RWA data can help the WQST assign appropriate ALU and standards to water bodies potentially affected by proposed or existing wastewater discharges. By studying the areas of the stream upstream of an existing discharge or downstream of a proposed discharge, it is possible to determine the appropriate ALU for the stream that would be expected to be maintained in the areas affected by the discharge.

For existing wastewater treatment plant (WWTP) discharges, the RWA must be conducted upstream of the discharge. A reach beginning approximately 30 m upstream of the discharge point must be selected. If this area is not accessible, or is not representative of conditions downstream of the discharge, the reach may be moved 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 impact 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. It is typically the case with larger wastewater plants that 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 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 in Appendix B illustrates the RWA reach for an existing discharge of 3.6 million gallons per day (MGD) into an intermittent and perennial stream. Figure B-9 in Appendix B illustrates the RWA reach for a proposed 3.6 MGD discharge into an intermittent and perennial stream. RWAs should be planned in consultation with the TCEQ's WQST to ensure all necessary data are collected.

Ideally, RWAs should be conducted during summer low-flow conditions, or 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 due to permit action necessity. Whenever possible, RWAs must be completed six months before the wastewater permit action (renewal or amendment) date. 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 requirements for RWA sampling are found in Table B-16 in Appendix B.

Use Attainability Analysis

As part of the triennial revision of the TSWQS, UAAs are primarily used by the WQST to review site-specific standards for classified waterbodies. 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. UAAs are conducted on classified water bodies and those unclassified water bodies that are already listed in Appendix D and have established ALU and DO criteria. The purpose is to determine if the existing designated ALU and associated DO criterion are appropriate and, if not, to develop designated use and/or criteria adjustment information. UAAs require a significant amount of coordination with the WQST. A UAA considers the physical, chemical, and biological characteristics of a water body, as wells as economic factors to determine the existing and attainable uses. Completed UAAs are submitted to the USEPA for technical approval. If the UAAs are technically approved, the changes are incorporated into the next triennial review of the TSWQS after public notice and full public participation. The details of the required biological events and number of events for a UAA are outlined in Appendix E, Table E-4.

Site and reach selection must ensure that sufficient sites and reaches are monitored to provide adequate data to accurately characterize the ALU for the entire study area. To accomplish this, sampling of multiple sites/reaches will be required for most water bodies. Land use/land cover analysis of the proposed sites is strongly recommended prior to selecting the UAA sites. Data collection efforts are required for each site/reach. As each water body is different in terms of the number of sites necessary to adequately characterize the water body, coordination with the WQST is crucial in determining the appropriate number of sites. Detailed requirements for UAA sampling are found in Table B-17 in Appendix B.

Large River Monitoring and Assessment

Collecting and assessing fish assemblages in predominantly large, runoff-dominated streams and rivers present substantially greater complexity and potential for problems than work in wadeable 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 wadeable streams in terms of discharge and scale. However, most have significant reaches that are not primarily wadeable, 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/or irrigation withdrawals. Instead, sampling periods should be site and collection-method specific and meet the objectives of the study.

Reference streams used for comparisons may not be available given man-induced modifications to larger waterways and the lack of streams of similar size and faunal composition for comparison. Aside from issues associated with establishing a comparative baseline, large streams and rivers require different equipment or application of equipment than wadeable 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 collection gears must be employed to obtain a representative sample.

Analytical tools for evaluating biological collections need consideration when sampling large streams and rivers. The assessment tools and regionalized indices of biotic integrity (IBI) described by Linam et al. (2002) are a starting point, but were designed for wadeable streams and

may not be directly applicable in all situations and depend on the system being sampled. A test of the adequacy of wadeable stream sampling methods may be whether at least 50 percent of the reach can be sampled by wading methods (for example: backpack electrofisher and seines).

Many reaches may be marginally wadeable, whereas others are predominately deep except for the stream margins. The former might be adequately sampled using a combination of a backpack electrofisher and seines as in the wadeable stream protocols, whereas boat electrofishing equipment would be more appropriate in others. In the latter case, seines must still be used as a complementary tool for sampling.

Other gears (for example: gill nets, hoop nets) may be required depending on the objectives of the study and stream conditions. Sampling duration may vary depending upon the system scale. USEPA has proposed 40 to 100 times the wetted stream width as a reach length to be covered 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. Given the aforementioned complexity in sampling and assessment, staff 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, nonwadeable streams and rivers.

Lakes and Reservoir Monitoring and Assessment

The freshwater stream sampling index period, as described above, may not be appropriate for lakes and reservoirs. In these types of habitat, the appropriate sampling period should be study and collection-method specific. 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—would be considered a critical period. In these situations, written explanation of how appropriate sample windows were established must be provided along with results. In other words, the sampling period should be assessment-study and collection-method specific. For example, the TPWD inland fisheries assessment procedures allow for collection methods (boat mounted elecrofishing, gill netting, and trap netting) to be conducted 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 to December) and in the spring (March to May). Gill netting is conducted during January to 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 a reservoir can complicate regulatory processes by making it difficult to first determine what the biological community and structure must comprise for a reservoir to meet its designated ALU. 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. Preliminary work in the development of procedures for assessing the biological and habitat integrity of lakes and reservoirs has begun (TPWD 2002), 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 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 USEPA 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 USEPA has also published a technical guidance document for the development of lake and reservoir bioassessment and biocriteria programs (USEPA 1998).

Before conducting any biological monitoring activities on 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.

Saltwater Biological Monitoring Categories

There are three categories of biological monitoring that may occur in salt water. Each is designed to serve the same regulatory purpose as those for freshwater. They are:

- Aquatic Life Monitoring (ALM)
- Aquatic Life Assessment (ALA)
- Use Attainability Analysis (UAA)

While the purposes for conducting these assessments in salt water 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 assigned tidal streams the ranking of “high” or “exceptional” aquatic life uses, based on best professional judgment. As development occurs along the coast, UAAs have begun to be conducted 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 considered, including profiles, since the water column is often stratified due to temperature and salinity. Samples are collected for analysis of routine water chemistry and carbonaceous biochemical oxygen demand, 5-day ($CBOD_5$).

Flow. Tidal stream hydrology is very different from freshwater stream hydrology. The multi-directional nature these flows is very important to tidal stream and estuary communities. Technologies to measure multi-directional stream flows must be considered to provide information about the hydrology in these systems.

Biological. Important biological components of tidal streams and estuaries include nekton, benthic macroinvertebrates, aquatic insects, and plankton.

Habitat. Both in-stream habitat and riparian habitat must be considered for tidal streams. Bottom structure and sediment must be sampled in estuaries.

Dissolved oxygen. DO measurements, collected at frequencies of 24 hours or greater, are important in tidal streams. The nature of the hydrology in these streams makes it more likely that low DO concentrations will occur.

Land-use and land-cover analysis. This type of analysis provides 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 which 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 *non-critical* portion of the index period may not be so easily defined and may be related to fish migration patterns, high runoff and inflow periods, as well as tidal patterns and temperature. TCEQ SWQM or WQST should be consulted prior to establishing the sample regime in these systems. Written explanation of how appropriate sample windows were established should be provided 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. However, beyond the minimum efforts outlined below, sampling will always continue until no additional species are collected despite covering new areas of the reach. Sample all available habitats and combinations of habitats. Be prepared to preserve some specimens for later identification and verification with a 10 percent formalin (one part full-strength formalin and nine parts water) solution.

Scientific Collection Permit

Anyone conducting fish surveys in Texas must possess or be listed on a valid TPWD Scientific Collection Permit (SCP). To apply for a SCP, contact TPWD at 512/389-4491. All TCEQ regional office and WQST staff are included on the SWQM SCP. Any TCEQ staff who need to be added to this permit should contact the central office SWQM Team.

The permittee, or sub-permittees, must notify TPWD Austin main office dispatcher (512/389-4848 or 512/389-4491) or regional law enforcement office not less than 24 hours in advance of a fish collection event. The SCP number and information on who, when, and where fish will be collected are required when notifying TPWD. The TPWD requires that the original permit be carried with the permittee during collections. A sub-permittee must carry a photocopy of the original permit if the permittee is not present. If persons who will be collecting fish are not listed on a valid permit, fax a letter with the names of the unpermitted individuals and assistants participating in the collection to the TPWD dispatcher 24 hours in advance.

Records

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

Field logbook. For each sample event, information including the date and time of sample collection, location of the sample site (Station ID), collectors name(s), method of collection, number and type of samples collected, number of sample containers, and preservative used must be entered in the field logbook. Field notes must describe the collection methods employed, equipment used, areas sampled, the way equipment was used, time spent sampling, a description of the sampled habitat(s), and any unusual site characteristics. It is imperative that complete and accurate notes be kept on every aspect of the sampling, from effort expended for each method used to which specimens were collected and which processing procedures and preservation were used.

Sample tracking logbook. A sample tracking logbook containing the information described in the QA chapter of this manual must be maintained. This logbook documents when samples arrive at the laboratory or headquarters facility, when each sample enters each sample processing step, and who has custody or responsibility for the sample.

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

Sample Collection

The method used to collect nekton samples will depend on several factors, including water body characteristics, 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 are included in Appendix C. Electronic copies of all the tables in the appendices can be furnished by Internet, E-mail, or by other means. Definitions of technical terms are located in the Glossary in Appendix F.

In most streams, fish are collected using multiple gear types, such as seines and electrofishers, employed separately. Both electrofishing and seining are required for fish sample collection. If unable to employ multiple gear types, indicate the reason and increase effort with the gear employed. For instance, if seining is not possible because of an abundance of heavy debris, indicate the reason and increase electrofishing effort. It must be noted that some seining is usually possible. If electrofishing is not possible, increase seining effort. 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 habitat, fish, and benthic macroinvertebrate sampling crew leaders have agreed where sampling will be conducted, the habitat crew marks the ends of the reach with bright survey flagging. Sampling from areas outside those boundaries is discouraged.

Electrofishing

Electrofisher capabilities vary by manufacturer and model. Each model is effective under certain specific conductance ranges. 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 if the unit will be consistently operated at higher conductivities. A smaller ring anode may be an option in those circumstances. Alternately, the larger ring may be covered with electrician's tape in a "candy cane" pattern. Note that battery life is shortened when electrofishers are used 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 or selectivity may occur (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 a minimum, two people are required when electrofishing (one to carry the backpack and the other to net fishes), though three people make an optimum crew. Caution must always be practiced while using electrofishing equipment. All participants must wear rubber linemans 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 the backpack electrofisher. Use a backpack electrofisher in small wadeable streams, where conductivity falls within the range specified in the user manual supplied with the electrofisher. After reaching the stream,

- power up the unit and set controls for ambient stream conditions,
- set the initial frequency at 60 Hz at 6 ms (setting I5 on the newer Smith-Root backpacks) and the voltage at 100 volts, and
- 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 provides general recommendations for voltage in waters of differing specific conductance—100 to 300 volts for specific 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.

Collecting. Once the controls are adjusted, reset the timer per the backpack model instructions. The collector carrying the backpack wades in an upstream direction to eliminate the effects of turbidity caused by disturbing bottom sediment. Electricity is discontinuously applied as fishes outside of the field may be herded and will not be susceptible to collection. For example, electrical current could be applied along the length of an undercut bank and then turned off until another discreet habitat type is encountered. The netters must follow and attempt to capture all stunned fishes. Wear polarized sunglasses to facilitate spotting organisms. In particularly turbid water, pull a small seine behind the electrofisher to capture stunned fishes that will be difficult to observe.

Where to sample. Sample all available habitat and cover types. In contrast to routine sampling for benthic macroinvertebrates, during which only one habitat type is usually sampled, an attempt must be made 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.

Actual shocking (trigger) time as recorded by the backpack timer must not be less than 900 seconds. 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 electrofishing equipment, safety is extremely important when using boat-mounted electrofisher equipment. Use only commercially produced electrofishing equipment. A minimum of three people are required when electrofishing out of a boat. Everyone on the boat must wear rubber, nonconductive gloves and knee boots or waders. Every effort should be made to keep the gloves dry. All individuals on the boat must wear personal floatation devices. The procedures for setting output is the same as for backpack electrofishers. Once the controls are adjusted, the samplers reset the timer. Electricity is then discontinuously applied. As with the backpack electrofisher, catches can usually be increased in areas of submerged cover by moving in with power on but circuit off and then energizing electrodes for an element of surprise. All habitat and cover types must be electrofished. If elevated conductivities are encountered, observe manufacturer's recommendations about the equipment's limits.

Collecting. In larger streams, or in reservoirs and lakes, use boat-mounted electrofishers. The length of sampling effort is 900 seconds of actual shock time.

When sampling in streams and rivers, boat-mounted electrofishers may be used moving either upstream or downstream depending on a variety of factors (for example: stream velocity, amount of debris in the river that one has to navigate around, turbidity, conductivity, sampling equipment). The collectors will determine if an upstream or downstream approach is be best. If the boat speed is slightly slower than the flow, it increases the chances that fishes will float to the surface and stay close enough to the boat for capture.

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 the most complete unit. Record both the distance and time of the lap. A slow, deliberate capture style is safer than fish chasing, and also yields more representative samples.

If elevated conductivities are encountered, observe manufacturer's recommendations about the equipment's limits. Electrofisher capabilities vary by manufacturer and model. Each model is effective under certain specific conductance ranges. Check the manufacturer's specifications for optimal operating procedures and consult with the manufacturer if the unit is consistently operated at higher conductivities.

Seine

Seining is a required collection technique in all sampling habitats. Seining is often more successful in areas 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 fish capture method that is used to capture mainly smaller fish and juveniles. Seines can be hard to deploy 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 must be repaired or replaced prior to each use.

In wadeable streams, use a 6 ft to 30 ft straight seine depending on stream size. One end of the seine is positioned near the bank. The seine is positioned perpendicularly from the bank. With the net fully extended (or rolled to make taut), both persons pull the net parallel to bank with person on bank slightly behind person in the channel. The person in the channel proceeds to the bank with seine extended. Both persons pull seine onto bank. Be sure the lead line remains on bottom until seine is pulled out of the water.

Count and record all organisms collected by the seine or put them in a container with fixative 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.

Repeat this process until a minimum of six replicates is collected.

Collection Method

Seine Types

Several different seines are used, depending on the habitats. Deep pools may be sampled with a 30 ft or 15 ft by 6 ft by ¼ in mesh seine, whereas riffles, runs, and small pools are usually sampled using a 15 ft or 6 ft by 6 ft by 3/16 in mesh seine. All are straight seines constructed of delta weave mesh with double lead weights on the bottom line.

Collecting. A seining crew consists of a minimum of two persons, but is more effective with three. Attempt a minimum of six effective seine hauls covering a distance of at least 60 m. It may take more than six seine hauls to cover the required minimum of 60 meters. 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. Seining may be conducted in either an upstream or downstream direction depending on current velocity and habitat. Record the number of effective seine hauls and an estimate of the distance.

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

Sample Preservation and Processing

Field Processing

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 fishes caught using either method back into the stream until all sampling is completed!** Maintain the fishes in some type of holding bucket or tank with adequate aeration.

Retain two representatives 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. Retain all but the most readily identifiable fishes for laboratory identification.

Use of Digital Photos as Fish Vouchers

An exception to the requirement that a minimum of two representatives of each species should be retained as vouchers is to allow 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*.

For fish this size, photographic vouchering is a relatively economical method which will reduce the overall volume of hazardous chemicals needed for tissue fixation and preservation and storage space needs, and will eliminate the need to obtain storage containers large enough to maintain such large specimens.

Stauffer et al. (2001) provide detailed guidance for producing photographic vouchers including discussions of the following factors that they consider critical:

- Choosing the right camera
- Photographing specimens
- 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. When selecting a camera, Stauffer et al. (2001) identify the following camera capabilities as critical:

- Color photographs
- High pixel density CCD or CMOS
- Macro capability
- Built-in or external flash

The color capability of digital cameras is expressed as color depth, which is the number of colors or shades comprising an image. The color depth is expressed as bits. A camera capable of producing 16-bit or greater digital images should be used to produce photographic vouchers (Stauffer et al. 2001) to ensure that the image adequately represents the actual coloration of the specimen. Stauffer et al. (2001) recommend that images have a minimum resolution of 1024 by 768 pixels for producing photographic vouchers.

Even on large fish it may be necessary to photograph small characters such as fins, gills, spines, etc. 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 conditions. If possible, move specimens from shade to full sun in order to use natural light. However, in situations where it is not possible to improve 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. A means of estimating size such as a tape measure, meter stick, or some calibrated device should be included in each photograph.

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 collector(s)
- Name of identifier if different from collector

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

A name, typically consisting of a combination of alpha and/or numeric characters, is automatically assigned to an image when captured by the camera. This automatically assigned name gives no indication of the file contents. Therefore, a system should be developed to name photographic voucher images as files that can be saved and subsequently retrieved as efficiently as possible. Stauffer et al. (2001) recommend that the file name 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 for that species.

Rules for Capturing Voucher Images of Fishes

For the voucher images to convey the most information and enable identification/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 done on preserved fishes is done on the right side, often damaging tissues and blemishing the specimen's appearance. Therefore, every effort should be made to conform to the convention of photographing the left side of the fish. Also, if there are more than one species expected to co-occur, the photograph(s) should clearly show the character which allows identification of each species in the photo.

Table 3-1 summarizes guidelines for the appropriate view for the photographic image of the common freshwater fish families. An assumption is made that the collector is able to use the photograph to identify a fish to family level. Table 3-1 includes families where individuals are expected to reach a minimum of 12 in total length.

Table 3-1. Guidelines for Photographing the Appropriate View of Fish Specimens

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

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. Take care to avoid breathing, or contact with, formalin.

> **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. Care must be taken in storage and handling.
>
> Check the alcohol and formalin solution material safety data sheets for proper handling requirements.

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
- Collection method (for example: seine or electrofishing)
- Preservative used

- Name of collector(s)
- Container replicate number (for example: 1 of 2 or 2 of 2), if needed

Laboratory Processing

Upon return to the laboratory, assign a unique sample tracking number to the jar(s) containing the fish specimens according to the sequence in the fish sample tracking logbook. For example, a system of numbering may look like F 040 04, where “F” refers to “fish,” “040” refers to sample number 40, and “04” refers to the year 2004.

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 staff 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 the purposes of identifying Texas freshwater fishes, the primary reference is Hubbs et al. (1991), with complementary sources employed as required.

Sample Tracking Log

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

- Sample tracking number
- Date and time of collection
- Station number and location description
- Name of collector(s)
- Collection method (for example: seine or electrofishing)
- Preservative used
- Number of containers in sample

Laboratory Sample Processing

Keep specimens in 10 percent formalin (one part full-strength formalin and nine parts water) 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 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. Complete information on curation of 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 a single sample at a time. For QA purposes, maintain a record of who made the identifications. Chapter 11 provides a complete listing of required and recommended freshwater fish identification references.

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 that include the following:

- Station number and location description
- State
- County
- River basin
- Name of collector(s)
- 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 label to the outside of the container—*but never on the lid* —with the sample tracking number and container replicate number. Make sure container is dry, and wrap with clear tape to ensure the label will not come off.

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

A minimum of 5 percent of all identifications are subject to a blind re-check by another biological expert. Selection of samples for re-check must be randomized. A record of re-checks must be kept for quality control (QC) purposes. If identifications done by a particular individual have an error rate of more than 10 percent, re-identify all specimens. Laboratories must be cognizant 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 a minimum of five years or until the conclusion of applicable regulatory decision (whichever is longer) to allow identification verification if necessary. Voucher specimens serve as long-term physical representations that substantiate the names applied to organisms collected as part of the TCEQ database. Voucher specimens ensure the credibility of TCEQ bioassessment data by documenting the identity of the organisms and making them available for review by the general scientific community.

Take the following into consideration when storing voucher specimens:

- Long-term maintenance of wet (alcohol-preserved) and mounted specimens
- Adequate quantity and quality of space to store specimens
- An effective mechanism for locating and retrieving specimens upon request
- Personnel experience in 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 wadeable, freshwater streams are described in the document *Regionalization of the Index of Biotic Integrity for Texas Streams* (Linam et al. 2002), as cited in the reference section. The report outlines regional IBIs and their application for assessing aquatic life uses, and provides detailed explanations of 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 staff. The full report can be found on the TPWD web site at: <www.tpwd.state.tx.us/publications/pwdpubs/media/pwd_rp_t3200_1086.pdf>. The metric sets are also found in Tables B-3 through B-9 in Appendix B of this document.

Collection and Assessment Methods for Large Rivers

Collecting and assessing fish assemblages in predominantly large, runoff-dominated streams and rivers present substantially greater complexity and potential problems than work in wadeable 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 wadeable streams in terms of discharge and scale. However, most have significant reaches that are not primarily wadeable, have substantial flow, and may pass through multiple ecoregions. Unlike smaller water bodies that are normally replicated across a given region or basin, large rivers are typically unique (Emery et al. 2003).

Reference streams used for comparisons may not be available given man-induced modifications to larger waterways (for example: mainstem reservoirs, wastewater and agricultural return flows) and the lack of streams of similar size and faunal composition for comparison. Aside from issues associated with establishing a comparative baseline, large streams and rivers require different equipment or application of equipment than wadeable 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.

The summer index period may not be appropriate in large rivers depending on issues such as system hydrology including seasonal releases from reservoirs, and/or irrigation withdrawals. Instead, sampling periods should be site and collection-method specific and meet the objectives of the study.

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 collection gears must be employed to obtain a representative sample.

Analytical tools for evaluating biological collections need consideration when sampling large streams and rivers. The assessment tools and regionalized IBIs described by Linam et al. (2002) are a starting point, but were designed for wadeable streams and may not be directly applicable in all situations and depend on the system being sampled. A test of the adequacy of wadeable stream sampling methods may be whether at least 50 percent of the reach can be sampled by wading methods (for example: backpack electrofisher and seines).

Many reaches may be marginally wadeable, whereas others are predominately deep except for the stream margins. The former might be adequately sampled using a combination of a backpack electrofisher and seines as in the wadeable stream protocols, whereas in others boat electrofishing equipment would be more appropriate. In the latter case, seines must still be used as a complementary tool for sampling.

Other gears (for example: gill nets, hoop nets) may be required depending on the objectives of the study and stream conditions. Sampling duration may vary depending upon the system scale. The USEPA has proposed 40 to 100 times the wetted stream width as a reach length to be covered 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 long enough to characterize 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, staff 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, nonwadeable streams and rivers.

Collection and Assessment Methods for Lakes and Reservoirs

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 a reservoir can complicate regulatory processes by making it difficult to first determine what the biological community and structure must comprise for a reservoir to meet its designated ALU. 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 has begun (TPWD 2002), 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 USEPA have developed methodologies for data collection and assessment of the biological integrity of lakes or reservoirs (USEPA 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 uses a similar approach to what has been developed for stream assessment (Dycus and Baker 2001). The USEPA has also published a technical guidance document for the development of lake and reservoir bioassessment and biocriteria programs (USEPA 1998).

Before conducting any biological monitoring activities on 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 addition to seining and electrofishing, gill netting, hook and line, and trap netting may be incorporated in the sampling effort. The TPWD has inland fishery assessment procedures specifically designed to estimate abundance and population structure for game and forage fish species (TPWD 2002). The TPWD inland fishery-assessment 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 the boat mounted electrofisher collection procedures on page 3-3 of this chapter 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 in 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 gears and techniques, the data will be valuable, not only for the given study, but also to address the development of assessment methodologies for these salt water bodies.

Any study employing saltwater fish collection must have clearly defined objectives. Careful consideration of the end uses of the data are essential. The specific methods, gears, and level of effort must be chosen 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 provide fish community data must include at least one active gear type, generally seines or trawls. Passive gears, such as gill nets or trap nets, must be used in conjunction with active gears.

Scientific Collection Permit

Anyone conducting fish surveys in Texas must possess or be listed on a valid TPWD SCP. To apply for a SCP, contact the TPWD at 512/389-4491. All TCEQ regional office and WQST staff are included on the SWQM SCP. Any TCEQ staff that need to be added to this permit should contact the central office SWQM Team. The permittee or sub-permittees must notify the TPWD Austin main office dispatcher (512/389-4848) or regional law enforcement office not less than 24 hours in advance of a fish collection event. The TPWD La Porte office telephone number is 281/842-8100.

The SCP number and information regarding when and where fish will be collected and who will be collecting the fish are required when notifying the TPWD. The TPWD requires that the original permit be carried with the permittee during collections. A sub-permittee must carry a photocopy of the original permit if the permittee is not present. In the event that persons collecting fish are not listed on a valid permit, fax a letter listing the names of the unpermitted individuals and assistants participating in the collection to the TPWD dispatcher 24 hours in advance.

Records

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

Field logbook. For each sample event all relevant information including the date and time of sample collection, location of the sample site (Station ID), collectors name(s), method of

collection, number and type of samples collected, number of sample containers, and preservative used must be entered in the field logbook. Field notes must describe the collection methods employed, equipment used, areas sampled, the way equipment was used, time spent sampling, a description of the sampled habitat(s), and any unusual site characteristics.

Sample tracking logbook. A sample tracking logbook, containing the information described in the QA chapter of this manual, must be maintained. This logbook documents when samples arrive at the laboratory or headquarters facility, when each sample enters each sample processing step, and who has custody or responsibility for the sample.

Laboratory bench sheets. Laboratory bench sheets must be maintained at the location where specimen identification and enumeration occurs. These bench sheets provide documentation of the raw counts of numbers of individuals for each taxon and notes relevant to the identification and enumeration process.

Sample Collection

The method used to collect nekton samples will depend on the habitat type. Saltwater fish collections will generally be conducted in one of three major habitat types, tidal streams, open bay, or Gulf waters. Each of these areas requires a slightly different sampling approach. The coastal bays and nearshore Gulf waters are sampled extensively by the TPWD Coastal Fisheries Division to understand status and trends of selected fin fish 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 amount of guidance available for assessing fish data from tidal streams for regulatory purposes, it is important to consult with the TCEQ WQST staff before any sampling of fish is conducted.

The concept of critical and index periods as used for freshwater streams may not be directly applicable 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 staff to ensure that timing and level of effort are appropriate for the type of assessment that is being undertaken.

The field equipment and materials necessary to collect fish are listed in Appendix A. Forms needed for biological assessments are included in Appendix C and are posted on the TCEQ SWQM web page. Definitions of technical terms are located in the Glossary in Appendix F.

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 gears.

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. Tidal streams are usually too saline for effective electrofishing, so this method is discouraged in tidal streams. In bayous where the salinity may be low in places, it may be possible to employ 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 and bottom-dwelling

organisms. As the net (trawl) is dragged along the bottom, fish enter the net and are captured. They collect in the end of the net (cod end), which is tied shut. After retrieving the net from the water the cod end may be untied to easily remove the fish for inspection. 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 the net as it is towed forward. It may not be possible to use a trawl in environments with a lot of rocky or woody debris on the bottom.

In tidal streams, use a 10 ft otter trawl.

The required reference for trawling is:

Murphy, B.R. and D.W. Willis, (eds.)1996. *Fisheries Techniques*. 2nd Edition. American Fisheries Society, Bethesda, Maryland. 732 pp.

This reference fully describes the appropriate methods for deploying and collecting biological specimens with trawls.

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

Do not pull trawl in marked navigation channels.

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

Seine

Seining is an active fish capture method that is used near the shore to capture mainly smaller fish and juveniles. Seines can be hard to deploy 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 must be 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 allow a 15 ft straight seine to be deployed. One alternative in this situation is for one worker to deploy 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 person on shore slightly behind person offshore. At 8 m, the person at the shore remains stationary. The person offshore proceeds to shore with seine extended. Both persons pull seine onto shore. Be sure the lead line remains on bottom until 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 a minimum of six 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. A minimum of three people are required when electrofishing out of a boat. Rubber, nonconductive gloves and knee boots or waders must be worn by all people on the boat. Every effort should be made to keep the gloves dry. All individuals in the boat must wear personal floatation devices. 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 power on but circuit off and then energizing electrodes for an element of surprise. All habitat and cover types must be electrofished. Record distance and actual shocking time. A slow, deliberate capture style is safer than fish chasing, and also yields more representative samples.

Collecting. Electrofishing is likely to be an ineffective method for collecting nekton except in areas where the salinity is quite low, such as in the upper end of tidal streams or bayous. 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, not enough data exist to determine their effectiveness. The length of sampling effort is 900 seconds of actual shock time.

If elevated conductivities are encountered, observe manufacturer's recommendations about the equipment's limits. Electrofisher capabilities vary by manufacturer and model. Each model is effective under certain specific conductance ranges. Check the manufacturer's specifications for optimal operating procedures and consult with the manufacturer if the unit is consistently operated at higher conductivities.

Gill Net

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 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.

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

The required reference for gill netting is:

Murphy, B.R. and D.W. Willis, (eds.)1996. *Fisheries Techniques*. 2nd Edition. American Fisheries Society, Bethesda, Maryland. 732 pp.

This reference fully describes the appropriate methods for deploying gill nets and collecting the fish.

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

Sample Preservation and Processing

Field Processing

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 fishes back into the stream from either method until all sampling is completed!** Maintain the fishes in some type of holding bucket or tank with adequate aeration.

Retain two representatives of each species collected for positive identification in the laboratory. This does not apply to fishes greater than 0.3 m total length. Retain all but the most readily identifiable fishes for laboratory identification.

Use of Digital Photos as Fish Vouchers

Refer to the Chapter 3, Freshwater Fish, for procedures on 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. Take care to avoid breathing or contact with formalin.

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

Container Label

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 collector(s)
- Container replicate number (for example: 1 of 2 or 2 of 2), if needed

Laboratory Processing

Upon return to the laboratory, assign a unique sample tracking number to the jar(s) containing the fish specimens according to the sequence in the fish sample tracking logbook. For example, a system of numbering may look like F 040 04, where “F” refers to “Fish”, “040” refers to sample number 40, and “04” refers to the year 2004.

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 staff 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 the purposes of identifying Texas saltwater fishes, the primary reference is Hoese and Moore (1998), with complementary sources employed as required. Many estuarine and freshwater fishes, often collected in tidal streams, can be identified by reference to Hubbs et al. (1991). Chapter 11 provides a complete listing of required and recommended saltwater fish identification references.

Sample Tracking Log

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

- Sample tracking number
- Date and time of collection
- Station number and location description
- Name of collector(s)
- Collection method (for example: trawl, gill net, seine)
- Preservative used
- Number of containers in sample

Laboratory Sample Processing

Specimens are to remain in 10 percent formalin (one part full-strength formalin and nine parts water) for at least one week and then soaked in water for three days with a change of water each day. Take care to avoid breathing or exposing yourself to the formalin. Transfer to 45 percent isopropyl alcohol or 70 percent ethanol prior to 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 and 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. Complete information on curation of 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 a single sample at a time. For QA purposes, maintain a record of who made the identifications.

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 gear type with appropriate labels that include the following:

- State
- County
- River basin
- Station number and location description
- Name of collector(s)
- Collection method (for example: trawl, gill net, seine)
- Species name (not common name)
- Number of specimens in container
- 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 *Opisthonema oglinum*.

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

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.

Quality Control Checks

A minimum of 5 percent of all identifications are subject to a blind re-check by another biological expert. Selection of samples for re-check must be randomized. A record of re-checks must be kept for QC purposes. If identifications done by a particular individual have an error rate of more than 10 percent, re-identify all specimens. Laboratories must be cognizant 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 a minimum of five years or until the conclusion of applicable regulatory decision (whichever is longer) to allow identification verification if necessary. Voucher specimens serve as long-term physical representations that substantiate the names applied to organisms collected as part of the TCEQ SWQM Program. Voucher specimens ensure the credibility of TCEQ bioassessment data by documenting the identity of the organisms and making them available for review by the general scientific community.

Take the following into consideration when storing voucher specimens:

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

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 the sampled habitat(s), and any unusual site characteristics.

Data Evaluation

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

CHAPTER 5
FRESHWATER BENTHIC MACROINVERTEBRATES

Objective

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

Scientific Collection Permit

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

Overview of Sample Collection Methods

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

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

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

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

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

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

Equipment

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

Records

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

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

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

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

Wadeable Streams and Rivers

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

Where to Collect Samples

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

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

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

Riffles

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

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

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

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

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

Runs and Glides

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

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

Pools

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

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

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

Rapid Bioassessment Protocols

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

RBP Kicknet Sample Collection Procedures

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

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

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

Equipment

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

Collecting a Sample

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

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

Processing a Sample in the Field

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

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

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

Processing a Sample in the Lab

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

RBP Snag Sample Collection Procedures

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

When to Use the Snag Sample Collection Method

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

Selecting Snags

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

Collecting a Sample

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

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

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

Processing a Sample in the Field

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

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

Processing a Sample in the Lab

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

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

Benthic Macroinvertebrate Sample Preservation Procedures for RBP Samples

Preservation for Processing a Sample in the Field

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

Labeling the Sample

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

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

Preservation for Processing a Sample in the Lab

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

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

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

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

Labeling the Field Sample for Laboratory Processing

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

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

Sample Processing Procedures for RBP Kicknet or Snag Samples

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

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

Field Processing RBP Kicknet or RBP Snag Samples

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

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

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

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

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

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

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

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

Sample Tracking Requirements for RBP Kicknet or RBP Snag Samples

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

The sample log will contain the following information:

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

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

Laboratory Processing RBP Kicknet or RBP Snag Samples

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

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

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

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

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

Voucher Specimens

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

Take the following into consideration when storing voucher specimens:

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

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

Data Evaluation Procedures for RBP Kicknet or RBP Snag Samples

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

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

The criteria set includes 12 metrics.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Surber Sampler Protocols

Sample Collection Procedures

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

Where to Collect Samples

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

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

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

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

Collecting a Surber Sample

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

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

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

Quantitative Snag Samples

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

Selecting Snags

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

Collecting a Sample

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

Field Processing and Preserving a Surber Sample

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

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

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

Labeling the Sample Container

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

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

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

Sample Tracking Requirements for Surber Samples

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

The sample log will contain the following information:

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

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

Laboratory Processing for Surber Samples

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

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

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

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

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

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

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

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

Laboratory Processing for Quantitative Snag Samples

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

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

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

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

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

Voucher Specimens

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

Take the following into consideration when storing voucher specimens:

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

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

Data Evaluation Procedures for Surber Samples

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

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

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

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

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

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

The draft criteria set includes the following 11 metrics:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Sample Collection Procedures

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

Collecting a Sample

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

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

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

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

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

Preserving a Sample

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

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

Labeling the Sample Container

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

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

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

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

Laboratory Processing

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

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

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

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

Labeling the Sample Container

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

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

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

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

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

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

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

Records

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

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

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

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

Voucher Specimens

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

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

Take the following into consideration when storing voucher specimens:

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

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

CHAPTER 6
SALTWATER BENTHIC MACROINVERTEBRATES

Disclaimer

Methodologies for assessing ALU 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

The objective of this chapter is to describe the methods used by the TCEQ for the collection and assessment of benthic macroinvertebrate samples from saltwater systems. In general, the TCEQ will use benthic macroinvertebrate samples collected according to these methods in conjunction with fish community surveys and physical habitat assessments in an attempt to provide a holistic evaluation of the health of biological assemblages and to develop future indices of aquatic life use for these waters. Sampling saltwater benthic macroinvertebrates also facilitates the collection of 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 gears and techniques, the data will be valuable, not only for the given study, but also to the development of assessment methodologies for these salt water bodies.

Any study employing saltwater benthic macroinvertebrate collection must have clearly defined objectives. Careful consideration of the end uses of the data is essential. Methods, gears, and levels of effort must be chosen with the goal of meeting the study objectives. 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.

Scientific Collection Permit

Aquatic insects are not protected under state law; however, an SCP is required for the collection of 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.

Sample Site Selection

Marine macrobenthos 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 do otherwise. In choosing a sample location, some trial dredge hauls will help determine an area of 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 it may be

necessary to use a heavier dredge, 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 them carefully to ensure that all parts are in good operational condition. The following collection methods refer to the Ekman dredge but, with only minor exceptions, apply to 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 Ekman 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 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.

Sample Washing

To minimize damage to organisms, homogenize the sample by hand. Wash the sediments overboard through a #30 (mesh size $\leq$ 595 μm) or #35 (mesh size = 500 μm) sieve bucket by dunking the bucket gently, or gently washing with a deck pump. If a deck pump is used, 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 narcotizer must cover the sample without washing the sample out of the sieve. Narcotizer is 7 percent magnesium chloride ($MgCl_2$) in sea water (75 g of $MgCl_2$ per liter). After the sample is narcotized for 0.5 to1.0 hr, remove the sieve the from 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 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 attempt to use alcohol for a fixative with marine organisms.

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

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

Labeling the 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 collector(s)
- Container replicate number (for example: 1 of 2 or 2 of 2), if needed

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 collector(s)
- Collection method (for example: Ekman dredge)
- Sieve type
- Number of containers in each replicate sample

> **Safety Note:** To reduce your exposure to formalin, rinse the sample with water in a sieve with mesh size $\leq$ 595 µm 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.

Within two weeks of sample 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. Rose Bengal vital stain (or another appropriate stain) may be added to the samples to aid in sample 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 fixature with the sample.

Sorting in the Lab

The objective of processing an estuarine benthic macroinvertebrate sample in the laboratory is to count and identify every individual benthic macroinvertebrate 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 will allow 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 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 (2x), pick *all macroinvertebrates* visible from the sample and place in a sample bottle or vial containing 70 percent ethanol or isopropyl alcohol and a label. It is usually necessary to place a small portion 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 no organisms were missed. Check at least 10 percent of samples for missed organisms. Record the date and identity of the sorter(s) in the sample tracking logbook.

Labeling a 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 collector(s)
- 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—*but never on the lid*. Make sure the container is dry, and wrap with clear tape to ensure the label will not come off.

Laboratory Procedures for Identification of Specimens

Identification and enumeration of estuarine benthic macroinvertebrates must be conducted by individuals with appropriate expertise, training, and knowledge of the literature. Using appropriate reference(s), a stereo dissecting microscope, and compound microscope, identify the organisms to the lowest practical taxonomic level—*species* in most cases. Chapter 11 provides a complete listing of required and recommended saltwater benthic macroinvertebrate identification references. 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

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

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

Sample tracking logbook. Maintain a sample tracking logbook that contains the information described in the QA chapter of this manual. This logbook documents when samples arrive at the

laboratory or headquarters facility, when each sample enters each sample processing step, and who has custody or responsibility for the sample.

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

Voucher Specimens

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

Take the following into consideration when storing voucher specimens:

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

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

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 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 wadeable freshwater systems. Benthic algae are an important component of the periphyton 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 (USEPA 1976).

The purpose of this sampling is to determine the kinds and total number—or relative abundances—of benthic algae present. Depending on the sample location, there may be macroscopic and microscopic benthic algae (periphyton), microscopic plankton (algae and other organisms suspended in the water column), and possibly visible surface "scums." Record general types and abundances of these algae on a field data sheet. The type of sampling and analysis of algal assemblages performed will depend on the purpose of the study.

Overview of Benthic Algal Sample Collection Methods

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

Equipment

Benthic algal sample collection equipment is minimal. Qualitative sample collection requires a sample collection jar, a pocket knife 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.

Records

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

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

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

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

Wadeable Streams and Rivers

Benthic Algal Qualitative Sample Collection Procedures

For a synoptic analysis of the benthic algal community, collect a qualitative composite sample from all available habitats. 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 macro-algae, green and blue-green algal mats, and diatom mats located in the sample reach.

Collect macro-algae with forceps and place in a separate sample jar. The macro-algae can be later identified and not lost during the diatom sample processing. Continue sampling until 20 to 50 mL of algal material has been collected.

Hard substrates (epilithic algae). Sample hard substrates, such as rocks, boulders, turtle shells, or mollusc shells by scraping with a knife or stiff brush and rinsing into the sample jar.

Woody debris (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 (epipsammic or epipelic algae). In depositional areas with no current, sample algal mats growing on top of fine sediments by using the sharp edge of a pocket knife or micro-spatula and gently lifting the top layer into the sample jar. A pipette can also be used to suction algae from the surface of fine sediments.

Macrophytes, roots wads, mosses (epiphytic algae). Rub algae from plant material with fingertips and place it in the sample jar. Squeeze water and algae from mosses into sample jar. Place bits of plant material into 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.

Benthic Algal Sample Preservation

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

Safety Note: 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 formalin solution and glutaraldehyde Material Safety Data Sheets for proper handling requirements.

Labeling the Container

Affix a label to the outside of the container, making sure the container is dry, and wrap with clear tape to ensure the label will not come off. 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 collector(s)
- Sample type
- Container replicate number (for example: 1 of 2 or 2 of 2), if needed

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

Field Notes

Record the following information 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

Laboratory Procedures for Qualitative Benthic Algal Sample Processing and Identification

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 macro- 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 “Sample Analysis” section of this chapter.

Chapter 11 provides a complete listing of required and recommended algal identification references.

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 mix it and dislodge epiphytes from filamentous algae. Using fine-tipped forceps, pick representative macro-algae from the mixture and place them on a microscope slide with a few drops of water. Cover with a cover slip and identify the filaments under a compound microscope that is equipped with 10x, 20x, and 40x objectives. Do not attempt to examine a wet mount using oil and an oil immersion objective.

2. Mix the sample again and pipette a few drops onto a new slide to identify the 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 a minimum of 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 counting the algae in the sample 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 an algae sample. 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 mounting media 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 Ehrlenmeyer 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 hotplate 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 at least a 2000 mL flask to prevent acid from boiling over.

4. After oxidation overnight, or after sample is cooled if the boiling method is used, fill the flask with distilled water. Allow the sample to settle overnight.

5. Decant or siphon off the supernatant, and refill the flask. Allow to settle overnight again.

6. Siphon off the supernatant and pour the cleaned sample into a 1000 mL glass cylinder. Fill with distilled water and allow to settle overnight again, or at least four hours, until all the diatom frustules have settled to the bottom of the cylinder.

7. 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 diatom sample for at least 60 seconds.

2. Pipette 2 to 3 drops of sample onto a cover slip placed on a cool hotplate 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 hotplate next to it. Place a drop of Naphrax or another high refractive index mounting medium onto the slide and invert the cover slip onto it. Turn the hotplate on low and heat until the slide begins to bubble.

4. Remove the hot slide from the hotplate with flat bladed forceps and set on a heat resistant surface (a piece of corrugated cardboard is suitable) to cool.

5. After the slide is cool and hardened, scrape any excess mounting medium from the slide.

6. Permanently label the slide. Slides with a frosted end are preferred, 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 100x 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. Identification of the diatoms in the sample in this way will speed the counting process.

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. Once counting has begun, 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 at the end of this chapter.

Benthic Algal Data Evaluation for Qualitative Samples

Non-diatom Benthic Algal Evaluation

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 Assemblage Evaluation

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 river water monitoring studies. 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.
- Total number of taxa and individuals found at any given site is usually high enough 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 have rapid response and recovery 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).

Verification of diatoms performed by outside experts will be circled on the slide with a diamond pencil and labeled appropriately with the taxon name.

Multiple Metric Analysis

The diatom community lends itself to multiple metric 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 gathered, 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).

1. **Taxa richness**. High species richness is assumed to be the case in a 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). The Shannon index is calculated as:

   $$H' = -\sum_{i=1}^{s} P_i \log P_i$$

   where s is the number of species and P_i is the proportion of individuals in the ith species.

   While higher values for this metric have historically been assumed to indicate higher water quality, this interpretation can be misleading if taxa richness 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).

3. **Percent dominance**. Recently, the diversity index has been replaced by indices that more directly measure the two components of the original index, taxa richness (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)**. Calculated using the formula,

   $PTI = \sum n_i \times t_i / N$

   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 macroinvertebrates (Hilsenhoff 1987), with the exception that tolerance values range from 1to 4, and increasing numbers signify increased sensitivity. Tolerance values were generated 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 (1977-1993) and data collections by the Kentucky Nature Preserves Commission (1979-1986) 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. ***Cymbella* group taxa richness**. 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. The formula for calculating PCSI is:

   $PCSI\ (AB) = 100-0.5 \sum |a-b|$ where a is the percentage of species a in sample A and b is the percentage of species a in sample B

   Higher percent similarity to the reference site may indicate higher water quality, assuming the reference site is of high quality.

Benthic Algal Quantitative Sample Collection Procedures

Quantitative sampling of the benthic algae are not part of a routine monitoring event; however, special studies may require quantitative analyses. Sampling methods for quantitative analysis depend on the type of study and must follow general guidelines described in this section.

For example, quantitative sampling of the benthic algae may be necessary to determine if nuisance periphyton levels are present. 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). Future studies on nutrient enrichment and algal biomass may require quantitative sampling of the benthic algal community. These samples may include chlorophyll *a* and/or other biomass estimates as well as qualitative counts of algal abundance and distribution.

Quantitative Sample Collection Procedure 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. Collect replicate samples from a riffle or run along a transect across the stream as follows:

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 five equal increments. Start sampling at the midpoint of the first increment.

5. Move to the first increment, and without looking, reach down and select the first rock touched for a cobble sample, or sample that area of bedrock using appropriate sample collection methods as outlined below.

6. Move to the midpoint of the next increment and collect the second replicate. Repeat until the required number of replicates is collected.

Note: If distribution of periphyton is extremely patchy, more than one transect of five replicate samples may be necessary to accurately represent the sample site.

Sample Collection Methods

Use the sample collection method that is most appropriate for the habitat, making modifications as professional judgment deems necessary. In all cases, measure the surface area of the bedrock or cobble sampled as accurately as possible and attempt to collect all the algal material within that selected area. 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 pool or depositional area sample collection.

Bedrock or Boulder. After setting up the transect (see above), sample known areas of rock or bedrock with a brush and suction device as follows:

1. Press a 4 in to 6 in length of 4-in-diameter PVC pipe fitted with a neoprene gasket against the substrate. Seal the gasket tightly against the substrate so that water and dislodged algae does not leak out of the enclosed area.

2. Scrape and remove as much filamentous algae as possible from within the enclosed area and place it into the sample jar. If the algal filaments are particularly long, it helps to cut them with small scissors.

3. Brush the remaining algal material from the substrate with a stiff brush. A toothbrush bent at the head at a 90 degree angle or a stiff artist's paint brush is suitable.

4. Keeping the 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 a hand-operated vacuum pump.

5. Repeat this process along the transect across the riffle or run until five separate replicates are collected.

Cobble. After setting up the transect (see above), sample cobble riffle habitat as follows:

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 must 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 surface area and sample volume for later biomass calculations.

5. Keep replicate samples separate.

Note: A smaller suction device, such as a 30 mL syringe with the end cut off, may be appropriate for sampling cobble (USGS 2002). However, more effort is required for a representative sample. If using the USGS sampling device, samples from five separate cobbles from each transect interval must be composited for each replicate. This will total 25 separate syringe samples–five cobbles per replicate times five replicates. Label and analyze replicates separately, do not composite them.

Quantitative Sample Collection in Streams with Clay, Silt, or Sandy Substrates, or Non-wadeable Streams

If the stream has a clay, silt, or sand substrate, the above methods are not appropriate. In this case, the following collection methods may apply.

Macrophytes and snags. Cut sections of submersed plant material or woody snags and wash the algal material from them 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 submersed in the stream long enough to have developed a natural algal community; it should not be material that has 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 must be colonized by filamentous algae or diatom mats.

Artificial substrates. Use artificial substrates if there is no feasible or safe way to collect a sample otherwise. While the benthic algal community that colonizes artificial substrates is usually not representative of the community that colonizes a naturally occurring substrate, a sample using an artificial substrate 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 vandalization, redeploy fresh ones.

Phytoplankton. See Chapter 8 for phytoplankton sample collection methods.

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.

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 cool dark container 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 Note**: 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 formalin solution and glutaraldehyde Material Safety Data Sheets for proper handling requirements.

Labeling the Sample Container

Affix the label to the outside of the container making sure the container is dry. Wrap with clear tape to make sure the label stays fixed to 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 collector(s)
- Sample type
- Container replicate number (for example: 1 of 2 or 2 of 2), if needed

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

Field Notes

Field notes comprised of 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.

Laboratory Procedures for Quantitative Benthic Algal Sample Processing and Identification

Biomass Estimation

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 1999). Samples for chlorophyll *a* analysis must be processed and filtered within 24 hours of collection. Filters can then be retained, frozen in a dark container—such as a 35 mm film canister—for 28 days before extraction. While the methods described in *Standard Methods* are written for samples from artificial substrates, they can be easily adapted for qualitative samples from natural substrates. Ash-free weight and chlorophyll contentanalysis methods are described in methods 10030.C.5 and 10030.C.6. If fluorometric analysis of chlorophyll is to be performed, use USEPA method 445.0 (USEPA 1997).

Taxonomic Analysis

Chapter 11 provides a complete listing of required and recommended algal identification references.

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 will have to be made to report the data as cells per mm^2. Record on a laboratory bench sheet 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, then proceed to count strips or fields. See *Standard Methods* (APHA 1999) for details on using counting chambers.

Identify algae to genus whenever possible, counting 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 from *Standard Methods*.

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

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)

Benthic Algal Data Evaluation for Quantitative Samples

Periphyton Biomass Evaluation

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 (for example: algae, cyanobacteria, and moss) and heterotrophs (for example: 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).

Benthic Algal Assemblage Evaluation

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

The purpose of macrophyte sampling is to illustrate short- and long-term changes in water quality. Select a sample site that is representative of the attached vegetation found at a monitoring station and which is readily accessible for future sampling. Collect samples by hand, or using a rake or grapple. Report data as number of the individuals per unit area. Data can also be used to describe presence or absence of nuisance growths of aquatic plants.

CHAPTER 8
PLANKTON

Disclaimer

Methodologies for assessing ALU 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-Wadeable Streams, Rivers, Lakes, Reservoirs, and Bays

The objective of this chapter is to describe methods recommended by the TCEQ for the collection and assessment of plankton assemblages.

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 fish kill or other harmful algal bloom event. Special handling may be required for red tide, golden alga, or cyanobacteria blooms. Contact the laboratory that will be analyzing the samples before sample collection for specific instructions.

Netplankton Collection Method

1. Collect a minimum of two vertical net tows per station.
2. Lower a clean plankton net with bucket attached to a depth of 1 m above the substrate.
3. Keeping the net line as vertical as possible, raise the net through the water column at a rate of 0.6 m/sec.

Low Abundance

Low netplankton abundance is indicated by the net bucket draining rapidly and freely as the net is lifted from the water. In this circumstance, rinse the plankton on the surface of the net down into the bucket. This can be accomplished by holding the net upright and dunking it several times into the water, up to the mouth. Alternatively, water can be splashed on the outside of the net and the plankton will be washed down to the bucket. Disconnect the bucket and rinse into a clean waterproof container using a rinse bottle filled with either 3 to 5 percent formalin (three to five parts full-strength formalin and 97 to 95 parts water) or ambient water prefiltered through the plankton net. If formalin is used, add several grams of borax to buffer the formalin solution.

High Abundance

High netplankton abundance is indicated by the net bucket draining slowly in a restricted manner as the net is lifted from the water. Discard the net sample and collect water samples at specified depths with a Kemmerer or Van Dorn sampler. Pour the water samples into the plankton net. Sample depths are:

- 0.3 m below the surface
- 1 m above bottom
- At 3 m intervals between surface and bottom (a minimum of three samples are collected)

Sample Processing

Once all the water samples have been poured through the plankton net, wash the plankton down into the bucket and then rinse the net and bucket into a clean, waterproof container using a rinse bottle filled with ambient water prefiltered through the plankton net.

Sample Preservation

Preserve by adding 1 mL of Lugol's solution per 100 mL of final sample volume. The Lugol's solution is prepared by combining 180 mL of deionized water, 20 mL of glacial acetic acid, 20 g of potassium iodide, and 10 g of iodine crystals (APHA 1999).

> **Safety Note**: Label 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

Affix the label to the outside of the container making sure the container is dry. Wrap with clear tape to make sure the label stays fixed to the container. Labels must contain the following information:

- Station number and location description
- Date and time of collection
- Preservative used
- Name of collector(s)
- Sample type
- Container replicate number (for example: 1 of 2 or 2 of 2), if needed

Additional labeling is necessary depending on the method of collection.

For net hauls include:
- Number of net hauls
- Depth of net hauls
- Diameter of net mouth

For Kemmerer (or Van Dorn) samples, subsequently filtered with a net, 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.

Nannoplankton Collection Method

Collect a minimum of 500 mL of sample per station.

Measure and record Secchi disk transparency for the location (see *Secchi Disk Transparency* in RG-415, Chapter 3).

Using a Kemmerer, Van Dorn, or other type of sampler capable of collecting water samples from specific depths, collect samples from the following depths.

- 0.3 m
- 1 x Secchi disk transparency
- 2 x Secchi disk transparency
- 3 x Secchi disk transparency

Note: Do not collect samples closer than 1 m above the substrate.

Upon retrieving the sampler from the water, quickly pour 200 mL of sample into a clean, graduated cylinder. Empty the graduated cylinder into a clean, 1 L cubitainer.

Repeat until samples are collected from the required depths and 200 mL from each sample is composited into the same cubitainer.

As an alternative to compositing water from various depths, a plastic tube can be used to take a continuous sample to a depth equal to three times the Secchi disk transparency. Slowly lower a weighted tube (of the same continuous inner diameter) to the desired depth, cap the end, and pull up the tube, full of water. Release the water into the sample container or net. Repeat as necessary to obtain at least 500 mL of sample.

Sample Preservation

If the sample cannot be analyzed within 24 hours, preserve it with Lugol's solution, i.e., 1 mL of Lugol's solution per 100 mL of sample (APHA,1999). If the sample will be analyzed within 24 hours, store the sample in a cool, dark location with 5 cm of air space in the container. Do not refrigerate or place a live sample on ice.

Labeling Sample Containers

Affix the label to the outside of the container making sure the container is dry. Wrap with clear tape to make sure the label stays fixed to the container. 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
- Secchi disk transparency
- Volume (mL) composited from each depth
- Preservative used
- Name of collector(s)
- Sample type
- Container replicate number (for example: 1 of 2 or 2 of 2), if needed

Sample Storage

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

CHAPTER 9
PHYSICAL HABITAT OF AQUATIC ECOSYSTEMS

Objective

The objective of this chapter is to describe the methods used by the TCEQ for the collection and assessment of physical habitat data in wadeable freshwater streams. In general, the TCEQ will use habitat data collected according to these methods, in conjunction with fish and benthic macroinvertebrate community surveys, to provide a holistic evaluation of 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 fish and benthic macroinvertebrate community integrity. Characteristics of physical stream habitat such as the presence or absence of instream cover, substrate characteristics, and riparian integrity have important affects 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

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, data are summarized and averaged from the worksheets to complete the "Summary of Physical Characteristics of Water Body–Part II." The "Habitat Quality Index (HQI)–Part III" is then scored and calculated based on the values summarized in Part II. Locate transect locations on a USGS topographic quadrangle map and attach to the forms. For RWAs, also locate the existing or proposed discharge point(s) on the map. See Appendix C for Part I, II, and III forms.

Habitat Assessment Requirements for Biological 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 habitat reach placement requirements are the same and are outlined in Chapter 2, "Biological Monitoring Requirements." It is very important that the habitat assessment reach covers the areas where fish and benthic macroinvertebrate samples are collected. Effort must be made to ensure the reach is not close to a bridge overpass. Occasionally a bridge crossing is strewn with rip-rap or other debris that forms the only "riffle" in the reach. Sampling from these artificial riffles is discouraged and should only be conducted when all other sampling efforts outlined in Chapter 5, "Methods for Collecting and Assessing Freshwater Benthic Macroinvertebrates" have been exhausted. Once the habitat, fish, and benthic macroinvertebrate sampling crew leaders have agreed where sampling will be conducted, the habitat crew marks the ends of the reach with bright survey flagging. Sampling from areas outside those boundaries is discouraged.

Habitat Assessment Requirements for UAA, ALA, and ALM Monitoring During Second Event in an Index Period

In any one year, most ALMs, ALAs, and UAAs will involve two sampling events conducted 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 it is demonstrated that conditions have 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 conduct habitat assessment at both events. These allowances apply only to two habitat events conducted within one index period.

Receiving Water Assessments

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 from a point starting 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.

Reach Length Determinations

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 (or boating) 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.” The stream reach encompasses the biological and chemical collection areas and includes as many different geomorphic channel units as possible. Examples of geomorphic units include riffles, runs, glides, and pools.

Wadeable Streams

Streams are considered wadeable if most of the stream channel is accessible by wading during normal flow conditions. Generally, these streams are third order or less. Pool areas or high-flow conditions may cause the stream to be inaccessible to wading in certain places or at certain times; however, the stream would still be considered wadeable when determining reach length. The reach length of a wadeable stream is based on 40 times the average stream width, but not less than 150 m. For most wadeable streams, this will result in a reach length of approximately 200 to 300 m. The maximum reach length for wadeable streams is 500 m.

Non-Wadeable Streams

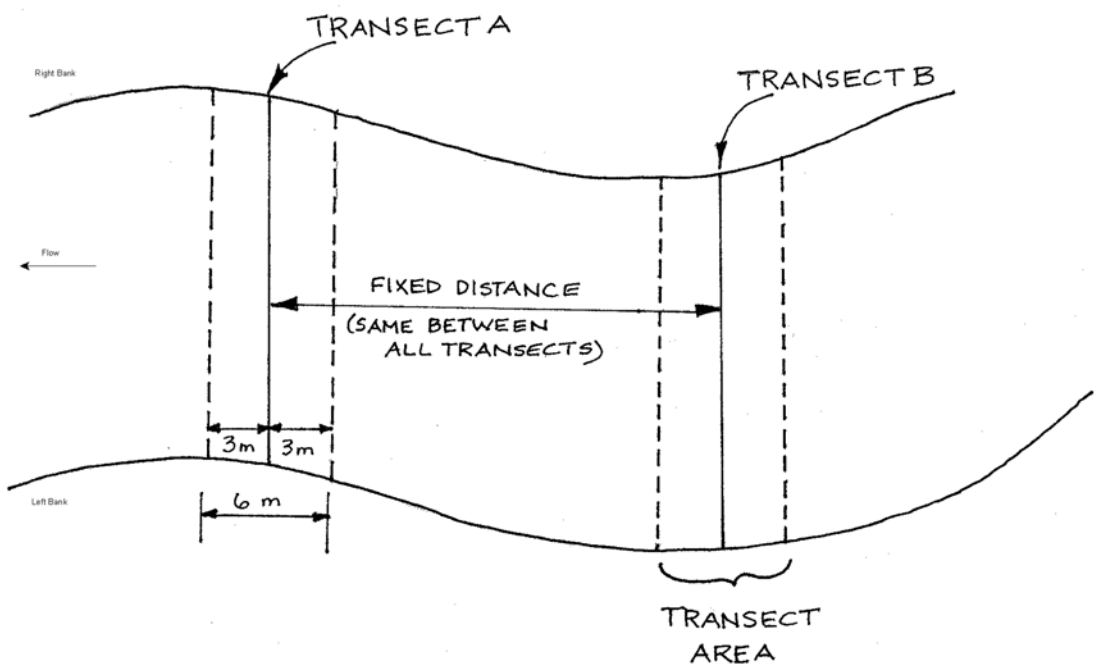
Streams are considered non-wadeable if water depth in the stream channel prohibits wading and requires use of a floatation device (boat or tube) during normal flow conditions. Generally, these are fourth order streams or larger and are usually considered rivers. Riffle areas or low-flow

conditions may cause the stream to be accessible to wading in certain places or at certain times; however, the stream would still be considered non-wadeable when determining reach length.

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

The minimum reach length for a non-wadeable 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.

Figure 9-1. Transect Placement



Transect Placement

For purposes of 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 11 evenly-spaced intervals along the reach, as shown in Figure 9-1.

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 9-1. Reach boundaries are included as transects. It is preferable to begin with the transect furthest downstream as this allows the biological sampling to be conducted ahead of the habitat assessment, thereby minimizing disturbance to the biota from the habitat measurement activities. The distance between transects is uniform and this distance must be measured with a measuring tape, hip chain, or range finder. The placement of transects within the reach is as follows:

Wadeable Streams

For reach lengths of 150 to 300 m, place five evenly-spaced transects throughout the length of the reach and include the reach boundaries as transects. The distance between transects is no greater than 75 m.

For reach lengths of 301 to 500 m, place six evenly-spaced transects throughout the length of the reach and include the reach boundaries as transects. The distance between transects is no greater than 100 m.

Non-Wadeable Streams

For reach lengths of 500 m to 1 km, place a minimum of six and a maximum of 11 evenly-spaced transects over the reach length and include the reach boundaries as transects. Select an appropriate number of transects so that transects are no greater than 100 m apart.

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 stream habitat assessment. If the stream contains standing perennial pools in which aquatic life is found and sampled, conduct a stream habitat assessment in the same manner described in this document with the following modifications.

Determine the reach length in the same manner as stated above using the stream 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, conduct the habitat assessment according to the procedures outlined 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, riparian information, etc. If the pools cover less than 50 percent of the reach length and pools 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 further 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 was water in the stream. Characterize only those pools in the reach with a surface area greater than 1 m^2 and deeper than 10 cm.

Part I: Stream Physical Characteristics Worksheet

The Stream Physical Characteristics Worksheet is used to record primary, secondary, and tertiary attributes for each transect or for the entire reach. Instream channel measurements (primary), stream morphology (secondary), and riparian environment (tertiary) information is recorded 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, 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. In order 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 $\geq$ 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 water surface. 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.

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 characterized by slow-moving laminar flow, similar to that found in a shallow canal. Water surface gradient over a glide is nearly zero, so velocity is slow, 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 slow 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 habitat assessment reach. 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 it as 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 —
- Large Boulders > 45 cm
- Boulders > 25 to 45 cm
- Cobble > 6 to 25 cm
- Gravel > 2 to 60 mm
- Sand 0.06 to 2 mm
- Mud and Silt < 0.06 mm

The size composition can be assessed visually or by obtaining one or more small samples by hand or grab.

Percent Gravel or Larger

Estimate the percentage of the substrate that is > 2 mm in size along and 3 m on either side of the transect. 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 abundance.

Instream Cover Types

Instream cover refers to physical structures which provide shelter for fish and benthic macroinvertebrates. It includes, but is not limited to, logs, tree stumps, woody debris, root wads, leaf packs, gravel or larger-sized substrates, boulders, artificial cover (for example: tires, 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 water 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 percentage 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.

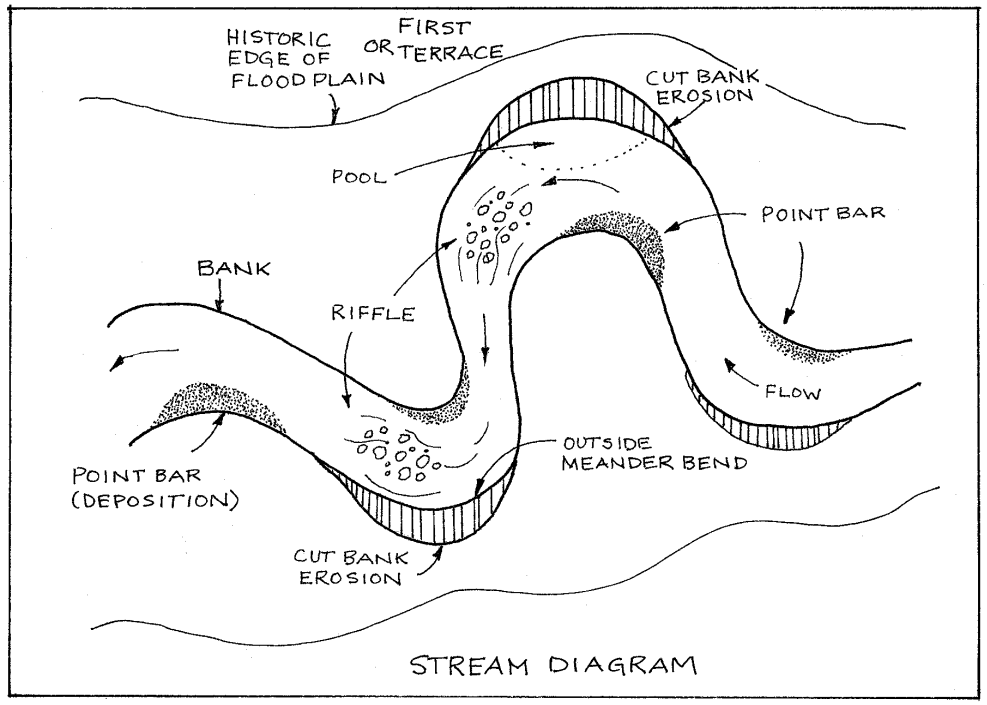


Figure 9-2. Stream Morphology

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 9-2 depicts a typical stream channel with a well developed stream pattern.

Stream Bends

Count the number of stream bends and determine their definition (well, moderate, poor). Figure 9-3 illustrates stream-bend classifications.

A high degree of sinuosity produces diverse habitat and fauna, and the 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 provides shelter for benthic macroinvertebrates and fish.

A well defined bend will usually have a point bar at the inside of the bend and a cut bank on the outside of the bend with flow directed toward the cut bank side. Eddy currents are usually found in these bends. Moderately defined stream bends have somewhat less sinuosity and the bends and

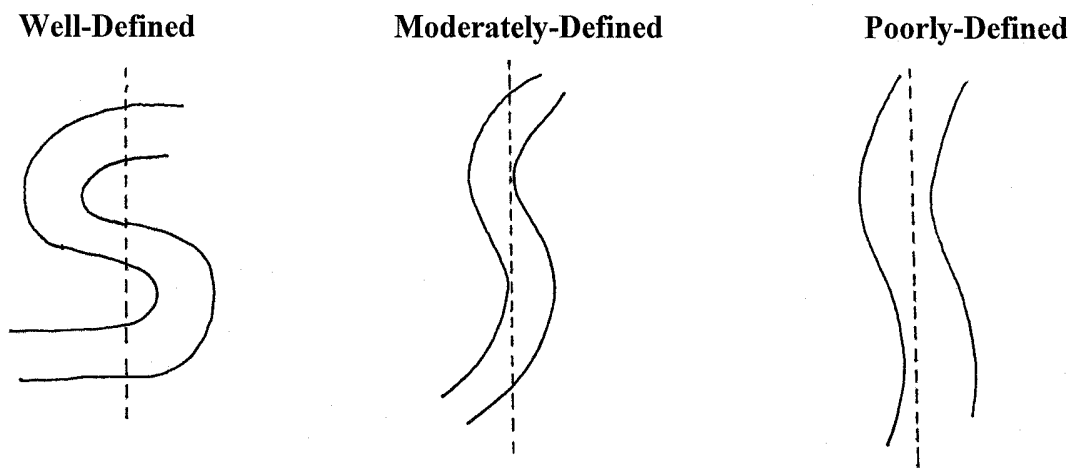


Figure 9-3. Stream Bends

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 viewing 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.

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 rip-rap within the reach; note whether these modifications are natural or man-induced.

Channel Flow Status

Channel flow status 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 the TCEQ's *Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue*, RG-415 (TCEQ 2003). Flow severity is a visual assessment of the amount of flowing water in the channel relative to base flow or "normal" flow conditions. Look for 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 present 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 flow 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 would 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 greater 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.

No flow: Very little water in the channel; mostly present as standing pools or 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. Water's edge can be defined as the point where stream materials, such as rocks, are no longer surrounded by water. Record this width in meters.

It is important to 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 wadeable 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-wadeable 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 wadeable streams. In non-wadeable streams, best profession judgement 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 flood waters from the stream. Interaction between this terrestrial zone and the stream is vital for the health of the stream.

Natural Vegetative Buffer

The natural vegetative buffer refers to 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 man-altered settings, the natural vegetative buffer limit would be at the point of man's 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 purposes of qualifying potential stream disturbances.

Aesthetics

Circle the descriptor that most adequately describes the reach as a whole. Make only one selection. The descriptors are defined as follows:

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 man-made alterations to landscape. Water clarity may be exceptional.

Natural area (2): Trees and/or native vegetation are common. Some development or alteration to the landscape by man may be evident, but is usually minimal, such as fields, pastures, or rural dwellings. Water may be discolored or slightly turbid.

Common setting (3): Landscape and stream are fairly altered by man, but the alteration is not offensive. This category could include an urban park setting. Water clarity is often colored or turbid.

Offensive (4): Stream does not enhance the aesthetics of the landscape. Stream is littered with trash, highly developed, or is 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 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 9-4 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 “bank.” In these instances, measure the slope of this “bank” from the water’s edge.

A *vertical bank* has a bank angle of 90°. If the vertical portion of the bank is $\geq$ 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 up against the roof of the undercut bank and in as far as possible. Turn the clinometer over and take the reading and subtract it from 180°.

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 these angles separately.

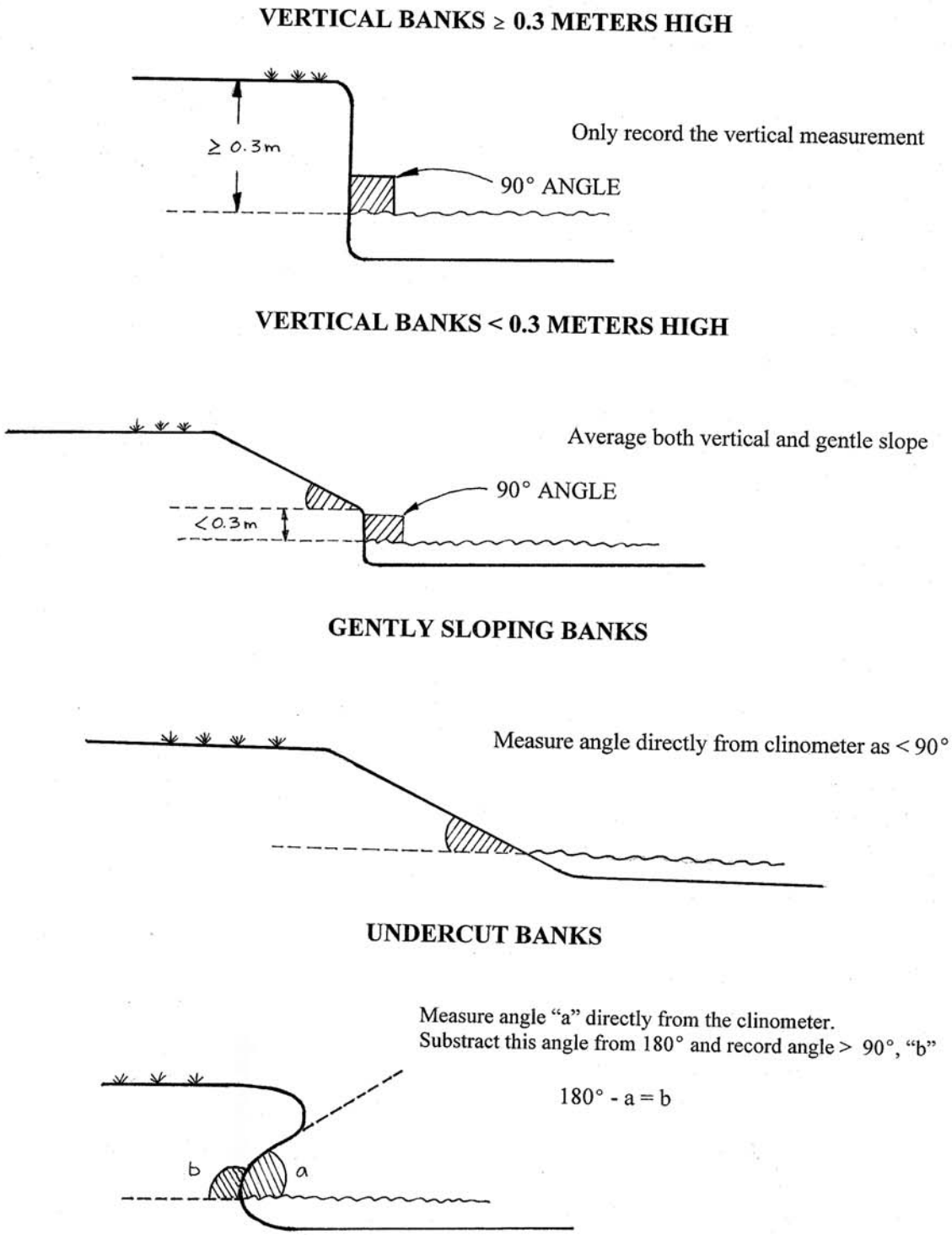


Figure 9-4. Bank Angle Measurements

Bank Erosion

Estimate the percentage of the areas of the stream bank which 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. Possible measurement range is from 0 percent (totally open) to 100 percent (totally closed canopy cover). See Figure 9-5, Densiometer Diagram.

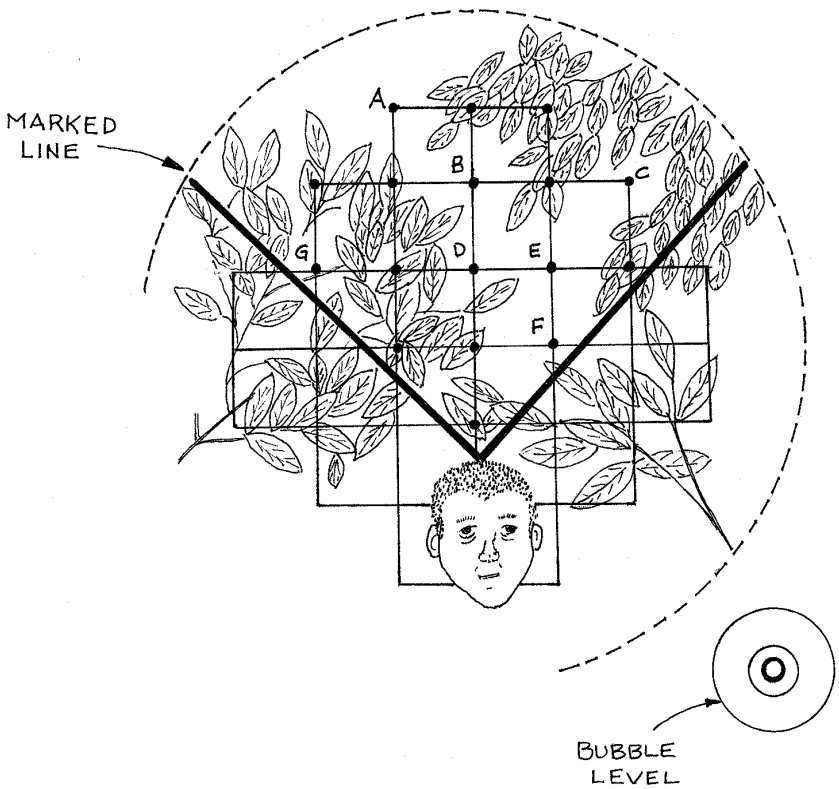


Figure 9-5. Convex Spherical Densiometer Diagram, (From Mulvey et al., 1992)

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 facingthe right bank.

Note: This is a change from previous procedures that specified a concave densiometer and only two measurements in mid-channel.

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 9-5, 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.

Note: This is a change in procedure from the one that specified holding the densiometer at waist level.

- 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.

- Two densiometer readings are taken at mid-stream, 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:

$$9 \div 17 = 0.53 \text{ or } 53 \text{ percent}$$

- 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 edge of 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 stream flow. See Chapter 3 of the SWQM Procedure, Volume 1 for details on measuring flow.

Take photographs of the stream reach from mid-channel, facing upstream and downstream. Ideally, photographs are taken at each transect from mid-channel facing left bank, right bank, upstream, and downstream.

Part II—Summary of Physical Characteristics of Water Body

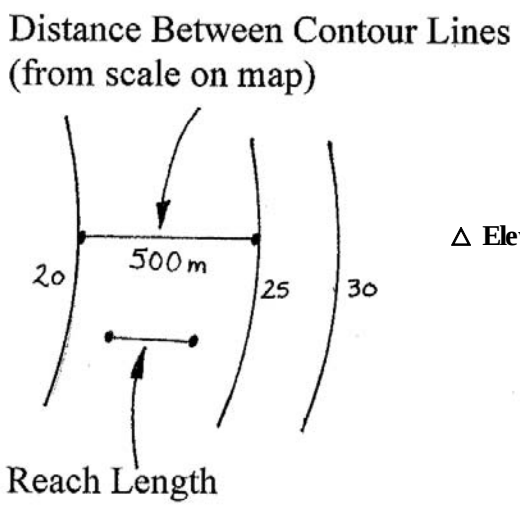
Once the field worksheet (Part I) has been completed, summarize the measurements on the summary sheet (Part II) in preparation for calculating the habitat metrics. Use information from all transects and measurements in Part I, as well as from other sources, to complete this form. This summary is used 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 is listed in parentheses after each descriptor heading.

Streambed Slope (72051)

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 1000 to get m/km.

Example: 10 ft/250 m = 3.048 m/250 m x 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 9-6). 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.



△ Elevation ÷ Distance Between Contour Lines x 1000 = Slope

5 meters ÷ 500 meters x 1000 = 10

Figure 9-6. Stream Slope

Drainage Area (89859)

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

Stream Order (84161)

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; and where two second order streams join, a third order stream is formed, and so on. Figure 9-7 depicts a typical stream order pattern.

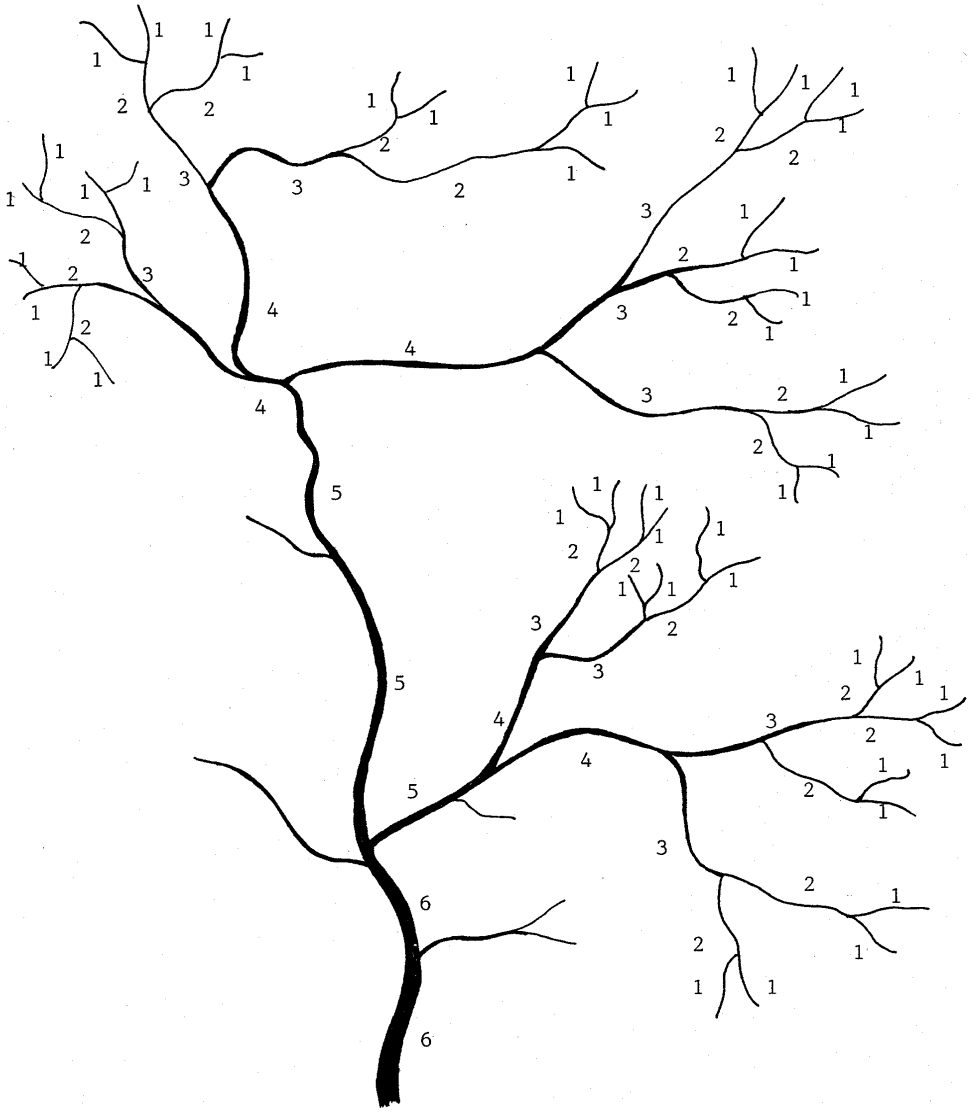


Figure 9-7. Stream Order

Length of Stream Evaluated (89860)

From Part I. Record in meters.

Number of Lateral Transects Made (89832)

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

Average Stream Width (89861)

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

Average Stream Depth (89862)

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

Instantaneous Stream Flow (00061)

Record the measured stream flow 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 stream flow gauge nearby. Record in ft^3/sec.

Flow Measurement Method (89835)

Indicate the type of equipment used to measure flow.

Channel Flow Status (89848)

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

Maximum Pool Width (89864)

Record the maximum width of the largest pool encountered in the reach. This is usually done when making "General Observations" on the walk back after performing the last transect measurement. Record in meters.

Maximum Pool Depth (89865)

Record the maximum depth of the largest pool encountered in the reach. This is usually done when making "General Observations" on the walk back after performing the last transect measurement. Record in meters.

Total Number of Bends (89839)

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

- Well defined bends (89840)
- Moderately defined bends (89841)
- Poorly defined bends (89842)

Total Number of Riffles (89843)

Record number of riffles from Part I. These are usually tallied during the "General Observations" portion of the assessment.

Dominant Substrate Type (89844)

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-size or Larger (89845)

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

Average Percent Instream Cover (84159)

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

Average Percent Stream Bank Erosion (89846)

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 as a percentage.

Average Stream Bank Angle (89847)

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, the average is calculated from all 10 individual bank angle measurements. Record in degrees.

Average Width of Natural Buffer Vegetation (89866)

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 was 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 (89849)
- Average percent shrubs as riparian vegetation (89850)
- Average percent grasses as riparian vegetation (89851)
- Average percent cultivated fields as riparian vegetation (89852)
- Average percent other as riparian vegetation (89853)

Average Percent Tree Canopy Coverage (89854)

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

Overall Aesthetic Appraisal of the Stream (89867)

Record your assessment from Part I.

Part III—Habitat Quality Index

After completing the summary of physical characteristics of water body form (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 “three 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**
$\leq$ 13	**Limited**

Assessing the Habitat of Lakes and Reservoirs

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

Preliminary work has begun to determine what habitat attributes are important for reservoirs. Some of the attributes that are being studied include: aquatic macrophyte coverage, shoreline habitat, human disturbance, and volumetric surveys. The USEPA has a field operation manual for environmental monitoring and assessment for lakes (USEPA 1997) and a guidance document for lake and reservoir bioassessment and biocriteria (USEPA 1998) that may provide 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-wadeable) tidal streams is Section 6 of the USEPA EMAP protocol for non-wadeable streams (USEPA 2000).

CHAPTER 10
BIOLOGICAL ASSESSMENT REPORTING REQUIREMENTS

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 habitat 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 for Biological Assessments

Data Type	ALM	ALA	RWA	UAA
Field multiprobe parameters	X	X	X	X
Diel (24-hour) measurements	●	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
Stream physical habitat survey	X	X	X	X
Field notes (copied pages of field data logbook)	X	X	X	X
Latitude/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 UAA report outline in Appendix C)	X	X	X	X

X = Required ● = Recommended

CHAPTER 11
QUALITY ASSURANCE AND QUALITY CONTROL

Biological monitoring programs that contribute data to the TCEQ must conform to all quality assurance measures outlined in this chapter and all quality control 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 the annual completion of a self-audit report; an annual technical systems audit (TSA), both in the field and laboratory; and by a TCEQ-approved Quality Assurance Project Plan (QAPP).

Self-Audit Reports

TCEQ Biological Monitoring Self-Audit Reporting Forms (in Appendix C) will be sent annually to all programs contributing biological data to the TCEQ. These reports will be completed by the entity and will be returned to the TCEQ SWQM Program. The self-audit report is designed to enable the entity to report on the administrative and record-keeping details of its biological monitoring program. The self-audit report requests information on educational background and training of staff performing biological monitoring, record-keeping practices, voucher specimens, etc. The information collected in the self-audit report will be used to assess the risk of a biological monitoring program and will help determine whether a TSA will be conducted that year.

Technical Systems Audits

A risk assessment of all biological monitoring programs contributing biological data to the TCEQ is conducted annually. TSAs are then conducted, if necessary, based on this risk assessment. Information used in determining the relative risk of a program contributing biological data will come from the self-audit report as well as from other sources. If it is determined that a TSA is needed for a TCEQ regional office, the TSA will be conducted during the fiscal year and will be performed separately from a TSA on other monitoring activities, such as routine monitoring. If it is determined that a TSA is needed for a CRP partner or other cooperator, a TSA is performed during that entity'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 entity submitting biological data.

Biological Sample Records

Records that must be maintained for reporting in a self-audit report and that must be kept available for inspection during a TSA include:

- Field notes containing sampling station location and number, date and time of collection, details of collection efforts including area and duration of sampling, raw counts of specimens collected, and photographs of any large specimens released after identification.

- Sample tracking log that details the event and sample information. Assign each sample a unique sample tracking number, such as BM 020 04—Benthic Macroinvertebrate, #020, year 2004.

- Laboratory identification notes and bench sheets. The laboratory bench sheet must contain the label information, unique sample tracking number from the sample logbook, date of identification, name of identifier, scientific name for each taxon, number of individuals in each taxon, and other comments that may pertain to identification.

- Appropriately labeled sample jars 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 will be available to TCEQ staff and other cooperators furnishing biological or habitat data to the TCEQ. These trainings will provide QA for the biological monitoring program and will be required regardless of level of expertise. In other words, even experienced field biologists will be required to attend periodic trainings to ensure they are practicing current methodologies. Staff attendance at biological monitoring training events will be factored in to the risk assessment of entities submitting biological or habitat data to the TCEQ. This risk assessment helps determine the TSA schedule each year as discussed above.

Approval of Deviation of Methods

Biological collection methods for wadeable streams are currently documented in this manual and any variation from those sampling protocols must be approved in advance. It is imperative that monitoring initiatives on water bodies that do not have prescribed protocols, such as reservoirs or tidal streams, be discussed at the beginning of study plan development with either TCEQ SWQM Team or WQST 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 a series of TSAs of personnel involved in the collection of biological data.

Sample Tracking Procedures

Proper sample custody is a joint effort of the sampling crew, sample transporter, and the laboratory staff (may include sorters and those performing taxa identification). The main sample documentation is an identifying data label, that includes:

- County
- River basin
- Stream name
- Station ID or location of nearest landmark (for example: a road crossing)
- Time and date of collection
- Collector names
- Collection methods used
- Type of preservative used

Place biological samples in jars with screw-top lids. Place the identifying data labels, written in either waterproof ink or pencil, in the sample jar.

Maintain a *sample tracking log* that contains the following for each sample:

- Unique sample tracking number
- Name of person entering information in sample tracking log
- Name of sample collector(s)
- Location of collection

- Date of collection
- Date entered in log
- Date identification and enumeration began
- Date identification and enumeration completed

For each sample, enter this information in the sample tracking log immediately upon returning to the lab. After completing the sample tracking log entries, inspect the *sample label* to ensure that the label is in good shape, legible, and includes the following information:

- Collector(s) name(s)
- Station location
- Station number if applicable
- Date and time of collection
- Collection method
- Preservative

Note: Replace the label if deterioration is apparent.

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

- Sample number from sample tracking log
- Name of identifier
- Location of collection
- Date of collection
- Date entered in log
- Date identification and enumeration began
- Date identification and enumeration completed
- Scientific name for each taxon in sample
- Number of individuals in each taxon
- ID qualifiers (difficulties)

Consistent with voucher specimen guidelines, maintain *separate specimen vials* for each taxon in the sample. Preserve specimens in 70 percent ethanol or isopropyl alcohol. Each vial must contain a label that includes the following information:

- Collector(s) name(s)
- Name of identifier(s)
- Station location
- Station number if applicable
- Date and time of collection
- Collection method
- Preservative
- Scientific name of taxon contained in vial

If it is necessary to make slide mounts of specimens (or parts of specimens) to examine them at higher magnification using the compound microscope in order to complete the identification, the slides must be labeled with the scientific name of the taxon, the initials of the identifier, and the sample number from the sample tracking log.

General Quality Assurance

To minimize misidentification of biological samples, the following steps will be taken:

Vouchers

Retain voucher specimens of all species of fishes and invertebrates as well as any algae samples and permanent diatom slides for a minimum of five years or until the conclusion of applicable regulatory decision (whichever is longer).

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

Take the following into consideration when storing voucher specimens:

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

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

Identification Re-checks

Identifications will be subject to a 5 percent blind re-check by another in-house biological expert. For example, if 200 fish are collected, then 10 would be re-checked through random selection. Fish would be numbered for selection; for example, 1-21 species A, 22 species B, 23-61 species C, and so on.

Random numbers would be generated and appropriate fishes re-checked. In the previous example, if a random number was 18, then a member of species A would be checked. The second biological expert may be a staff person within the program or an outside expert. Selection of samples for re-check must be randomized. A record of re-checks must be kept for QC purposes.

If a particular staff member demonstrates more than a 10 percent error rate, then all specimens will be re-identified. Laboratories must be cognizant of the potential for systematic errors, such as consistent errors in identification of a particular family, genus, or species.

Desktop QA

Desktop QA methods will be used to ensure identifications are correct. Those submitting biological data will check samples against known distributional information to determine if out-of-range organisms were identified. If the out-of-range determinations prove incorrect upon review, review and re-check the collection for other similar species.

Fish

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, only those with appropriate taxonomic training may perform species identifications.

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 the purposes of identifying Texas freshwater fishes, the primary reference is Hubbs et al. (1991), with complementary sources used as required.

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 (one part full-strength formalin to nine parts water). 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.

Take care to avoid breathing or contact with formalin. Each field container must include an internal label that includes the date, collection locality, names of collector(s), and sampling method. This paper must be of a high rag content and notations must be in pencil or waterproof ink.

Equipment Requirements

Proper identification and enumeration of fish requires, at a minimum, the following equipment:

- Stereo dissecting microscope, total magnification variable 7x to 30x; recommended 7x to 110x
- Jeweler's forceps
- Petri dishes
- Preservative: 70 percent ethanol
- Ruler

Taxonomic Keys

(FW = Freshwater, SW = Salt water)

Required:

FW: 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.

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

Other recommended keys include:

FW: Douglas, N.H. 1974. *Freshwater Fishes of Louisiana*. Claitor's Publishing Division, Baton Rouge, Louisiana. 443 pp.

FW/SW: Hubbs, C., J.D. McEachran, C.R. Smith, and N.T. Travis, eds. 1994. *Freshwater and Marine Fishes of Texas and the Northwestern Gulf of Mexico*. xviii+ 270 pp., 3 maps. Texas System of Natural Laboratories, Inc., Austin, Texas.

FW: Kuehne, R.A. and R.W. Barbour. 1983. *The American Darters*. The University Press of Kentucky. 177 pp.

FW: Lee, D.S., C.R. Gilbert., C.H. Hocutt, R.E. Jenkins, D.E. McAllister, and J.R. Stauffer, Jr. 1980. *Atlas of North American Fresh Water Fishes.* North Carolina State Museum of Natural History, Publication #1980-12 of the North Carolina Biological Survey. Raleigh, North Carolina. 854 pp.

FW: McGowan, N., R.J. Kemp, Jr. and R. McCune. 1971. *Freshwater Fishes of Texas*. Texas Parks and Wildlife Department, Austin, Texas. Bulletin 5-A. 40 pp.

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

SW: Murdy, E.O. 1995. *Saltwater Fishes of Texas. A Dichotomous Key*. TAMU-SG-83-607. Texas A & M University Sea Grant College Program. College Station, Texas. 158 pp.

FW: Nelson, J. S., E. J. Crossman, H. Espinosa-Pérez, L. T. Findley, C. R. Gilbert, R. N. Lea, and J.D. Williams. 2004. *Common and Scientific Names of Fishes from the United States, Canada, and Mexico*. American Fisheries Society, Special Publication 29, Bethesda, Maryland.

FW: Page, L.M. and B.M. Burr. 1991. *A Field Guide to Freshwater Fishes*. The Peterson Field Guide Series. Houghton Mifflin Company. Boston, Massachusetts. 432 pp.

FW: Pflieger, W.L. 1975. *The Fishes of Missouri*. Missouri Department of Conservation, Jefferson City, Missouri. 343 pp.

FW: Robison, H.W. and T.M. Buchanan. 1988. *Fishes of Arkansas*. The University of Arkansas Press, Fayetteville, Arkansas. 536 pp.

SW: Shipp, R.L. 1999. *Dr. Bob Shipp's Guide to the Fishes of the Gulf of Mexico*. KME Seabooks. Mobile, Alabama. 256 pp.

FW: Sublette, J.E., M.D. Hatch, and M. Sublette. 1990. *The Fishes of New Mexico*. University of New Mexico Press, Albuquerque, New Mexico. 393 pp.

FW: Tomelleri, J.R. and M.E. Eberle. 1990. *Fishes of the Central United States*. University Press of Kansas, Lawrence, Kansas. 226 pp.

Benthic Macroinvertebrates

Identification and enumeration of benthic macroinvertebrates must be conducted by individuals with appropriate expertise, training, and knowledge of the literature.

Identification and enumeration of benthic macroinvertebrates must be done consistently among samples and the taxonomic expertise of the identifier must, at a minimum, be adequate to allow identification of all specimens for each sample according to the appropriate taxonomic level identified on pages 5-9 in Chapter 5: Freshwater Benthic Macroinvertebrates.

Equipment Requirements

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

- Stereo dissecting microscope, total magnification variable 7x to 30x; recommended 7x to 110x
- Stereo compound microscope, total magnification 400x
- Jeweler's forceps
- Petri dishes
- Preservative: 70 percent ethanol or 70 percent isopropyl alcohol
- Microscope slides

Taxonomic Keys

(FW = Freshwater, SW = Salt water)

Required:

FW: Merritt, R.W. and K.W. Cummins (eds). 1996. *An Introduction to the Aquatic Insects of North America,* 3rd Edition. Kendall/Hunt Publishing Co., Dubuque, Iowa.

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

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

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

SW: Andrews, J. 1977. *Shells and Shores of Texas*. University of Texas Press. 365 pp.

SW: Fauchald, K. 1977. *The Polychaete Worms*. Definitions and Keys to the Orders, Families, and Genera. Natural History Museum of Los Angeles Science Series # 28. Los Angeles, California.

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

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

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

Other recommended keys include:

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

FW: Edmondson, W.T. ed. 1959. *Ward and Whipple Freshwater Biology 2nd Edition*. John Wiley and Sons, Inc., New York, New York. 1248 pp.

FW: McCafferty, W.P. 1983. *Aquatic Entomology*. Jones and Bartlett Publishers, Inc., Boston, Massachusetts.

FW: Usinger, R.L. (ed). 1968. *Aquatic Insects of California*. University of California Press, Berkley, California.

SW: Abbott, R.T. 1974. *American Seashells*, 2nd edition. Van Nostrand Reinhold Co., New York, New York.

SW: Barnes, R.D. 1987. *Invertebrate Zoology*, Fifth edition. CBS College Publishing, New York, New York.

SW: Farfante, I.P. 1988. *Illustrated Key to Penaeoid Shrimps of Commerce in the Americas.* National Oceanic and Atmospheric Association Report. NMFS 64. 32 pp.

SW: Williams, Austin B. 1965. *Marine decapod crustaceans of the Carolinas.* United States Fish and Wildlife Service, Fishery Bulletin 65(1): 1-298.

SW: Wood, Carl E. 1974. *Key to the natantia (crustacean, decapoda) of the coastal waters on the Texas coast.* Contributions in Marine Science 18: 35-56.

Benthic Algae and Plankton

Identification of algae and plankton samples must be conducted by individuals with proper expertise, training, and knowledge of the literature. Identification must include, at a minimum, genus-level for non-diatom algae, and 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; 10x oculars with 10x to 100x (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

(FW = Freshwater, SW = Salt water)

Required:

FW: Prescott, G. W. 1978. *How to Know the Freshwater Algae*. 3rd edition. Wm. C. Brown Co., Dubuque, Iowa.

FW: Patrick, R. and C. W. Reimer. 1966 and 1975. *The Diatoms of the United States, exclusive of Alaska and Hawaii*. Monograph No. 13, Vols. 1 and 2. Acad. Nat. Sci., Phila., Philadelphia, Pennsylvania.

SW: Tomas, C. R. 1997. *Identifying Marine Phytoplankton*. Academic Press. San Diego, California. 858 pp.

Other recommended references include:

FW: Dillard, G. E. 1989-1993. *Freshwater Algae of the Southeastern United States. Parts 1-6*. Bibliotheca Phycologica.

FW: Krammer, K. and H. Lange-Bertalot. 1986-1991. *Susswasserflora von Mitteleuropa. Band 2. Parts 1-5. Bacillariophycea*. Gustav Fischer Verlag. Stuttgart, Germany.

FW: Prescott, G. W. 1962. *The Algae of the Western Great Lakes Area*. Wm. C. Brown Co., Dubuque, Iowa.

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

Aquatic Macrophytes

Taxonomic Keys

(FW = Freshwater, SW = Salt water)

Recommended references include:

FW: Prescott, G.W. 1969. *How to Know the Aquatic Plants*. Wm. C. Brown Co., Dubque, Iowa. 171 pp.

FW: Riemer, D.N. 1984. *Introduction to Freshwater Vegetation.* Van Nostrand and Reinhold Co., New York. 207 pp.

SW: Hotchkiss, N. 1972. *Common Marsh Plants of the United States and Canada*. Dover Publications, Inc., New York. 124 pp.

SW: Stutzenbaker, C. D. 1999. *Aquatic and Wetland Plants of the Western Gulf Coast*. University of Texas Press, Austin, Texas. 465 pp.

FW/SW: Tarver, D.P., J.A. Rodgers, M.J. Mahler, and R.L. Lazor. 1986. *Aquatic and Wetland Plants of Florida,* 3rd edition. Bureau of Aquatic Plant Research and Control, Florida Department of Natural Resources, Tallahassee, Florida.

CHAPTER 12
BIOLOGICAL DATA MANAGEMENT

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 re-checked after data entry to ensure correct transcription. The TCEQ will provide a standard format for submission of data. Collectors must ensure that all data necessary to calculate multi-metric indices are provided.

Contracting and Institutional Standards

Desktop QA methods will also be employed 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). This is not to suggest that new records cannot occur, but that they will be scrutinized and the appropriate vouchers required to ensure proper identification. If the out-of-range determinations prove incorrect upon review, then the collection will be reviewed for other similar species and those re-checked.

If a sample fails desktop QC checks and the error is uncorrectable, the data are invalid and will not be accepted. Possible consequences for failure include invalidating the data, requiring re-sampling, more frequent QA visits, and withholding payment.

Submitting Biological Data

Submit data associated with a biological sampling event (fish, macroinvertebrates, habitat, water chemistry, and field data) electronically according to standard procedures, described in the SWQM DMRG or specified by the TCEQ WQST. The SWQM DMRG can be found at: <www.tceq.state.tx.us/goto/swqm/dmrg>.

Additionally, hard copies of the data must be submitted as prescribed in the Biological Data Summary Packet found in Appendix C.

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Patrick, R. and C. W. Reimer. 1975. *The Diatoms of the United States, exclusive of Alaska and Hawaii.* Monograph No. 13, Vol. 2. Acad. Nat. Sci., Phila., Philadelphia, Pennsylvania. 13 ix+1-213

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APPENDIX A
LIST OF EQUIPMENT AND MATERIALS

(Items in italics are for estuarine waters)

Flow Measurement

- Flow meter
- Top-setting wading rod
- 100 ft tape measure (marked in tenths of a foot increments)
- Hip and chest waders
- Calculator
- Flow measurement recording forms
- *Boat*
- *Long rope*
- *Rangefinder*

Fish Sampling

- 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 sizes)
- Battery charger
- Seines (30' or 15' x 6' x 1/4" mesh, 15' or 6' x 6' x 3/16" mesh, and 6' x 6' x 1/8" mesh)
- *Seines 10', 15' or 30' long with 3/16" mesh (height and length determinations are per site requirements)*
- *Experimental gill nets (graduated mesh sizes)*
- Two holding buckets or tanks with aerators (one for in boat or in-stream while sampling, and one for maintaining fish on bank while sampling)
- 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 floatation device
- Fish identification manuals (for field and laboratory identification use, see Quality Assurance chapter)
- Stereo dissecting microscope
- *Trawl*
- Scale
- Data recording form(s)

Benthic Macroinvertebrate Sampling

- 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
- Sample jars (wide mouth) and vials (2 drams)
- Preservative—70% ethanol and 10% formalin
- Sample labeling material
- Hip and chest waders

- Benthic macroinvertebrate identification manuals (for field and laboratory identification use, see the “Quality Assurance” chapter)
- Stereo dissecting microscope
- Compound binocular microscope (10X and 15X eyepieces; 4X, 10X, 20X, and 45X objectives)
- Lopping shears for snag samples
- Surber sampler
- Ekman dredge
- Data recording form(s)

Benthic Algae Sampling

- Pocket knife or other scraping device
- Pipettes
- Sample collection jars (60 mL glass snap caps)
- Preservative (glutoraldehyde or formalin)
- Compound microscope
- Glass slides and coverslips
- Hot plate
- Mounting media
- Data recording form(s)

Plankton Sampling

- Plankton net
- Sample jars
- Lugol’s solution
- Compound microscope
- Glass slides and coverslips
- Sedgewick-Rafter counting chamber
- Data recording form(s)

Physical Stream Habitat Measurements

- 50 m tape measure (minimum)
- Clinometer (degrees and percent)
- Densiometer (convex)
- Metric survey rod
- Hip and chest waders
- Rangefinder or hip chain (metric)
- Survey tape or flags
- Floatation tube, small boat
- Tag line or sturdy rope (metric)
- 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 I of the SWQM Procedures (RG-415, 12/2003) for water chemistry, flow measurement, field parameters (including 24-hour DO), and tissue sampling
- Necessary laboratory forms (for submitting water chemistry samples)
- Winch

APPENDIX B
BIOLOGICAL ASSESSMENT REFERENCE MATERIALS AND CRITERIA

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[1]
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

[1] 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 as specified in 30 TAC §307.7(b)(3)(A)(I) of the Texas Surface Water Quality Standards (TNRCC, 1997)

Aquatic Life Use Subcategory	Dissolved Oxygen Criteria, mg/L	
	Freshwater mean/minimum	Freshwater in Spring 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

- Dissolved oxygen means are applied 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 on a site-specific basis, when natural daily fluctuations below the mean are greater than the difference between the mean and minima of the appropriate criteria.
- Spring criteria to protect fish spawning periods are applied 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 the standards implementation procedures.
- Dissolved oxygen analyses and computer models to establish effluent limits for permitted discharges will normally be applied to mean criteria at steady-state, critical conditions.
- Determination of standards attainment for dissolved oxygen criteria is specified in §307.9(d)(6) (relating to Determination of Standards Attainment).

Table B-3. Ecoregion 24 Metrics

	Metric	Scoring Criteria		
		5	**3**	**1**
1	Total number of fish species		See Figure B-1	
2	Number of native cyprinid species	> 4	3-4	< 3
3	Number of benthic invertivore species	> 1	1	0
4	Number of sunfish species	>1	1	0
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	Number of individuals in sample			
	a. Number of individuals/seine haul	> 160.4	80.2-160.4	< 80.2
	b. Number of ind/min electrofishing	> 26.5	13.3-26.5	< 13.3
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: ≥43 Exceptional; 37-42 High; 35-36 Intermediate; <35 Limited

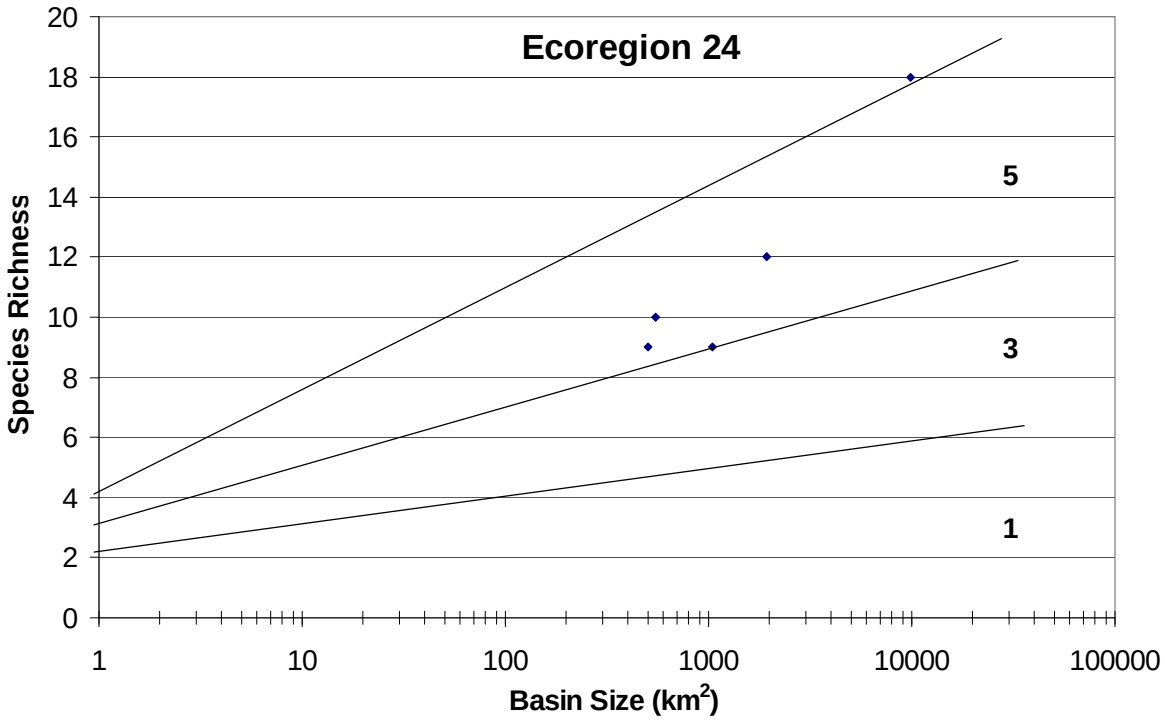

Figure B-1.

Table B-4. Ecoregions 25 and 26 Metrics

	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

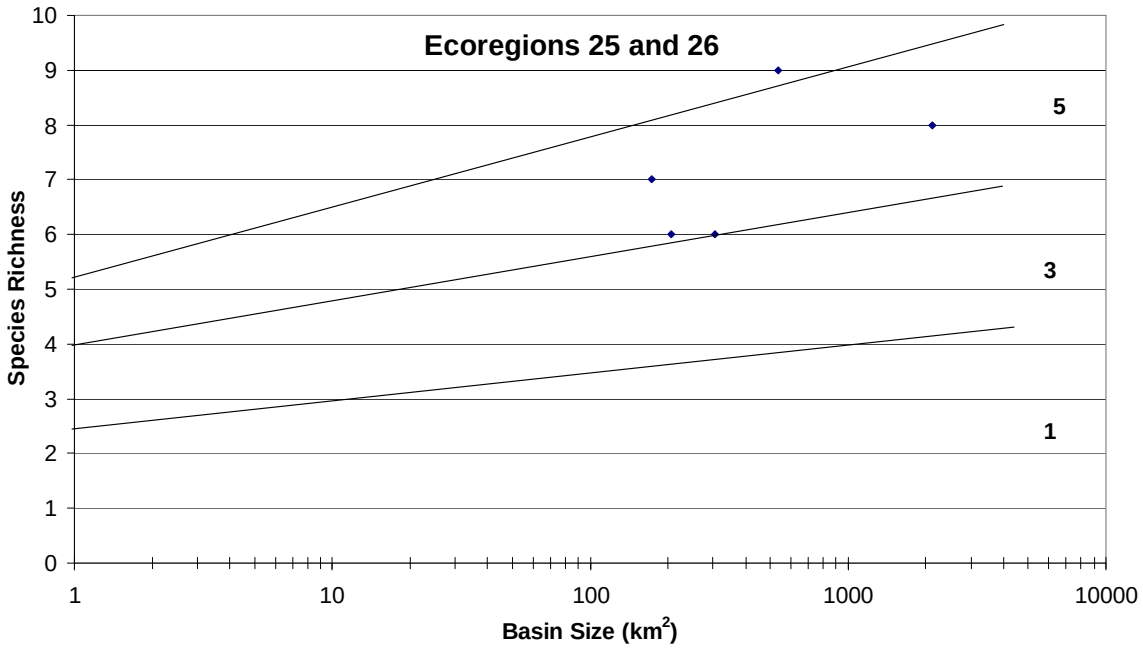

Figure B-2.

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

	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 ind/min 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

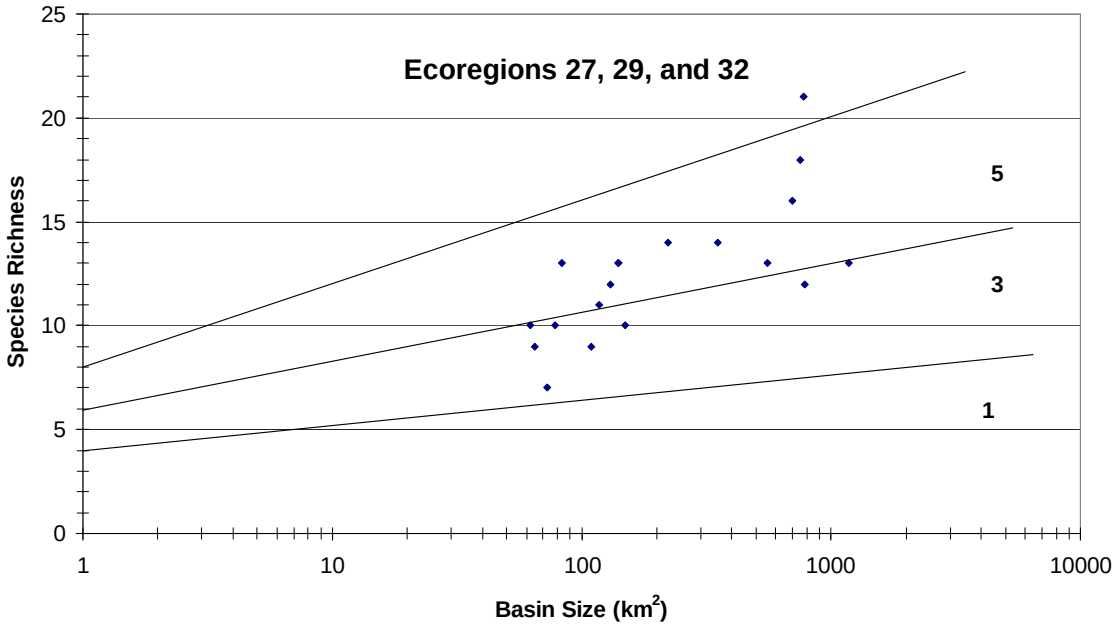

Figure B-3.

Table B-6. Ecoregion 30 Metrics

	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 ind/min 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

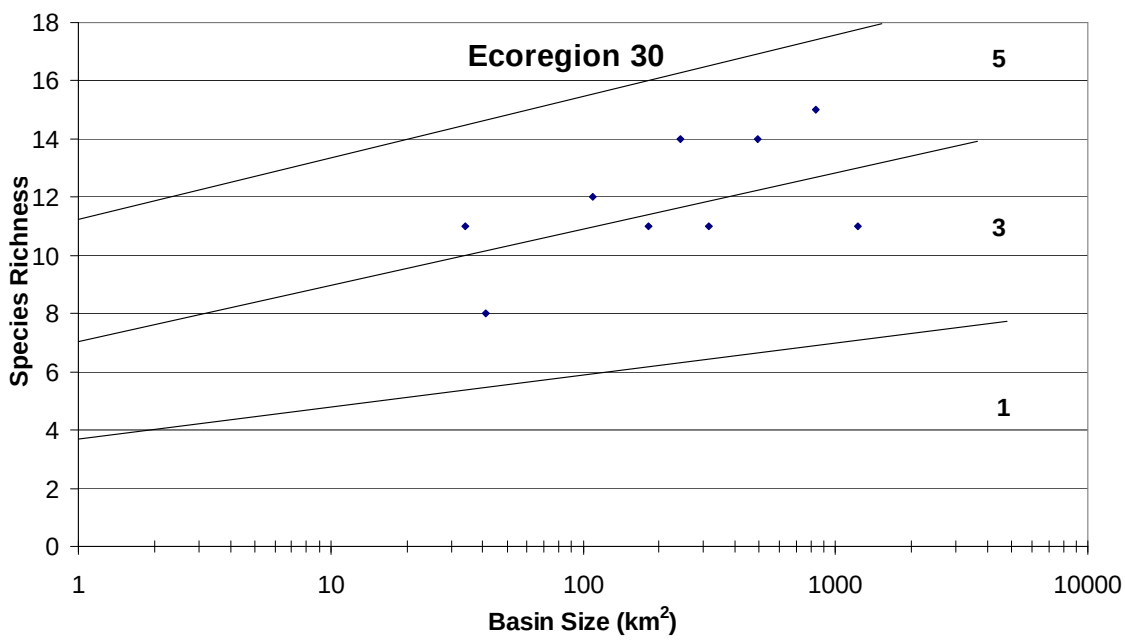

Figure B-4.

Table B-7. Ecoregion 31 Metrics

	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 ind/min 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

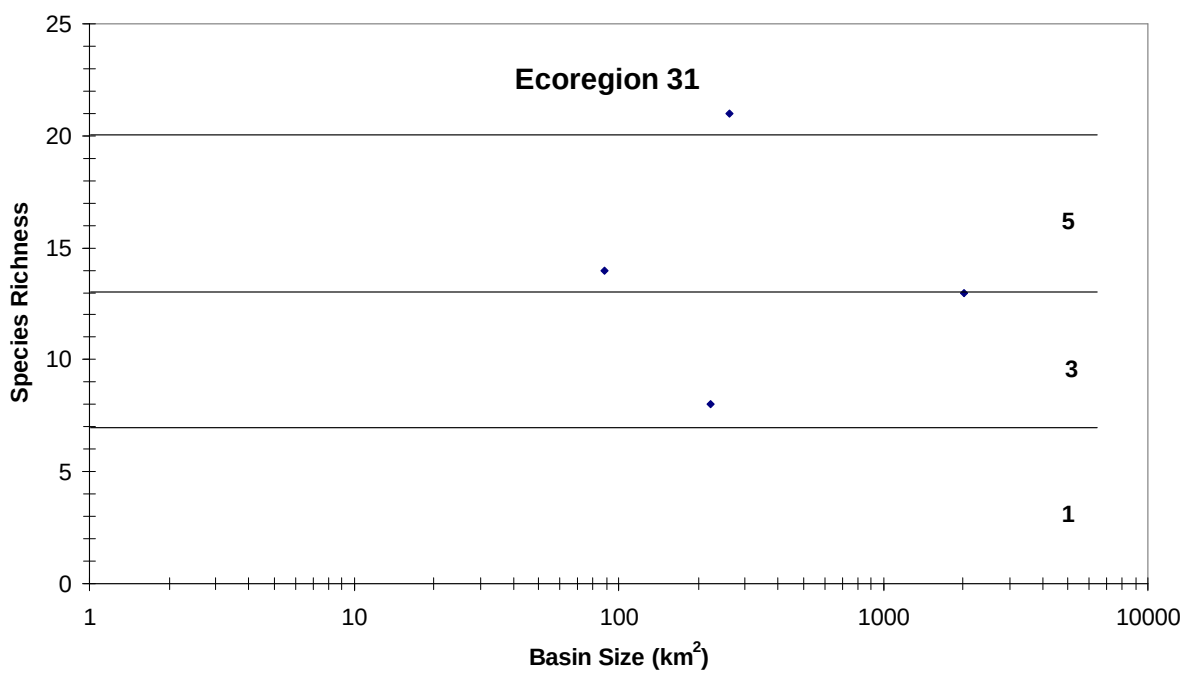

Figure B-5.

Table B-8. Ecoregions 33 and 35 Metrics

	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 ind/min 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

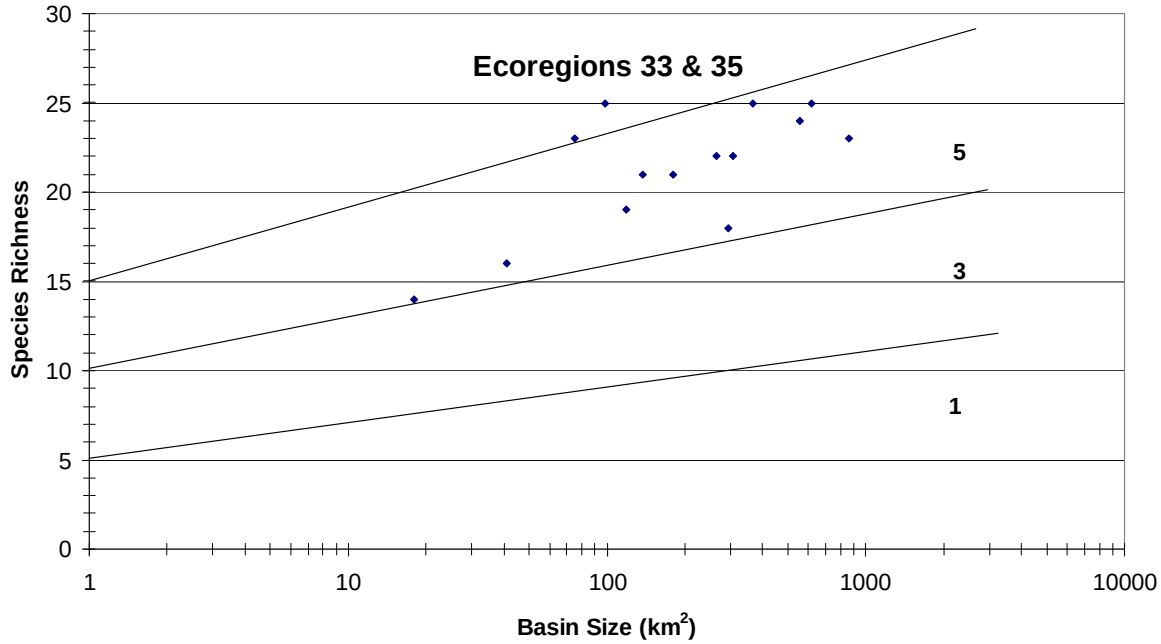

Figure B-6.

Table B-9. Ecoregion 34 Metrics

	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 ind/min 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

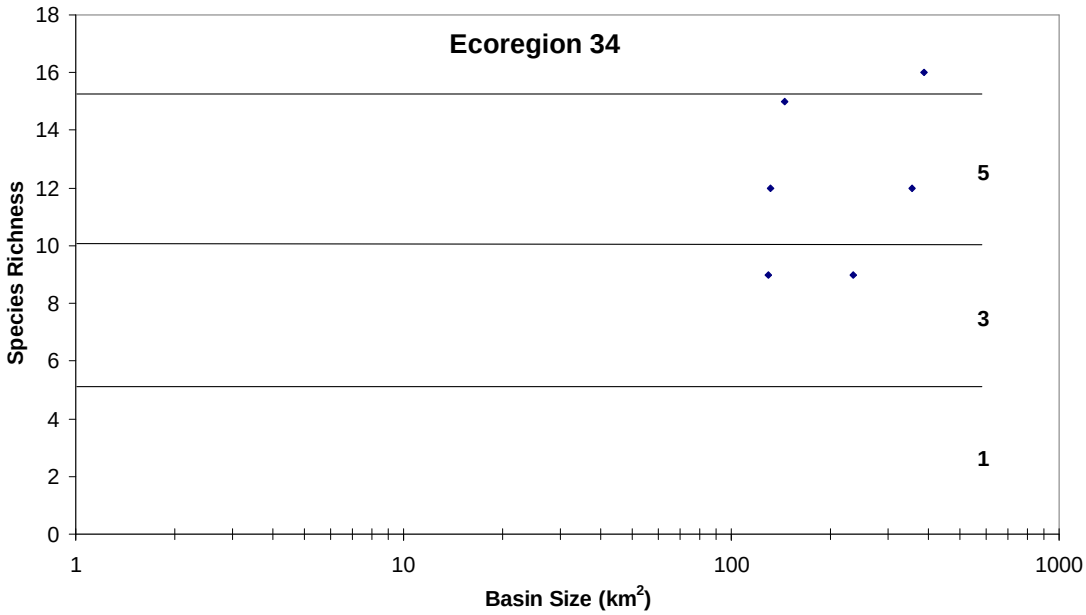

Figure B-7.

Table B-10.Classification of Texas Freshwater Fishes into Trophic and Tolerance Groups Adapted from Linam, G.W. and L.J. Kleinsasser (1998).

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

Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
Paddlefish	*Polyodon spathula*	98335	O	I
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
Bowfin	*Amia calva*	98347	P	T
American eel	*Anguilla rostrata*	98361	P	
Speckled worm eel	*Myrophis punctatus*	98388	P	
Chestnut lamprey	*Ichthyomyzon castaneus*	99297	P	I
Southern brook lamprey	*Ichthyomyzon gagei*	98013	None	I
Gizzard shad	*Dorosoma cepedianum*	98430	O	T
Threadfin shad	*Dorosoma petenense*	98429	O	
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	
Prairie chub	*Macrhybopsis australis*		IF	
Shoal chub	*Macrhybopsis hyostoma*		IF	
Burrhead chub	*Macrhybopsis marconis*		IF	
Peppered chub	*Macrhybopsis tetranema*		IF	

Table B-10.Classification of Texas Freshwater Fishes into Trophic and Tolerance Groups Adapted from Linam, G.W. and L.J. Kleinsasser (1998).

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

Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
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	
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	

Table B-10.Classification of Texas Freshwater Fishes into Trophic and Tolerance Groups Adapted from Linam, G.W. and L.J. Kleinsasser (1998).

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

Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
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	
Gray redhorse	*Moxostoma congestum*	98513	IF	
Golden redhorse	*Moxostoma erythrurum*	98514	IF	
Blacktail redhorse	*Moxostoma poecilurum*	98515	IF	
Mexican tetra	*Astyanax mexicanus*	98435	IF	
Black bullhead	*Ameiurus melas*	98563	O	T
Yellow bullhead	*Ameiurus natalis*	98564	O	
Gafftopsail catfish	*Bagre marinus*	98557	P	T
Suckermouth catfish	*Hypostomus plecostomus*	98553	H	T
Southern sailfin catfish	*Pterygoplichthys anisitsi*		H	T
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	
Hardhead catfish	*Ariopsis felis*	98559	IF	T
Southern striped Raphael	*Platydoras armatulus*			
Redfin pickerel	*Esox americanus*		P	
Chain pickerel	*Esox niger*	98405	P	
Rainbow trout	*Oncorhynchus mykiss*	98527	IF-Lotic	I
		98527	P-Lentic	I
Pirate perch	*Aphredoderus sayanus*	98773	IF	
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	

Table B-10.Classification of Texas Freshwater Fishes into Trophic and Tolerance Groups Adapted from Linam, G.W. and L.J. Kleinsasser (1998).

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

Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
Saltmarsh topminnow	*Fundulus jenkinsi*	98696	IF	
Blackstripe topminnow	*Fundulus notatus*	98677	IF	
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	
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
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	
White bass	*Morone chrysops*	99163	P	
Yellow bass	*Morone mississippiensis*	99164	P	
Striped bass	*Morone saxatilis*	99165	P	
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	

Table B-10.Classification of Texas Freshwater Fishes into Trophic and Tolerance Groups Adapted from Linam, G.W. and L.J. Kleinsasser (1998).

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

Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
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	
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	
Silver perch	*Bairdiella chrysoura*	98960	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*		P	
Walleye	*Sander vitreus*	99058	P	
Freshwater drum	*Aplodinotus grunniens*	98958	IF	T
Silver perch	*Bairdiella chrysoura*		IF	
Black drum	*Pogonias cromis*	98970	IF	
Red drum	*Sciaenops ocellatus*	98962	P	
Rio Grande cichlid	*Cichlasoma cyanoguttatum*	98953	IF	
Blue tilapia	*Oreochromis aureus*	98583	O	T
Mozambique tilapia	*Oreochromis mossambicus*	98565	O	

Table B-10.Classification of Texas Freshwater Fishes into Trophic and Tolerance Groups Adapted from Linam, G.W. and L.J. Kleinsasser (1998).

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

Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
Redbelly tilapia	*Tilapia zillii*	98584	O	
Fat sleeper	*Dormitator maculatus*		O	
Largescaled spinycheek sleeper	*Eleotris amblyopsis*		O	
Emerald sleeper	*Erotelis smaragdus*		IF	
Bigmouth sleeper	*Gobiomorus dormitor*		IF	
Striped mullet	*Mugil cephalus*	98793	O	
White mullet	*Mugil curema*		O	
Fine tooth shark	*Carcharhinus isodon*	98014	P	
Bull shark	*Carcharhinus leucas*	98280	P	
Small tooth sawfish	*Pristis pectinata*	98299	P	
Atlantic stingray	*Dasyatis sabina*	98318	IF	
Shovelnose sturgeon	*Scaphirynchus platorynchus*	98337	IF	
Goldeye	*Hiodon alosoides*	98408	IF	
Ladyfish	*Elops saurus*	98352	P	
Tarpon	*Megalops atlanticus*	98356	P	T
Skipjack herring	*Alosa chrysochloris*	98418	P	
Finescale menhaden	*Brevoortia gunteri*	98426	O	
Scaled sardine	*Harengula jaguana*	98015	IF	
Striped anchovy	*Anchoa hepsetus*	98410	IF	
Bay anchovy	*Anchoa mitchilli*	98412	IF	
Widemouth blindcat	*Satan eurystomus*	98572	IF	
Toothless blindcat	*Trogloglanis pattersoni*	98568	O	
Northern pike	*Esox lucius*	98406	P	I
Atlantic needlefish	*Strongylura marina*	98663	P	
Opposum pipefish	*Microphis brachyurus*	98857	IF	
Chain pipefish	*Syngnathus louisianae*	98757	IF	
Gulf pipefish	*Syngnathus scovelli*	98761	IF	
Smallscale fat snook	*Centropomus parallelus*	98806	P	
Common snook	*Centropomus undecimalis*	98989	P	I
Sauger	*Stizostedion canadense*	99057	P	I
Crevalle jack	*Caranx hippos*	98900	P	I
Irish pompano	*Diapterus auratus*	99047	IF	
Spotfin mojarra	*Eucinostomus argenteus*	99044	IF	
Flagfin mojarra	*Eucinostomus melanopterus*	98578	IF	
Barred grunt	*Conodon nobilis*	98993	IF	
Burro grunt	*Pomodasys crocro*		IF	
Sheepshead	*Archosargus probatocephalus*	99155	O	

Table B-10.Classification of Texas Freshwater Fishes into Trophic and Tolerance Groups Adapted from Linam, G.W. and L.J. Kleinsasser (1998).

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

Common Name	Scientific Name	Parameter Code	Trophic Group	Tolerance
Pinfish	*Lagodon rhomboides*	99153	O	
Atlantic threadfish	*Polydactylus octonemus*		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
Mountain mullet	*Agonostomus monticola*	98797	O	
Hogchoker	*Trinectes maculatus*	99218	IF	
Southern flounder	*Paralichthys lethostigma*	99246	P	
Fringed flounder	*Etropus crossotus*		IF	
Lined sole	*Achirus lineatus*		IF	
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	
Bay whiff	*Citharichthys spilopterus*		IF	
Least puffer	*Sphoeroides parvus*		IF	

Table B-11. 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: > 36 High: 29-36 Intermediate: 22-28 Limited: < 22			

Table B-12. Metrics and Scoring Criteria for Surber Samples—Benthic Macroinvertebrates (Davis, 1997).

	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
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 - discriminatory power was less than optimal for this bioregion, so metric was assigned only two scoring categories
b - 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 metric was assigned only two categories

Table B-13. Tolerance Values and Functional Group Classification for Benthic Macroinvertebrates.

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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/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 sp.*	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
91633	*Heptagenia sp.*	3	SCR	Ephemeroptera	Heptageniidae
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
91557	*Neochoroterpes sp.*	2	CG/SCR	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
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	FC	Trichoptera	Hydropsychidae
92305	*Potamyia sp.*	4	FC	Trichoptera	Hydropsychidae
92308	*Smicridea sp.*	4	FC	Trichoptera	Hydropsychidae
92376	*Helicopsyche sp.*	2	SCR	Trichoptera	Helicopsychidae

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Parameter Code	Genus/Species	Tolerance Value	Functional Group	Order	Family
92371	*Pycnopsyche sp.*	2	SHR	Trichoptera	Limnophilidae
92268	*Chimarra sp.*	2	FC	Trichoptera	Philopotamidae
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
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

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Parameter Code	Genus/Species	Tolerance Value	Functional Group	Order	Family
92211	*Psephenus sp.*	4	SCR	Coleoptera	Psephenidae
92090	*Dineutus sp.*	5	P	Coleoptera	Gyrinidae
92092	*Gyretes sp.*	6	P	Coleoptera	Gyrinidae
92093	*Gyrinus sp.*	6	P	Coleoptera	Gyrinidae
92153	Hydrophilidae	5	L=P; A=CG	Coleoptera	Hydrophilidae
92154	*Berosus sp.*	9	L=P; A=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	Hydrophilidae
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*	10	P	Coleoptera	Hydrophilidae
92223	*Lutrochus sp.*			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 sp.*	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*	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	Coleptera	Noteridae
91683	*Argia sp.*	6	P	Odonata	Coenagrionidae

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Parameter Code	Genus/Species	Tolerance Value	Functional Group	Order	Family
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
91669	*Hetaerina sp.*	6	P	Odonata	Calopterygidae
91769	*Macromia sp.*	3	P	Odonata	Macromiidae
91741	*Aeshna sp.*	4	P	Odonata	Aeschnidae
91745	*Basiaeschna*	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	Corduliidae
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 sp.*	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	P	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
92106	*Corixidae sp.*	9	P/CG	Hemiptera	Corixidae
92009	*Palmacorixa sp.*		P/CG	Hemiptera	Corixidae
92044	*Trichocorixa sp.*		P/CG	Hemiptera	Corixidae
92053	Naucoridae		P	Hemiptera	Naucoridae
92054	*Ambrysus sp.*		P	Hemiptera	Naucoridae
92057	*Cryphocricos sp.*		P	Hemiptera	Naucoridae

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Parameter Code	Genus/Species	Tolerance Value	Functional Group	Order	Family
92060	*Limnocoris sp.*		P	Hemiptera	Naucoridae
92059	*Pelocoris sp.*		P	Hemiptera	Naucoridae
91951	*Aquarius sp.*	5	P	Hemiptera	Gerridae
91953	*Mesovelia*		P	Hemiptera	Mesoveliidae
91919	*Microvelia sp.*		P	Hemiptera	Veliidae
91923	*Rhagovelia sp.*		P	Hemiptera	Veliidae
91944	*Rheumatobates sp.*		P	Hemiptera	Gerridae
91946	*Trepobates sp.*		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
92511	*Tribelos sp.*	5	CG	Diptera	Chironomidae
Chironominae: Pseudochironomini					
92538	*Pseudochironomus sp.*	5	CG	Diptera	Chironomidae

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Parameter Code	Genus/Species	Tolerance Value	Functional Group	Order	Family
92540	*Stenochironomus sp.*	6	CG/SHR	Diptera	Chironomidae
91901	*Stelechomyia sp.*	5			
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
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: Orthocladini					
91897	*Acricotopus sp.*	6	CG	Diptera	Chironomidae
92570	*Brillia sp.*	5	SHR/CG		
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					
92374	*Alotanypus sp.*		P		
90984	*Tanypodinae*	6	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
91864	*Djalmabatista sp.*	6	P	Diptera	Chironomidae
92495	*Procladius sp.*	9	CG/P	Diptera	Chironomidae
92505	*Psectrotanypus sp.*	8	P	Diptera	Chironomidae
Tanypodinae: Natarsini					
91862	*Natarsia sp.*	10	P	Diptera	Chironomidae

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Parameter Code	Genus/Species	Tolerance Value	Functional Group	Order	Family
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		
90976	*Thienemannimyia sp.*	6	P	Diptera	Chironomidae
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.*			Diptera	Ceratopogonidae
92367	*Sphaeromias sp.*	5	P	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		
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	CG	Diptera	Tabanidae
92622	*Tabanus sp.*	7	P	Diptera	Tabanidae
91851	*Ochthera sp.*	8	P	Diptera	Ephydridae
92627	*Empididae*			Diptera	Empididae
92628	*Hemerodromia sp.*	6	P/CG	Diptera	Empididae

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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
91227	*Lirceus sp.*	9	CG/SHR	Isopoda	Asellidae
91056	*Ostracoda*		CG/SCAV	Podocopa	Cypridae (all?)
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.*	5	CG	Decapoda	Cambaridae
91428	*Orconectes sp.*	5	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	SCR	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*	7	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.*	8	SCR	Limnophila	Planorbidae
92887	*Helisoma sp.*	7	SCR	Limnophila	Planorbidae
92892	*Planorbella sp.*		SCR	Limnophila	Planorbidae
92891	*Planorbula sp.*	7	P	Limnophila	Planorbidae
92874	*Physella sp.*	9	SCR	Limnophila	Physidae
92783	Hydrobiidae		SCR	Mesogastropoda	Hydrobiidae
92763	*Amnicola sp.*	5	SCR	Mesogastropoda	Hydrobiidae
92779	*Somatogyrus sp.*		SCR	Mesogastropoda	Hydrobiidae
92780	*Elimia sp.*	2.5	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

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Parameter Code	Genus/Species	Tolerance Value	Functional Group	Order	Family
92757	*Viviparus sp.*	1	SCR	Mesogastropoda	Viviparidae
91525	*Hydracarina*	6	P		
90913	*Hirudinea*	8	P		
90967	*Erpobdella sp.*	8	P		
93095	*Mooreobdella sp.*	7.8	P		
90931	*Placobdella sp.*	6	P		
90382	*Oligochaeta*	8	CG		
90075	*Dugesia sp.*	7.5	P		Planariidae
90291	*Nematomorpha sp.*				
90196	*Nematoda*	5	P		

Site/Reach Selection for RWAs

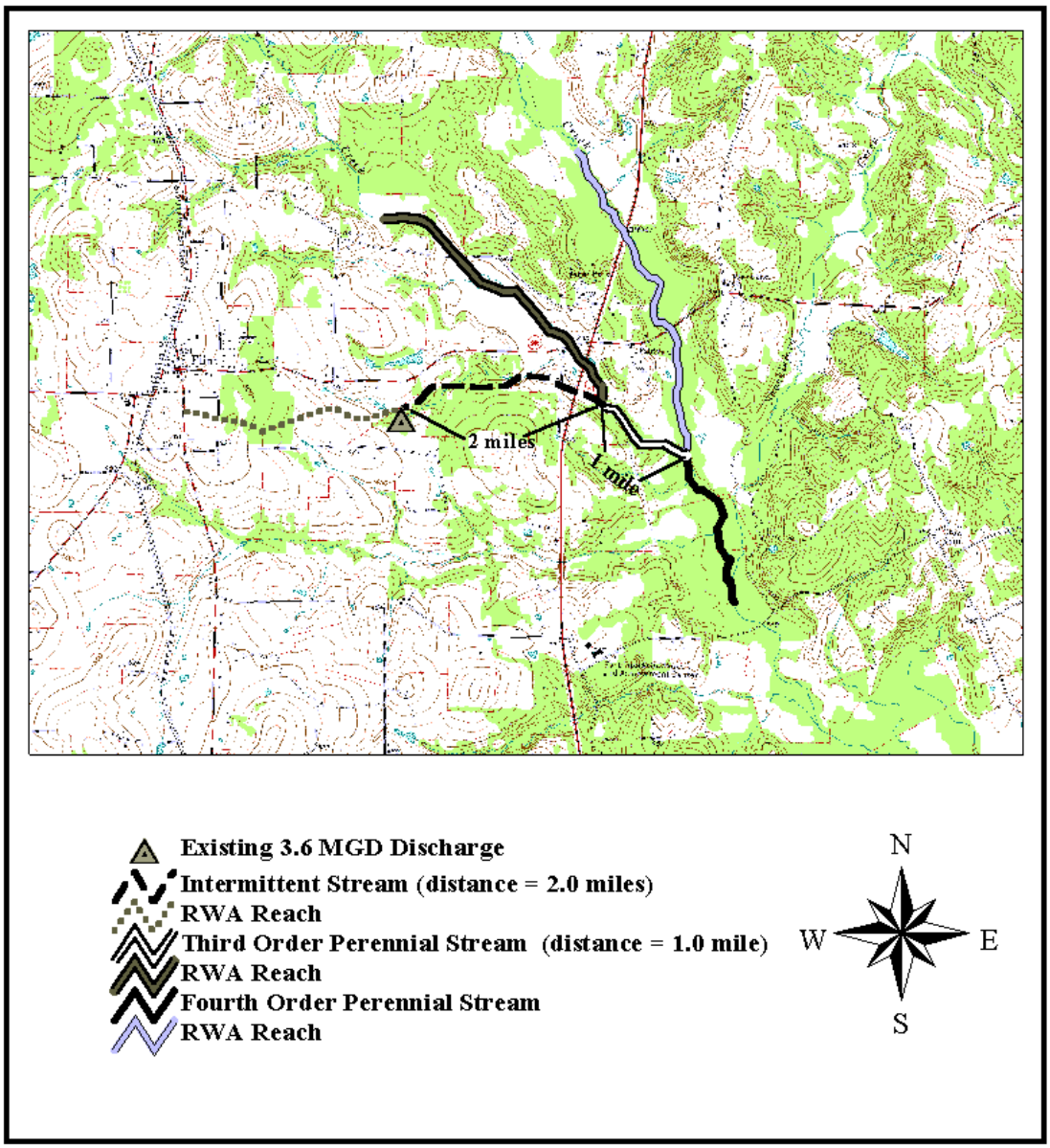


Figure B-8. Example of <u>Existing</u> 3.6 MGD Discharge to Intermittent and Perennial Stream. ***Extent of downstream impact on DO is 4.5 miles. Impact extends below the second confluence.***

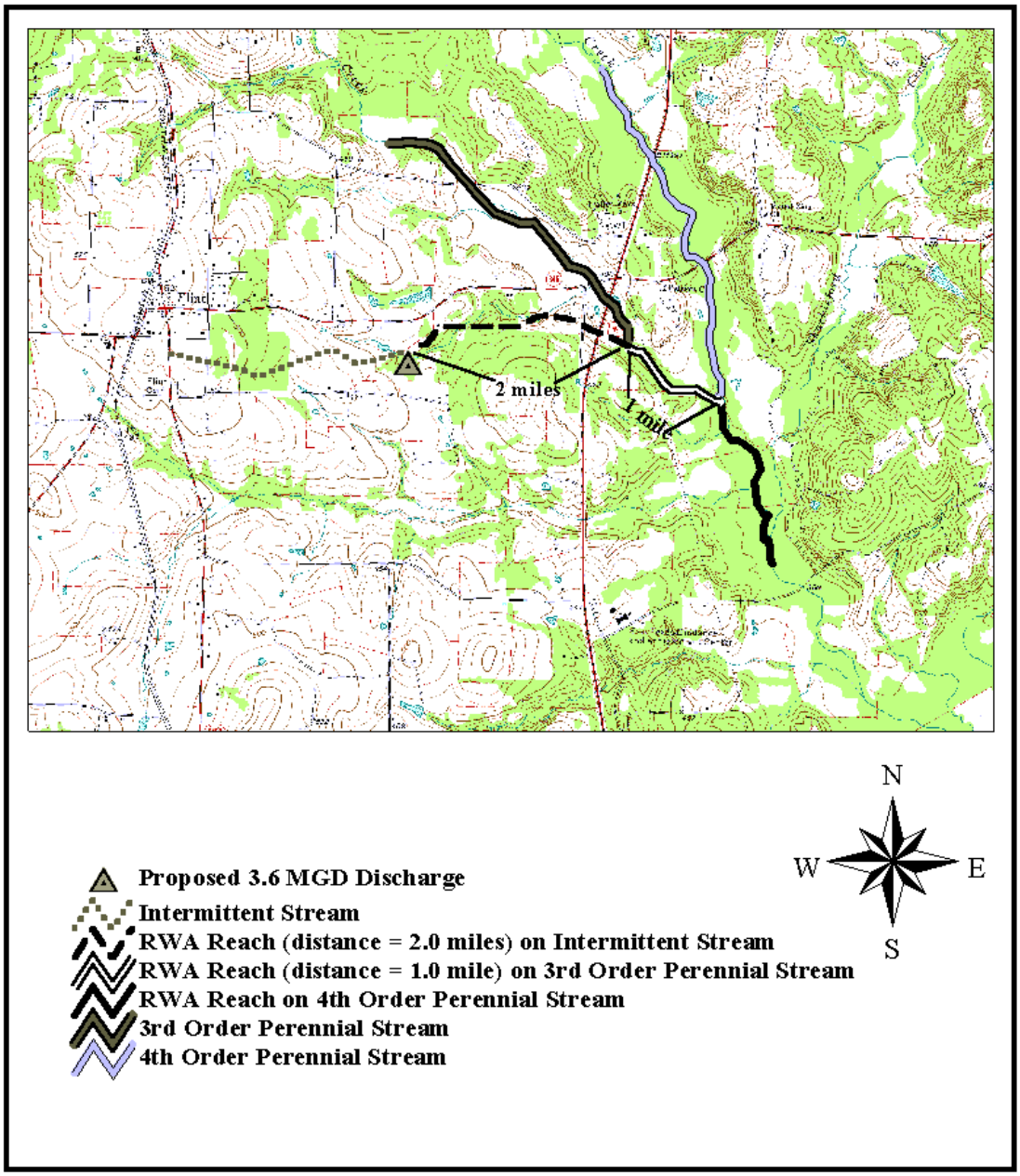
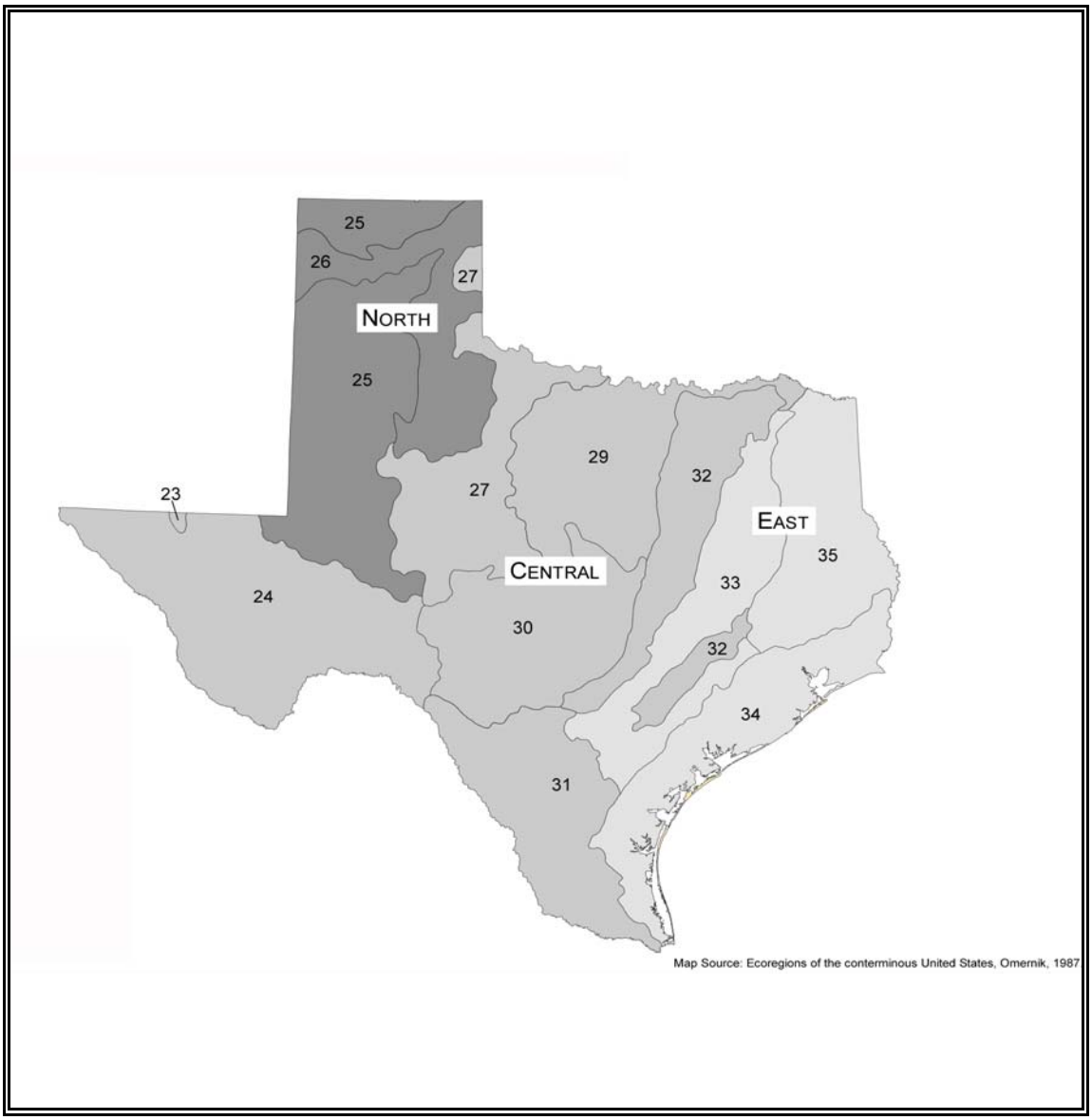


Figure B-9. Example of <u>Proposed</u> 3.6 MGD Discharge to Intermittent and Perennial Stream.
Extent of downstream impact on DO is 4.5 miles. Impact extends below second confluence.



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

Figure B-10. Macrobenthic Bioregions (North, Central, East) and Ecoregions of Texas.

APPENDIX C
FORMS FOR BIOLOGICAL MONITORING PACKETS

The forms included in this appendix should be used when preparing a biological monitoring packet to be submitted to the TCEQ. Some of the forms will be used in every biological monitoring packet and some forms 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 ALM, ALU, UAA, and RWA. For guidance in the *collection* of the biological data consult the TCEQ *Surface Water Quality Monitoring Procedures Volume 2: Methods for Collecting and Analyzing Biological Assemblage and Habitat Data* (RG-416) in conjunction with the current approved version of the *Guidance for Assessing Texas Surface and Finished Drinking Water Quality Data*.

The minimum data which should be submitted to the TCEQ as part of any biological assessment must contain the data in items 1 through 4 below. These items should be compiled in a packet and submitted to the TCEQ. If submitting the data as part of a UAA, please also utilize 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 entities should submit packets to the appropriate TCEQ CRP or appropriate Project Manager. Item 5 is optional.

1. Aquatic life monitoring and habitat assessment checklist.

2. Biological assessment
   - TCEQ nekton biological data reporting form or equivalent for seining efforts.
   - TCEQ nekton biological data reporting form or equivalent for electrofishing efforts.
   - 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 (RBA)
     - Scoring criteria for benthic macroinvertebrate quantitative samples (Surber)
     - Part III: Habitat quality index

Aquatic Life Monitoring and Habitat Assessment Checklist

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 #: _____
(TCEQ regional biologists only)

Field data (CRP partners only): Tag #: _____

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 (03/15 to 06/30 and 10/01 to 10/15) and critical period (07/01-09/30).

Stream characterization event 1, date:

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

Note: If sampling event for a 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 which may have adversely affected stream during each sampling event (for example, recent rains, drought, 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:

Indicate method(s) used:
Rapid bioassessment (5–minute kicknet or snags): _____
Quantitative (Surber, snags, or dredge): _____

Habitat assessment event 1:

TCEQ habitat protocols: Yes No

Stream flow 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 no, please describe why:

Benthic macroinvertebrate sampling event 2:

Indicate 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, flow, wetted channel width, photographs, description of bank conditions relative to first event, and description of canopy cover conditions relative to first event must be provided in this packet.

Stream flow 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

Appendices

Biological Monitoring Data Reporting Packet

The following pages comprise the TCEQ's standard biological monitoring data reporting packet. These forms are available online at
www.tnrcc.state.tx.us/water/quality/data/wqm/swqm_resources.html#guidance

Texas Commission on Environmental Quality
Surface Water Quality Monitoring Program

Nekton Data Reporting Form

RTAG# | REGION | EMAIL-ID: | COLLECTOR

STATION ID | SEGMENT | SEQUENCE | DATA SOURCE

Station Description_____

Composite - Most biological samples will be: 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. Code (<) if less than value, and (>) if greater than value, other wise leave this column blank. Continue if necessary, on additional worksheets. Codes to describe the habitat sampling effort are listed on the back.

CODE	(<) or (>)	Value	Description

Choose the most characteristic location and report data from this location as representative of the entire reach.

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Nekton Parameter Codes

NOTE: All measurements reported in metric units

Nekton Parameter Codes					
98005		Nekton, None Captured	98003		Total # Fish Species (Richness)
89944		Electrofishing Effort, Duration of Shocking (sec.)	98008		Total # of Sunfish Species (except bass)
89947		Seining Effort (# of Seine Hauls)	98010		Total # 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 # of Individuals, Seining
89943		Electrofishing Method (1= boat, 2=backpack, 3=tote barge)	98040		Total # of Individuals, Electrofishing
89976		Area Seined (m^2)	98062		# of individuals per seine haul
89961		Ecoregion (Texas Ecoregion Code)	98069		# of individuals per minute electrofishing
98032		Total # of Native Cyprinid Species	98052		Total # of Benthic Invertivore Species
98033		Individuals as Non-native Species (% of community)	98053		Total # of Benthic Species (catfish, suckers, and darters)
98030		Individuals with Disease / Anomalies (% of community)			
Additional Parameters					
89942		Net or Hook & Line Effort, Duration in Water (hrs)	89951		Cooling Water Intake Screen (1=revolving, 2=static)
89945		Castnetting Effort (# of casts)	89940		Intake Screen Collection, Duration (min.)
89907		Trawl, Otter, Duration (min.)	89953		Trawl, Otter, Width (meters)

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Texas Commission on Environmental Quality
Surface Water Quality Monitoring Program

Benthic Macroinvertebrate Data Reporting Form

RTAG# REGION EMAIL-ID: COLLECTOR

STATION ID SEGMENT SEQUENCE DATA SOURCE

Station Description_____

Composite - Most biological samples will be: 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. Code (<) if less than value, and (>) if greater than value, other wise leave this column blank. Continue if necessary, on additional worksheets. Codes to describe the habitat sampling effort are listed on the back.

CODE	(<) or (>)	Value	Description

Choose the most characteristic location and report data from this location as representative of the entire reach.

TCEQ-20151 (Rev. 05/14/2004) **Page 1 of 2**

Benthic Macroinvertebrate Parameter Codes

NOTE: All measurements reported 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= number of individuals from sub-sample; 2 = number of individuals/ft^2; 3 = number of individuals/m^2; 4 = total number 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 # Taxa
89950		Benthic Sampler (1=Surber, 2=Ekman, 3=kicknet, 4=Petersen, 5=Hester-Dendy)	90056		Total # of Diptera Taxa
89975		Area of snag surface sampled (m^2)	90057		Total # of Ephemeroptera Taxa
**89921		Percent undercut bank at sample point (%)	90058		Total # of Intolerant Taxa
**89922		Percent overhanging brush at sample point (%)	90060		EPT Taxa (% of communtiy)
**89923		Percent gravel substrate at sample point (%)	90062		Chironomidae (% of community)
**89924		Percent sand substrate at sample point (%)	90066		Tolerant Taxa (% of community), Benthos
**89925		Percent soft bottom at sample point (%)	90020		Benthic Grazers (% of community)
**89926		Percent macrophyte bed at sample point (%)	90025		Benthic Gatherers (% of community)
**89927		Percent snags and brush at sample point (%)	90030		Benthic Filterers (% of community)
**89928		Percent bedrock at sample point (%)	90067		Dominance (3 Taxa) (% of community)
RBAP Benthic Sample Descriptors					
89899		Biological Data Reporting Units (Values: 1= number of individuals from sub-sample; 2 = number of individuals/ft^2; 3 = number of individuals/m^2; 4 = total number in kicknet)	89946		Mesh size, sieve (diagonal measurements) (cm)
89950		Benthic Sampler (1=Surber, 2=Ekman, 3=kicknet, 4=Petersen, 5=Hester-Dendy)	89961		Ecoregion (Texas Ecoregion Code)
89902		Dip Net Effort, area swept (m^2)	84161		Stream Order
89903		Kicknet Effort, area kicked (m^2)	90005		Benthos Sampled–No Organisms Present
89904		Kicknet Effort, minutes kicked (min.)	90055		Total Taxa (Taxa Richness), Benthos, # Taxa
89905		Snags and Shoreline Sampling Effort, minutes picked	90008		EPT Taxa Abundance (# Taxa)
89906		Number of individuals in benthic RBA sub-sample (± 100)	90007		Biotic Index (HBI)
89950		Benthic Sampler (1=Surber, 2=Ekman, 3=kicknet, 4=Petersen, 5=Hester-Dendy)	90062		Chironomidae (% of community)
**89921		Percent undercut bank at sample point (%)	90042		Dominant Taxon, Benthos (% of community)
**89922		Percent overhanging brush at sample point (%)	90010		Dominant Functional Feeding Group (% of community)
**89923		Percent gravel substrate at sample point (%)	90036		Benthic Predators (% of community)
**89924		Percent sand substrate at sample point (%)	90050		Ratio of Intolerant: Tolerant Taxa
**89925		Percent soft bottom at sample point (%)	90069		% of Total Trichoptera as Hydropsychidae
**89926		Percent macrophyte bed at sample point (%)	90052		Total # Non-insect Taxa
**89927		Percent snags and brush at sample point (%)	90025		Benthic Collector-Gatherers (% of community)
**89928		Percent bedrock at sample point (%)	90054		% of Total # as Elmidae (% of community)

Texas Commission on Environmental Quality
Surface Water Quality Monitoring Program

Habitat Data Reporting Form

RTAG# REGION EMAIL-ID: COLLECTOR

STATION ID SEGMENT SEQUENCE DATA SOURCE

Station Description_____

Composite - Most biological samples will be: 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. Code (<) if less than value, and (>) if greater than value, other wise leave this column blank. Continue if necessary, on additional worksheets. Codes to describe the habitat sampling effort are listed on the back.

CODE	(<) or (>)	Value	Description

Choose the most characteristic location and report data from this location as representative of the entire reach.

TCEQ-20157 (Rev. 4-15-2004)

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 percent of substrate gravel size (> 2mm) or larger (%)
89860		Length of stream evaluated (km)	84159		Average percent instream cover (%)
89832		Number of lateral transects that were made	89929		Number of stream cover types
89861		Average stream width (m)	89846		Average percent 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/Flume **Note**: Use either 74069 or 00061, not both	89849		Average percent trees as riparian vegetation (%)
			89850		Average percent shrubs as riparian vegetation (%)
89848		Channel Flow Status 1=no flow, 2=low, 3=moderate, 4=high	89851		Average percent grasses and forbs as riparian vegetation (%)
89864		Maximum pool width at time of study (m)	89852		Average percent cultivated fields as riparian vegetation (%)
89865		Maximum pool depth in study area (m)	89853		Average percent other as riparian vegetation (%)
89839		Total number of stream bends	89854		Average percent 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		Ecoregion (Texas Ecoregion Code)
89843		Total number of riffles	89962		Land development impact (1=unimpacted, 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
# perennial pools evaluated	89914		

TCEQ-20157 (Rev. 4-05-2005)

Texas Commission on Environmental Quality
Surface Water Quality Monitoring Program

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 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		
	# of individuals as non-native species		% of individuals as non-native species		
	# of individuals with disease/anomaly		% of individuals with disease/anomaly		
			Index of biotic integrity numeric score:		

Note: This data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

[a] Excluding western mosquitofish

Aquatic life use:

Texas Commission on Environmental Quality
Surface Water Quality Monitoring Program

Quantitative Biological Scoring for Evaluating Aquatic Life Use Subcategories Regional Criteria Worksheets for Fish

Ecoregion 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		
	# of individuals as non-native species		% of individuals as non-native species		
	# of individuals with disease/anomaly		% of individuals with disease/anomaly		
			Index of biotic integrity numeric score:		

Note: This data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

Aquatic life use:

Texas Commission on Environmental Quality
Surface Water Quality Monitoring Program

Quantitative Biological Scoring for Evaluating Aquatic Life Use Subcategories Regional Criteria Worksheets for Fish

Ecoregion 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		
	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		
	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		
	# of individuals as non-native species		% of individuals as non-native species		
	# of individuals with disease/anomaly		% of individuals with disease/anomaly		
			Index of biotic integrity numeric score:		
			Aquatic life use:		

Note: This data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

[a] Excluding western mosquitofish

TCEQ-20155-C (Rev. 4-15-2004)

Texas Commission on Environmental Quality
Surface Water Quality Monitoring Program

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		
	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		
	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		
	# of individuals as non-native species		% of individuals as non-native species		
	# of individuals with disease/anomaly		% of individuals with disease/anomaly		
			Index of biotic integrity numeric score:		

Note: This data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

[a] Excluding western mosquitofish

Aquatic life use:

Texas Commission on Environmental Quality
Surface Water Quality Monitoring Program

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 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		
	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		
	# of individuals as non-native species		% of individuals as non-native species		
	# of individuals with disease/anomaly		% of individuals with disease/anomaly		
			Index of biotic integrity numeric score:		

Note: This data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

[a] Excluding western mosquitofish

Aquatic life use:

Texas Commission on Environmental Quality
Surface Water Quality Monitoring Program

Quantitative Biological Scoring for Evaluating Aquatic Life Use Subcategories Regional Criteria Worksheets for Fish

Ecoregion 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 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		
	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		
	# of individuals as non-native species		% of individuals as non-native species		
	# of individuals with disease/anomaly		% of individuals with disease/anomaly		
			Index of biotic integrity numeric score:		

Note: This data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

[a] Excluding western mosquitofish

Aquatic life use:

Texas Commission on Environmental Quality
Surface Water Quality Monitoring Program

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		
	# of individuals as non-native species		% of individuals as non-native species		
	# of individuals with disease/anomaly		% of individuals with disease/anomaly		
			Index of biotic integrity numeric score:		

Note: This data should be incorporated with water quality, habitat, and other available biological data to assign an overall stream score.

[a] Excluding western mosquitofish

Aquatic life use:

Texas Commission on Environmental Quality
Surface Water Quality Monitoring Program

Metrics and Scoring for Kick Samples, Rapid Bioassessment Protocol - Benthic Macroinvertebrates

Stream Name:				
Date:	**Collectors:**			
Location:				
County:	**Ecoregion #:**			
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. % of total trichoptera as Hydropsychidae				
10. # 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:				

Texas Commission on Environmental Quality
Surface Water Quality Monitoring Program

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:				

Stream Flow (Discharge) Measurement Form

Stream:____Date:_________

Station:____

Description:____

Time begin:____________Time end:____________ Meter type:_________________________

Observers:______________________Stream width*:_________ Section width (W):__________

Observations:____

Section Midpoint (ft) (m)	Section Depth (ft) (m) (cm) (D)	Observational Depth** (ft)(m)	Velocity (V)		Flow (Q) (m^3/s) (ft^3/s) Q = (W)(D)(V)
			At Point (ft/s)(m/s)	Average (ft/s)(m/s)	

$m^3/s \times 35.3 = ft^3/s$

Total Flow (Discharge)(∑Q)	

* Make a minimum of 10 measurements when the total width is > 5.0 ft, 20 measurements preferred.

** When water is < 2.5 ft deep take one measurement at each cross section. When water is > 2.5 ft deep, take two measurements at each cross section; one at ½ the total depth and the other at 2 x the total depth. Average the two velocity measurements. See SWQM Procedures Manual for a detailed flow measurement method.

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 w/ 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**
Trees		
Shrubs		
Grasses or forbs		
Cultivated fields		
Other		

Maximum Pool Depth:

Maximum Pool Width:

Notes

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 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 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 LB: RB:				Instream cover types:									% 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 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 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 LB:	RB:	Instream cover types:											% Instream cover	LB	
																	RB	

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 riparian vegetation percent composition by: (total to equal 100%)	
Trees	
Shrubs	
Grasses and Forbs	
Cultivated fields	
Other	
Average percent tree canopy coverage	
Overall aesthetic appraisal of the stream	

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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
Channel Flow Status	**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 and/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 <30°	**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; water clarity is usually exceptional	**Natural Area** Trees and/or native vegetation are common; some development evident (from fields, pastures, dwellings); water clarity may be slightly turbid	**Common Setting** Not offensive; area is developed, but uncluttered such as in an urban park; water clarity may be turbid or discolored	**Offensive** Stream does not enhance the aesthetics of the area; cluttered; highly developed; may be a dumping area; water clarity is usually turbid or discolored
Score_____	3	2	1	0
Total Score_____				

HABITAT QUALITY INDEX

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

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APPENDIX D
MISCELLANEOUS FORMS

The forms in this appendix are used in areas other than in the biological monitoring packet, such as for QA purposes.

TCEQ Biological Monitoring Self-Audit Reporting Form

Date	
Individual completing this report (Reporter)	
Reporter's contact information (address, phone, E-mail)	
Entity and program under whose QAPP biological data is collected (for example, Red River Authority–CRP and TCEQ Region 7– SWQM)	
Names of staff participating in biological sample collections and degree in aquatic or environmental sciences? (Yes or No)	
Names of staff participating in specimen identification (fish or benthic macroinvertebrates) and degree in aquatic or environmental sciences? (Yes or No)	
Have any staff attended a biological training event in the past year?	
If yes, name, date, and location of training event	

The following questions pertain to records management. All biological sampling records, such as sample logs and photographs, must be maintained and available for inspection during a TSA.

Are detailed field notes maintained for each biological sampling event?	
Are photographs taken and stored of each large specimen identified in the field and released?	
Are voucher specimens (at least two for minnows and shiners) preserved for each species collected at an event?	
Is a sample tracking system in place for easily identifying preserved samples in the lab?	
Are voucher specimen jars properly labeled with: collector(s) name(s), name of identifier(s), station location, station number if applicable, date and time of collection, collection method, preservative, scientific name of taxon contained in vial?	

TCEQ Biological Monitoring Self-Audit Form (continued)

Are detailed laboratory notes maintained on a bench sheet which include information on:

- Sample tracking log number
- Name of identifier
- Location of collection
- Date of collection
- Date entered into sample tracking log
- Date identification/enumeration started
- Date identification/enumeration completed
- Scientific name and counts of individuals
- Notes about peculiarities about the specimens or difficulties in identifying specimens

Did your program submit any specimens for identification confirmation during this past year?

If so, indicate the name and contact information of the individual performing this identification confirmation.

Are desktop quality assurance measures employed to ensure identifications are correct?

If so, which distribution lists or software are used?

List the keys which are used for the identification of fish and benthic macroinvertebrates.

Benthic Macroinvertebrate Laboratory Bench Sheet					
Stream Name		Location			
Station #	Log #	River Basin:		County:	
Date Collected:	Time:	Collector:			
Date Identified:		Identifier:			
Sample Type:				Rep # _____ of _____	
Riffle	**Multihabitat**	**Surber**	**Mulitplate**	Other	
Storet Code	**Taxon**		**No:**	**TV:**	**FFG:**
	Ephemeroptera				
	Plecoptera				
	Trichoptera				
	Odonata				
	Megaloptera				
	Hemiptera				
	Coleoptera				

Storet Code	Taxon		No:	TV:	FFG:
	Coleoptera (cont)				
	Diptera				
	Oligochaeta				
	Hirudinea				
	Isopoda				
	Amphipoda				
	Decapoda				
	Gastropoda				
	Bivalvia				
	Other				

Algal Identification Bench Sheet (Page 1 of ___)

Stream Name		Location	
Station #	Log #	River Basin:	County:
Date Collected:	Time:	Collector:	
Date Identified:	Identifier:		
Sample Type:		Rep # _______ of _______	
Natural Substrate		Artificial Substrate (Type):	

STORET	Division	Taxon	No:	TV:

Algal Identification Bench Sheet (Page ___ of ___)				
STORET	Division	Taxon	No:	TV:

APPENDIX E
BIOLOGICAL FACT SHEETS

Use Attainability Analysis (UAA)

A Use Attainability Analysis (UAA) is conducted on water bodies for which aquatic life uses and dissolved oxygen criteria have been established in the Texas Surface Water Quality Standards (including Appendix D), to determine if the existing designated aquatic life use/dissolved oxygen criterion is appropriate and, if not, to develop information to adjust the designated use and/or criterion. UAAs may also be conducted on previously unassessed and/or unclassified water bodies.

Biological Events	Number of Index Period Events	Number of Critical Period Events	Detail
Fish assemblage characterization	1	1st year: 1 2nd year: 1	Two year sampling period with, at least, one month between monitoring events. **Index period** (March 15 - October 15) **Critical period** (July 1 - September 30) Three **Biological Events** are required. Two of the events during the critical period with one during year 1, and one during year 2. The third event should be conducted during the non-critical portion of the index period (March 15 - June 30 or October 01 - October 15) in either year 1 or year 2. Site/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/reaches will be required for most water bodies. Site/reach selection should be done in consultation with TCEQ Water Quality Standards.
Benthic macroinvertebrate community characterization			
Stream physical habitat assessment			
Instantaneous field measurements			
Conventional water chemistry sample			
24-hour DO monitoring			
Flow discharge measurement			
Additional Diel Events			
24-hour DO monitoring	1	1	Besides the three monitoring events described above, a minimum of two **Additional Diel Events** must be conducted. One of the two additional events should be conducted during year 1 and the other during year 2. One must be collected during the critical period and one must be collected during the non-critical portion of the index period. If, 2 or more of the five 24-hr DO samples do not support the criteria, additional 24-hr DO data may be required. An effort should be made to collect all samples when flows are at, or above the critical low flow condition.
Flow discharge measurement			

Aquatic Life Assessment (ALA)

An Aquatic Life Assessment (ALA) is conducted on an unclassified water body not already included in Appendix D of the Texas Surface Water Quality Standards (TSWQS), and which has previously been assessed and determined not to attain the presumed aquatic life use and/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	Detail
Fish assemblage characterization	1st year: 1 2nd year: 1	1st year: 1 2nd year: 1	Two year sampling period with, at least, one month between monitoring events. **Index period** (March 15 - October 15) **Critical period** (July 1 - September 30) Four **Biological Events** are required*. Each year, one event is conducted during the critical period and one event conducted during the non-critical portion of the index period (March 15 - June 30 or October 01 - October 15). Site/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. Site/reach selection must be done in consultation with TCEQ Water Quality Standards. ***Exceptions to the number of Biological Events Required**: If an ALA was required based on the results of Aquatic Life Monitoring (ALM), that is, results indicate that *presumed use is not supported*; and the first year's samples from the ALA *indicate agreement* 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 Aquatic Life Monitoring (ALM), that is, results indicate that *presumed use is not supported*; and the first year's samples from the ALA *are not in agreement* 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.
Benthic macroinvertebrate community characterization			
Stream physical habitat assessment			
Instantaneous field measurements			
Conventional water chemistry sample			
24-hour DO monitoring			
Flow discharge measurement			
Additional Diel Events			
24-hour DO monitoring	1st year: 1* 2nd year: 1	1st year: 2* 2nd year: 2	A total of ten recent 24-hour DO events are needed to determine appropriate DO criteria, with no more than six in any one year (total six in the critical period, and four in the non-critical period.) If two or more of the five 24-hour DO events collected by the end of the first year of the ALA do not support the presumed criterion, then up to five 24-hr DO monitoring events must be collected in the second year, even if Biological Events are not required (to make a total of ten, including DO samples from ALM). An effort should be made to collect all samples when flows are at, or above the critical low flow condition. 7Q2. * A minimum of five 24-hour DO samples must be available by the end of the first year; from recent DO sampling plans, an ALM, and these ALA events. Samples are used to determine if additional DO monitoring is needed as described in the paragraph above.
Flow discharge measurement			

Receiving Water Assessment (RWA)

A Receiving Water Assessment (RWA) is conducted on unclassified water bodies that are the subject of a wastewater permitted activity. 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 criterion.

Biological Events	Number of Index Period Events	Number of Critical Period Events	Detail
Fish assemblage characterization		1	One year sampling period **Index period** (March 15 - October 15) **Critical period** (July 1 - September 30) The RWA typically involves a single site, located 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/reaches should be representative of the water body(ies) being evaluated and should be selected in consultation with TCEQ Water Quality Standards Team. One **Biological Event** is required but two are strongly recommended for determining the appropriate aquatic life use. An effort should be made to ensure that data are collected during the index period (March 15 - October 15), and , preferably within the critical period (July 1 - Sept. 30). The aquatic life use indicated by the RWA will be considered for *Appendix D* in the next TSWQS revision. * Conventional water chemistry and 24-hour DO monitoring are optional, but are strongly recommended for evaluation of the Biological Event.
Benthic macroinvertebrate community characterization			
Stream physical habitat assessment			
Instantaneous field measurements			
Flow discharge measurement			
* 24-hour DO monitoring			
* Conventional water chemistry sample			

Aquatic Life Monitoring (ALM)

Aquatic Life Monitoring (ALM) events are typically scheduled as part of the cooperative monitoring schedule and are conducted to provide baseline data on environmental conditions, and to determine if aquatic life uses/dissolved oxygen criteria are being attained. ALM samples can contribute to the establishment of an appropriate aquatic life use.

Biological Events	Number of Index Period Events	Number of Critical Period Events	Detail
Fish assemblage characterization	1	1	One year sampling period with, at least, one month between monitoring events. **Index period** (March 15 - October 15) **Critical period** (July 1 - September 30) Two **Biological Events** are required. One event to be conducted during the critical period and the other event to be conducted during the non-critical portion of the index period (March 15 - June 30 or October 01 - October 15). ALM is appropriate for routine monitoring sites, and should be representative of the water body being assessed * Conventional water chemistry and 24-hour DO monitoring are optional, but are strongly recommended for evaluation of the Biological Event. When the ALM is conducted on an unclassified water body (not in either *Appendix A* or *D* of the Texas Surface Water Quality Standards) and the samples indicate that the *presumed use is supported*, this will be adequate information to establish the appropriate aquatic life use. When the ALM is conducted on an unclassified water body (not 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 Aquatic Life Assessment (ALA) will be necessary to determine the appropriate aquatic life use. When the ALM is conducted on a classified water body and (established in either *Appendix A* or *D* of the Texas Surface Water Quality Standards) and the samples indicate that the *adopted use is not supported*, the water body will be listed on the 303(d) List.
Benthic macroinvertebrate community characterization			
Stream physical habitat assessment			
Instantaneous field measurements			
Flow discharge measurement			
* 24-hour DO monitoring			
* Conventional water chemistry sample			

APPENDIX F
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 in color, 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 macrophyte	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) submersed rooted aquatics, (2) floating leaved aquatics, (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 routine biological monitoring conducted to provide baseline data on environmental conditions and/or to determine if aquatic life use/dissolved oxygen criteria are being attained. This category also includes reference condition, or ecoregion monitoring.
Bank	The portion of the channel which tends to restrict lateral movement of water. It often has a slope less than 90° 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 at which the channel is filled to its top and just begins to spill out onto the flood plain. 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, on average, has a recurrence interval of 1.5 years.
Benthic organisms	Aquatic bottom-dwelling organisms including 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 and/or higher aquatic plants in a body of water. This is often related to pollutants that increase the rate of growth.
CBOD5	The quantity of oxygen utilized 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 containing 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	Classified water bodies, also referred to as *designated 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 of the Texas Surface Water Quality Standards. Classified waters include most rivers and their major tributaries, major reservoirs, and estuaries.
Criteria	Water quality conditions 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 cf volume of water travels 1 ft in 1 second.
Cut bank	The outside (concave) bank of a stream channel bend characterized by high erosion. Stream flow 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 man-made 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), prior to entering a water body.
Emergent vegetation	Aquatic macrophytes (plants) that are rooted in the sediment, near shore or in marshes, with nearly all of the leaves above the water surface (cattails).
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, duck weed, and water hyacinths).

Floodplain	Level land areas adjacent to rivers and streams that are subject to recurring inundation. They are formed by the deposition of sediment during periodic floods. Floodplains contain such features as levees, backswamps, delta plains, and oxbow lakes.
Fork length	Fish: Greatest distance in a straight line from tip of snout to center of fork in caudal fin.
Genus	A category of biological classification ranking between family and species, comprising structurally or phylogenetically (evolutionary relationship) related species and being 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 shore to shore without eddy development. A glide is too shallow to be a pool but the water velocity is too slow 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 organisms	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	Streams that may have periods of zero flow or a 7Q2 flow of less than 0.1 cfs, but maintain pools that create significant aquatic life uses.
Intolerant organism	Organisms that are 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	Running or flowing water systems— rivers and streams.
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	The natural vegetative buffer refers to an area of either natural or native vegetation which 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 man-altered settings, the natural vegetative buffer limit would be at the point of man's 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 purposes of quantifying potential stream impairments.
Nekton	Free swimming organisms (for example, fish, insects).

Nonpoint source	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 and/or cover for fish and benthic macroinvertebrates and/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 greater 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: from a pipe or a ship).
Pool	A portion of a stream where water velocity is slow 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 in which 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 (RBP)	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 stream bank and the area out onto the flood plain that is periodically inundated by the flood waters from the stream. The limit of the zone depends on many factors including native plant community makeup, soil moisture levels, and distance from the stream (or the limit of interaction between land and stream processes). It is periodically inundated by the flood waters from the stream. 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 Specific waters designated by the Texas Commission on Environmental Quality in the Texas Surface Water Quality Standards, 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 (Ex: 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 stream flow 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 µmhos/cm, of 1 cm^3 of water at 25°C. 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-Greatest distance in a straight line from tip of snout to base of caudal peduncle.

Stream bend Curved part of a stream. A well defined bend has a deep outside area (cut bank) and shallow inside area accentuated by point bar development. Due to sharp bending, stream flow 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 stream flow is generally not forced to one side.

Stream order A stream size classification scheme where the smallest, unbranched tributaries in a watershed are designated first order streams. Where two first-order streams join, a second-order stream is formed; and 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 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	Organisms that have 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 wing span. Squid: Posterior mantle margin to top of pen.
Transect line	A straight line, perpendicular to stream flow, between two points on opposite stream banks.
Tree canopy	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 canopy densiometer. Possible measurement range is from 0% (totally open) to 100% (totally closed canopy cover).
Tributary	A stream or river that flows into a larger stream or river.
Unclassified water body	Unclassified water bodies are those smaller water bodies that do not have site-specific water quality standards assigned to them (not included in Appendix D of the Texas Surface Water Quality Standards), but instead are protected by general standards that apply to all surface waters in the state.
Use attainability analyses (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. Watersheds are sometimes referred to as drainage basins or drainage areas.