## Chapter 3

## Freshwater Fish

## Objective

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

## Scientific Collection Permit

A Scientific Permit for Research is required to collect, salvage, band, or hold native Texas wildlife for scientific purposes. Scientific purposes include activities aimed at enhancing, protecting, conserving, or managing protected wildlife, or furthering scientific understanding of a resource or the environment. Refer to the TPWD website for application requirements and reporting forms: <www.tpwd.state.tx.us/business/permits/land/wildlife/research/>.
Notify each TPWD Law Enforcement Office in each region of your field activities by telephone at least 24 hours, but not more than 72 hours, before collection if collection techniques or devices being used are ordinarily illegal (e.g., using gill nets or electroshocking devices to collect fish, or hunting or collecting along public roads and rights-of-way). A confirmed response from the local game warden is required before collection if the sampling activities being conducted involve methods of capture ordinarily classified as illegal. In addition, please be advised that collecting in a wildlife management area is not authorized without prior written permission from the area manager.
For regional office location and telephone numbers, see <www.tpwd.state.tx.us/warden/connect/ offices> or call a Parks and Wildlife Communication Center: Austin-512-389-4848; Houston-281-842-8100.

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

## Records

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

## Field Logbook

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

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


## Sample-Tracking Logbook

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

## Laboratory Bench Sheets

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

## Sample Collection

The method used to collect nekton samples will depend on several factors, including water-body characteristics, the number of sampling personnel, and available sampling equipment. The field equipment and materials necessary to collect fish are listed in Appendix A. Forms needed for biological assessments appear in Appendix C. Examples of laboratory bench sheets are in Appendix H. Electronic copies of all the tables in the appendixes are available at the TCEQ website. Definitions of technical terms are in Appendix E.
In most streams, fish are collected using multiple gear types, with fishes counted separately. Both electrofishing and seining are required for collecting fish samples. If unable to employ multiple types of gear, indicate the reason in the field logbook and increase effort with the gear used. For example, if electrofishing is not possible because of elevated conductivity, increase your seining effort by conducting additional seine hauls to ensure all possible habitat types are sampled. Collections at each site in the study must be comparable. Consequently, collectors must
ensure that the sampling procedures, level of effort expended, and types of habitat sampled are similar in succeeding years.

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

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

## Electrofishing

Electrofisher capabilities vary by manufacturer and model. Each model is effective under certain ranges of specific conductance. For example, the Smith-Root Type 12 model is most effective at specific conductance levels less than $1,000 \mu \mathrm{~S} / \mathrm{cm}$, though it is rated to $1,600 \mu \mathrm{~S} / \mathrm{cm}$ (SmithRoot, 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 highconductivity 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 $1,600 \mu \mathrm{~S} / \mathrm{cm}, 400$ to 700 volts for 200 to $400 \mu \mathrm{~S} / \mathrm{cm}$, and 800 to 1,100 volts for $<200 \mu \mathrm{~S} / \mathrm{cm}$.

## 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 $60-120 \mathrm{~Hz}$. 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 $30 \mathrm{ft} \times 6 \mathrm{ft} \times 1 / 4 \mathrm{in}$ mesh seine, whereas riffles, runs, and small pools are usually sampled using a 15 ft or $6 \mathrm{ft} \times 6 \mathrm{ft} \times 3 / 16$ in mesh seine. All are straight seines constructed of delta-weave mesh with double lead weights on the bottom line. Seines must be inspected for any holes and repaired or replaced prior to each use.

## Collecting

A seining crew consists of at least two persons, but is more effective with three. Attempt at least six effective seine hauls covering at least 60 m . Use a 6,15 , or 30 ft straight seine, depending on stream size, current, and depth. One end of the seine is positioned near the bank. The seine is positioned perpendicular to the bank. With the net fully extended or rolled to make it taut (in areas were the sampling habitat is smaller or the stream channel narrows), two persons pull the
net parallel to the bank with a person on bank slightly behind a person in the channel. The person in the channel proceeds to the bank with seine extended. Both persons pull the seine onto the bank. Be sure the lead line remains on the bottom until the seine is pulled out of the water.
Given moderate velocity, seining may be most effective in a downstream direction, with fishers moving slightly faster than the current. Staking out the seine in swift water and having others kick into the net can also be effective in riffles.

Repeat this process until at least six replicates are collected, covering 60 m . One unit effort for each seine haul would average 10 m , but several short hauls may be required to make up one unit level of effort (e.g., riffle kicks). For a seine haul to be considered effective, evaluate whether the haul was negatively affected in any way. If the seine gets caught on woody debris or the net is lifted in a manner that may allow fish to escape, the haul must be considered ineffective and not counted as viable. Capturing no fish would not necessarily constitute an ineffective haul. Keep any fish collected even if the haul is ineffective.
As in backpack electrofishing, continue sampling until no new species are noted.
Count and record all organisms collected by the seine or put them in a container with preservative and attach a label. Often the organisms are so small and numerous that it is preferable to bring the entire catch back to the laboratory for identification and enumeration.

## Sample Preservation and Processing

## Field Processing

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

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

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

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

## Use of Digital Photos as Fish Vouchers

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

For large or protected fish, photographic vouchering is an economical method that reduces the overall volume of hazardous chemicals needed for preservation and eliminates the need for storage containers and space to maintain large specimens.
Stauffer et al. (2001) give detailed guidance for producing photographic vouchers, including discussions of photographing specimens and understanding the rules for capturing voucher images of fishes. This document is the primary reference for the discussion of photographic vouchers.
A digital camera is required to produce satisfactory photographic vouchers and the following camera capabilities are required:

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

Even on large fish it may be necessary to photograph small characters such as fins, gills, and spines. Thus, the camera should be capable of focusing on objects that are very close to the lens. Stauffer et al. (2001) recommend that the camera be able to focus on images as close as 4 cm .
It is not unusual to conduct fish assessments in low light. If possible, move specimens from shade to full sun. However, where it is not possible to sense natural light, the flash allows fast shutter speeds that produce crisp photos.

The primary considerations for image collection and data handling are:

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

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

Size is a key piece of information about the specimen and can be helpful in the identification or verification of vouchers. In each photograph, include a means of estimating size such as a tape measure, meter stick, or calibrated device.
Some type of text label should be included in the image of the specimen. This might be a small dry-erase or magnetic board that includes, at minimum:

- species
- location of collection
- date of collection
- sample or specimen ID number
- name of each collector
- name of identifier, if different from collector

Cameras can usually store images in compressed JPEG format. When selecting the degree of compression or size of the captured image, choose the physically largest image available (in horizontal and vertical resolution). Also, it is best to choose medium- to high-quality JPEG formats in order to preserve image quality.
The camera automatically assigns a filename, typically consisting of alphanumeric characters, to an image when captured. This automatically assigned name gives no indication of the file contents. Therefore, develop a system to name photographic voucher images as files that can be saved and subsequently retrieved as efficiently as possible. The filename should include:

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

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

## Rules for Capturing Voucher Images of Fishes

For the voucher images to convey the most information and enable identification or verification of the specimen, it is especially important that the viewing aspect is appropriate for each type of fish. By following the guidelines discussed below, Stauffer et al. (2001) suggest that most fish species can be successfully identified from digital images.
Physical work on preserved fishes is done on the right side, often damaging tissues and blemishing the specimen's appearance. Therefore, the convention is to photograph the left side. Also, if more than one species are expected to co-occur, photographs should clearly show the characters that allow identification of each species.
Table 3.1 summarizes guidelines for the appropriate view for the photographic image of fish in common freshwater families. It assumes that the collector can use the photograph to identify a fish to family level. Table 3.1 includes families where individuals are expected to reach at least 12 inches in total length.

## Field Preservation

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

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

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

| Acipenseridae (sturgeons) | lateral view |
| :--- | :--- |
| Amiidae (bowfin) | lateral |
| Anguillidae (freshwater eels) | lateral |
| Catostomidae (suckers) | lateral; ventral (head and jaw) |
| Centrarchidae (sunfish) | lateral |
| Clupeidae (herrings) | lateral |
| Cyprinidae (minnows) | lateral; ventral (head and jaw) |
| Esocidae (pikes) adults | lateral |
| Gobiidae (gobies) | lateral |
| Hiodontidae (mooneyes) | lateral |
| Ictaluridae (bullhead catfishes) | lateral (clear view of dorsal and caudal fins);ventral <br> (head and chin) |
| Lepisosteidae (gars) | lateral |
| Percichthyidae(temperate bass) | lateral with anal fin flared; close-up of flared <br> 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 nonwadable 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.

