



RG-416  
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# **Surface Water Quality Monitoring Procedures, Volume 2:**

## **Methods for Collecting and Analyzing Biological Assemblage and Habitat Data**



































# CHAPTER 2

## BIOLOGICAL MONITORING REQUIREMENTS

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

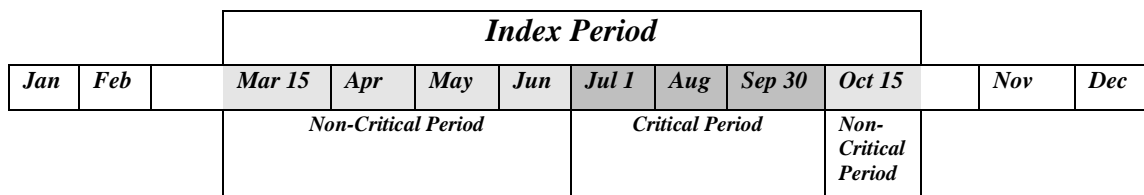
### Index Period

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

The index period was established to:

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

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



**Figure 2.1.** The index period.

## ***Scheduling Biological Monitor Events***

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

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

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

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

## **General Sampling Guidelines**

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

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

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

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

## ***Exceptions***

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

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

## ***Representativeness of Sites***

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

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

## ***Site Reconnaissance***

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

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

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

## ***Sampling Conditions***

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

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

## ***Low-Flow Monitoring***

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

<[www.tceq.texas.gov/goto/swqm-procedures](http://www.tceq.texas.gov/goto/swqm-procedures)>

## *Other Monitoring Requirements*

### **Documentation and Field Notes**

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

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

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

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

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

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

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

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

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

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

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

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

## Creating New Monitoring Stations

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

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

<[www.tceq.texas.gov/goto/dmrg](http://www.tceq.texas.gov/goto/dmrg)>

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

## Additional Latitude and Longitude Coordinates

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

## Non-Biological Parameters

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

## Monitoring Categories for Wadable Freshwater Streams

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

### ALM

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

## ***Ecoregion ALM***

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

### **Identifying Minimally Impacted Ecoregion Reference Streams**

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

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

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

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

## **Aquatic-Life Assessments**

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

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

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

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

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

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

## **Receiving-Water Assessments**

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

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

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

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

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

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

## **Use-Attainability Analyses**

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

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

## ***Monitoring and Assessment of Large Rivers***

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

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

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

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

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

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

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

## ***Monitoring and Assessment of Lakes and Reservoirs***

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

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

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

Before conducting any biological monitoring at a lake or reservoir, it is imperative to coordinate this work with the TCEQ and the TPWD. As methodologies and metrics are established, this manual will be updated to reflect those changes.

## *Categories of Saltwater Biological Monitoring*

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

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

### **Tidal Streams and Estuaries**

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

**Water quality sampling.** Instantaneous field measurements must be collected, including profiles, since the water column is often stratified due to temperature and salinity. Samples can be collected for analysis of routine water chemistry and carbonaceous biochemical oxygen demand, five-day (CBOD<sub>5</sub>). Profiles and grab samples should be collected at depths specified in Volume 1 (RG-415).

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

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

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

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

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

## **Sampling Index Period**

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

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

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

# CHAPTER 3

## FRESHWATER FISH

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### Objective

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

### Scientific Collection Permit

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

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

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

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

### Records

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

## ***Field Logbook***

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

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

## ***Sample-Tracking Logbook***

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

## ***Laboratory Bench Sheets***

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

## **Sample Collection**

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

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

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

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

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

## **Electrofishing**

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

### ***Collection Procedures***

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

### **Backpack Electrofisher**

#### ***Safety***

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

#### ***Adjusting a Backpack Electrofisher***

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

After reaching the stream,

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

- engage the unit and check the output

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

### ***Collection Method***

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

### ***Where to Sample***

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

### ***Sampling Time***

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

## **Boat-Mounted Electrofisher**

### ***Safety***

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

## ***Adjusting the Output***

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

## ***Collecting***

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

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

## ***Seine***

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

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

## ***Seine Types***

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

## ***Collecting***

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

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

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

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

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

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

## *Sample Preservation and Processing*

### **Field Processing**

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

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

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

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

### **Use of Digital Photos as Fish Vouchers**

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

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

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

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

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

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

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

The primary considerations for image collection and data handling are:

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

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

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

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

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

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

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

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

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

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

## **Rules for Capturing Voucher Images of Fishes**

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

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

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

## **Field Preservation**

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

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

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

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

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

**Alcohol** is highly flammable, requiring special care in storage and handling.

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

## Labeling a Field Sample

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

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

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

## ***Laboratory Processing***

### **Identification of Fish-Assemblage Samples**

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

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

### **Sample-Tracking Log**

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

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

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

### **Laboratory Sample Processing**

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

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

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

## **Sorting and Identification**

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

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

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

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

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

### ***Laboratory Bench Sheets***

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

### ***Quality-Control Checks***

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

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

## ***Voucher Specimens***

Retain at least one representative of each fish taxon collected as a voucher specimen for at least five years or until the conclusion of any applicable regulatory decision (whichever is longer) to allow verification of the identification if necessary. Voucher specimens serve as long-term physical proof that confirm the names applied to organisms stored in SWQMIS. Voucher specimens ensure the credibility of TCEQ biological data by documenting the identity of the organisms and making them available for review by the general scientific community.

Consider the following when storing voucher specimens.

- long-term maintenance of wet (alcohol-preserved) and mounted specimens
- adequate quantity and quality of space to store specimens
- an effective mechanism for locating and retrieving specimens upon request
- personnel experience in fish taxonomy

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

## **Data Evaluation**

The primary tools required for analyzing fish data for wadable freshwater streams are described in Linam et al. (2002). The report outlines regional indices of biotic integrity and their application for assessing aquatic-life uses, and explains in detail the individual metrics for the various regions. As noted in the section on large rivers, these indices may be suitable for evaluating fish assemblages in those water bodies as well, but that should be discussed with TCEQ personnel. The full report is online at <[https://www.tpwd.state.tx.us/publications/pwdpubs/media/pwd\\_rp\\_t3200\\_1086.pdf](https://www.tpwd.state.tx.us/publications/pwdpubs/media/pwd_rp_t3200_1086.pdf)>.

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

## **Large-River Monitoring and Assessment**

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

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

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

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

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

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

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

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

## **Reservoir Monitoring and Assessment**

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

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

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

Before any biological monitoring of a lake or reservoir, it is imperative to coordinate this work with the TCEQ and the TPWD. As methodologies and metrics are established, this manual will be updated to reflect those changes.

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

# CHAPTER 4

## SALTWATER NEKTON

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### Disclaimer

Methodologies for assessing ALUs have not been developed for Texas saltwater habitats—including Gulf waters, bays, estuaries, the Intracoastal Waterway, and tidal streams. Before conducting any biological monitoring activities on a saltwater body, it is imperative to coordinate this work with the TCEQ and the TPWD. As methodologies and metrics are established, this manual will be updated to reflect those changes.

### Objective

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

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

### Scientific Collection Permit

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

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

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

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

## Records

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

### *Field Logbook*

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

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

### *Sample-Tracking Logbook*

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

## ***Laboratory Bench Sheets***

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

## **Sample Collection**

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

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

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

## ***Collection Procedures***

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

## **Fish Collection in Tidal Streams and Bayous**

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

## **Trawl**

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

In tidal streams, use a 10 ft otter trawl.

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

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

Do not trawl in marked navigation channels.

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

## **Seine**

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

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

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

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

Repeat this process until six or more replicates are collected.

## **Boat-Mounted Electrofisher**

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

### ***Safety***

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

### ***Collecting***

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

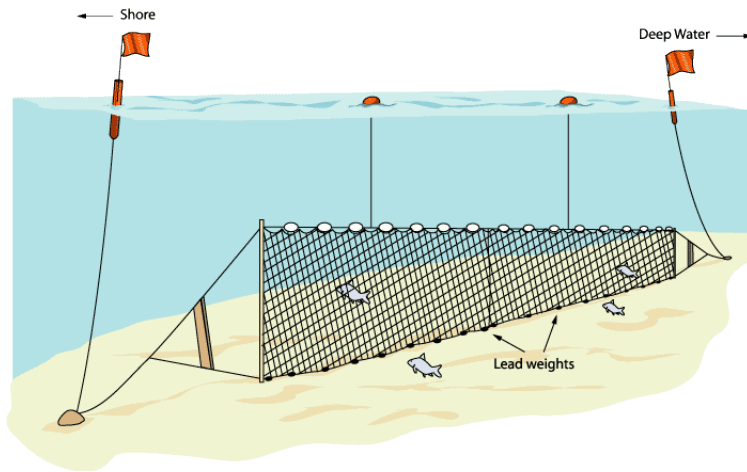
Frequency and voltage are set to maximize output for the water conditions. Once the controls are adjusted, the samplers reset the timer. Electricity is discontinuously applied. Catches usually can be increased in areas of submerged cover by moving in with the power on but the circuit off and then energizing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

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

## **Gill Netting**

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

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



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

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

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

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

## *Sample Preservation and Processing*

### **Field Processing**

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

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

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

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

### **Field Preservation**

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

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

### ***Safety***

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

Alcohol is highly flammable; take care in storage and handling.

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

### **Labeling a Field Sample**

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

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

### ***Laboratory Processing***

#### **Identification of Fish-Assemblage Samples**

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

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

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

#### **Sample-Tracking Log**

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

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

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

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

### ***Laboratory Sample Preservation***

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

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

### **Sample Sorting and Identification**

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

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

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

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

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

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

## **Laboratory Bench Sheets**

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

## **Quality-Control Checks**

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

## ***Voucher Specimens***

Retain at least one representative of each fish taxon collected as a voucher specimen for at least five years or until the conclusion of all applicable regulatory decisions (whichever is longer) to allow verification of identification if necessary. Voucher specimens serve as long-term physical proof that confirm the names applied to organisms stored in SWQMIS. Voucher specimens ensure the credibility of TCEQ bioassessment data by documenting the identity of the organisms and making them available for review by the general scientific community.

Consider the following when storing voucher specimens.

- long-term maintenance of wet (alcohol-preserved) and mounted specimens
- adequate quantity and quality of space to store specimens
- an effective mechanism for locating and retrieving specimens upon request
- personnel experience in fish taxonomy

The organization maintaining voucher specimens must have a history that demonstrates the ability to preserve the specimens into the future (USGS 2000). This could include

in-house provisions for sample maintenance or archiving in a university or museum natural-history collection.

## **Field Notes**

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

## **Data Evaluation**

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

# CHAPTER 5

## **FRESHWATER BENTHIC MACROINVERTEBRATES**

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### **Objective**

This chapter describes the methods the TCEQ uses for the collection and assessment of benthic macroinvertebrate samples from freshwater systems. In general, the TCEQ uses benthic macroinvertebrate samples collected according to these methods in combination with fish-community surveys (Chapter 3) and physical-habitat assessments (Chapter 9). These methods provide a holistic evaluation of the health of instream biological assemblages. Benthic macroinvertebrate samples collected from freshwater rivers and streams using the rapid bioassessment protocols (RBPs) are currently used in the biological assessments outlined in Chapter 1.

### **Scientific Collection Permit**

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

### **Overview of Sample-Collection Methods**

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

#### ***Riffles, Runs, and Glides in Wadable Streams and Rivers***

#### **Rapid Bioassessment Protocols**

##### ***5-minute Kicknet***

RBPs were originally developed as cost-effective screening tools for evaluating the biotic integrity of benthic macroinvertebrate assemblages. Benthic macroinvertebrate samples are usually collected with a D-frame kicknet, preferably from riffle habitat, or secondarily from run or glide habitats by kicking and disturbing the streambed, hence the name “kicknet.” Dislodged material and associated benthic macroinvertebrates are collected in the net.

##### ***Snag Sampling***

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











































































































































































































































































# Packet for Reporting Biological-Monitoring Data

These forms are available online at <[www.tceq.texas.gov/goto/biopacket](http://www.tceq.texas.gov/goto/biopacket)>.































































































































