



## **Guidelines for Aquatic Resource Relocation Plans for Fish and Shellfish, Including Freshwater Mussels**

Dewatering, maintenance, and construction related activities in rivers, creeks, streams, lakes, sloughs, reservoirs, bays, estuaries, stilling basins, and other flood control structures may negatively impact fish, shellfish, and other aquatic resources. The Texas Parks and Wildlife Department is the state agency with primary responsibility for protecting the state's fish and wildlife resources. The Texas Parks and Wildlife Code authorizes the department to investigate fish kills and any type of pollution that may cause loss of fish or wildlife resources, estimate the monetary value of lost resources, and seek restitution or restoration from the party responsible for the fish kill or pollution through suit in county or district court. The Texas Administrative Code requires the department to actively seek full restitution for and/or restoration of fish, wildlife, and habitat loss occurring as a result of human activities. The restitution value of lost resources can be significant, in particular for species classified as threatened or endangered. Restitution for each individual of a threatened species is at least \$500 and for each individual of an endangered species is at least \$1,000. In addition, the Texas Parks and Wildlife Code makes it a criminal offense to kill any fish or wildlife resources classified as threatened or endangered.

Besides potential impacts to other aquatic resources, the department is particularly concerned about declining freshwater mussel populations, reflected in the 2009 Texas Parks and Wildlife Commission's decision to list 15 species of freshwater mussels as threatened. In order to avoid adverse impacts to aquatic resources and potential civil and criminal liability, the department recommends entities coordinate with the department to develop a plan to avoid impacts to aquatic resources and, in some instances, relocate aquatic resources outside of the project area.

There are two steps to this planning process. First, an applicant develops a written Aquatic Resource Relocation Plan (ARRP) to control and limit the impacts of dewatering, maintenance, or construction related projects on aquatic resources and submits it to the appropriate TPWD representative. The plan should be submitted no less than four weeks prior to beginning the project. The applicant must receive formal approval of the ARRP by the department prior to initiating dewatering, maintenance, or construction related activities. See Attachment 1 below for the specific information necessary for the ARRP. The TPWD point of contact for the project location can be found in Attachment 5.

If state or federal threatened or endangered freshwater mussel species are present or likely to be present in the project area, a mussel survey may be necessary prior to department approval of the ARRP. Surveyors must obtain one of two authorizations from the department to handle threatened and endangered species: 1) a Relocation Letter of Authorization, or 2) a TPWD Scientific Research Permit (i.e., "collection permit"). Both the Relocation Letter of Authorization and the Scientific Research Permit are issued in the name(s) of the person(s) conducting the surveys, and may not be issued to organizations or other entities. Additional information on freshwater mussel surveys may be found in Attachment 4.

Second, an applicant must complete an "Application for Permit to Introduce Fish, Shellfish, or Aquatic Plants into Public Waters." Because the application is to be received 30 days prior to the

activity, it is suggested that both the ARRP and this permit application be submitted at the same time. The application can be obtained at the following web link:

[http://tpwd.texas.gov/publications/pwdforms/media/pwd\\_1019\\_t3200\\_app\\_permit\\_stock\\_public\\_waters.pdf](http://tpwd.texas.gov/publications/pwdforms/media/pwd_1019_t3200_app_permit_stock_public_waters.pdf)

There is no application fee for the ARRP or introduction permit. Once the department has issued the introduction permit, please have a copy available at the project site in case the local game warden or other department staff requests to see it. A department representative may be present during some or all of the proposed activity.

Please do not hesitate to contact your TPWD point of contact if you have any questions or require additional assistance.

Attachment 1:

Aquatic Resource Relocation Plan (ARRP). The ARRP should include the following information to be considered complete.

- 1) A description of the project and associated aquatic/instream activities with sufficient detail for department staff to evaluate the risk to aquatic resources.
- 2) A computer-generated map showing the project location and the relocation site, including the county, GPS coordinates, and the Texas Commission on Environmental Quality (TCEQ) water body segment number. For mussel relocations, provide the USFW stream group classification found in the Google Earth KML file on the USFW website at: [fws.gov/library/collections/texas-freshwater-mussel-sampling-protocol](https://fws.gov/library/collections/texas-freshwater-mussel-sampling-protocol)
- 3) Expected start dates of the project and the aquatic resource relocation. An applicant must submit any changes to the start date of aquatic resource relocation activities at least 72 hours prior to the revised start date.
- 4) Identify any state or federally threatened or endangered species that may occur. Explain what methods will be used to protect these species. If the project area contains any state or federally listed freshwater mussels, a mussel survey may be necessary prior to approval of the ARRP (see Attachment 4 for Freshwater Mussel Survey Protocols). The following web link may help in identifying the location of these species by county: <http://www.tpwd.texas.gov/gis/rtest/>
- 5) List all shellfish that may become stranded due to the operation. Explain what methods will be used to protect these shellfish including freshwater mussels (See Attachment 2 for Freshwater Fish and Shellfish Handling Protocols).
- 6) Identify List all known exotic and invasive species in the project area. Describe decontamination procedures for preventing the spread of exotic and invasive species. See link below for more information: <http://tpwd.texas.gov/huntwild/wild/species/exotic/>
- 7) Methods of collecting and relocating aquatic resources, including the types and sizes of containers used, the mode of transportation, and best management practices (BMPs) to protect aquatic resources. Provide an estimate of the time expected to complete the collection and relocation (See Attachment 2 for Freshwater Fish and Shellfish Handling Protocols). For freshwater mussel relocations, please describe the methods for surveys and relocations found in Attachment 4 and based on the waterbody's stream group classification. If the methods described in Attachment 4 are not possible for the project, explain why and describe an alternative method.
- 8) Describe how the receiving waters will be protective of aquatic life (i.e., sufficient dissolved oxygen levels, water body size and flow, and similar habitat as the source water).

- 9) Describe how dead fish and shellfish, as well as exotic and invasive species, will be disposed of and documented. Documentation should include no less than the species and number of individuals found dead, or disposed of, including the lengths (inches) of all fish for both native and non-native species. Please submit a final report after the completion of the relocation using the Excel Spreadsheet template that is issued with the introduction permit. For freshwater mussel relocations, submit a report that has all of the required information described in Attachment 4.
  
- 10) Identify best management practices (BMPs) to be used to prevent or minimize the risk of transporting any species, including aquatic invasive species (AIS) to new locations on equipment, boats, trailers, and vehicles. These BMPs should also ensure compliance with regulations that prohibit the possession and transport of certain AIS species. For more information see Attachment 3.



## Fish and Shellfish Handling Protocols

### Introduction

A key element in the survival of aquatic life such as fish and shellfish (oysters and freshwater mussels) which are caught and released is how they are handled during the process. Physiologically, these organisms experience many stressors during a catch and release and transport. By minimizing the amount of stress, the chance of survival after release improves greatly. During a catch and release event, fish, as well as shellfish, can experience a combination of many stress factors. Below is a list of some types of stressors that aquatic life can experience during catch and release.

- behavior stress – crowding
- handling stress – capture, struggle, confinement
- exercise stress – prolonged swimming, being chased
- temperature stress – change in temperature
- salinity stress – change in salinity
- hypoxial stress – removal from the water/low oxygen
- toxicity stress – exposure to ammonia

The primary response of stress is the releasing of hormones into the blood causing a disturbance to the physical state of the fish. The secondary stress responses are disturbances to osmoregulation, blood chemistry, metabolism, and immune system. These effects can reduce the fish's resistance to fungal and bacterial infections that lead to mortality in some cases.

In order to reduce these effects, proper care and procedures should be taken when catching and releasing aquatic life. In order to minimize these stressors, follow the recommendations listed below.

### Handling, Maintaining, and Transporting Aquatic Life

#### Fish

- Catch the fish fast and efficiently. As the fish resists capture, its oxygen demand increases. The fish will need oxygen to recuperate after the capture. Therefore, keep the water in the transport basin well aerated.

- If a landing net is used, rubber netting works best for minimizing mucous loss. Cloth and nylon type dip nets disrupt the protective mucous coating, disturb scales, and increase the possibility of injury or secondary infection that usually results in fish mortality.
- Help keep the protective mucous coat and scales of the fish from rubbing off by using wet hands when handling fish.
- Keep handling of the fish to a minimum. If at all possible, do not grab fish with hands. Instead, go directly to the transport basin. Avoid excess handling and/or dropping of the fish on the ground and the floor of the boat.
- Help keep the protective mucous coat and scales of the fish from rubbing off by using wet hands when handling fish. NovAqua ® or StressCoat ® can be added to the water in holding tanks to help mitigate the abrasive damage of capture and handling to the external mucous coating.
- Keep the fish in the water as much as possible to reduce stress. As a rule, keep the fish out of water no longer than you can hold your breath. Fish can suffer from brain damage from prolonged loss of oxygen.
- Water temperatures above 84° Fahrenheit tend to be stressful for warm water fish. Therefore, adding ice to the transport basin can minimize stress.
- Avoid overcrowding fish in the transport basin. A good rule of thumb to use would be to place no more than 5 fish in the 15"-20" range for a 120 quart cooler equipped with some type of an aeration system. Plan on 25% water exchange every 20-30 minutes. About 7.5 gallons (1.5 buckets if using a five gallon bucket). Use common sense, the more fish (>5) and the longer they sit in the transport basin, the more frequent water exchanges need to occur.
- Livewells or other holding tanks should be fitted with a water recirculation system. Oxygen cylinders are expensive, but provide the best aeration while maintaining water temperature.
- Run the aeration system continuously! Transport basins should be filled with ambient water to aid in acclimating the fish to the transport conditions.

When transporting saltwater fish, it is important to keep the transport water as close to the same salinity and temperature as the water from which the fish were collected. If possible, it is preferable to lower the water temperature a couple of degrees to reduce stress during the transport procedure. The oxygen concentration in the water should be between 5.0 - 7.0 mg/L. Water with oxygen levels lower than 4.0 mg/L can cause stress and eventually lead to a fish kill. The pH of the water should range between 8.0-8.3 for saltwater fish and 6.5-8.0 for freshwater fish.

## **Shellfish (freshwater mussels)**

- Handling protocols for freshwater mussels can be found in Attachment 4 (Texas Freshwater Mussel Survey Protocols), on page 7 under "Mussel Handling and Processing".



## Aquatic Surveys, Introductions, and Relocations: Best Management Practices to Prevent or Minimize Aquatic Invasive Species (AIS) Transfer

### Introduction

All permitted aquatic surveys, aquatic species introductions, and aquatic resource relocations are required to comply with regulations regarding possession, transport, and introduction of controlled exotic species into public waters. These regulations apply even to small fragments or seeds of these species, regardless of whether transfer is intentional or accidental. Implementation of Best Management Practices (BMPs) is necessary to prevent or minimize the risk of accidentally possessing or transferring controlled exotic species or pathogens.

Aquatic invasive species (AIS) cause or are likely to cause harm to our native ecosystems, both directly by competition or predation on native species and indirectly by altering the environment (e.g., reducing dissolved oxygen, shading). Many AIS cause significant economic harm in a number of ways—by damaging water transfer and hydroelectric infrastructure and increasing maintenance costs, clogging waterways and costing millions of dollars each year to manage, increasing evaporative water loss from reservoirs, and even lowering property values. These AIS can also impact human health and quality of life by helping to cause harmful algal blooms, impeding boater access, fouling beaches, and creating hazards for swimmers.

Because of these potential impacts, the legislature delegated to the Texas Parks and Wildlife Commission [TPW 66.007 & 66.0072] the authority to develop a list of Exotic Harmful or Potentially Harmful Fish, Shellfish, and Aquatic Plants that may not be possessed, transported, or introduced into public waters except as authorized by rule or permit issued by the department [31 TAC§57.112]. A complete list of these species, hereafter referred to as controlled AIS, can be found on the department website at

[http://tpwd.texas.gov/huntwild/wild/species/exotic/prohibited\\_aquatic.phtml](http://tpwd.texas.gov/huntwild/wild/species/exotic/prohibited_aquatic.phtml). Possession or transfer of controlled AIS—live or dead—or the eggs, seeds, or fragments thereof, is punishable as a Class C Misdemeanor (with a fine up to \$500); repeat violations can be elevated.

Examples of controlled AIS include zebra mussels (*Dreissena polymorpha*) and their microscopic larvae, tilapias (*Oreochromis* spp.), hydrilla (*Hydrilla verticillata*), giant and common salvinia (*Salvinia molesta*, *S. minima*), and Eurasian watermilfoil (*Myriophyllum spicatum*). Some controlled AIS are fairly widespread in Texas, but their prevalence increases, rather than negates, the risk of accidental transfers that could cause infestations in new areas. More information about most of the controlled AIS that have been found in Texas, including maps of where they have been

#### Abbreviations:

*AIS*: Aquatic Invasive Species

*TAC* –Texas Administrative Code; numbers before refer to title and numbers after to specific regulations

*TPW* –Texas Parks and Wildlife Code; numbers after TPW refer to specific statutes



found, is available on [www.TexasInvasives.org](http://www.TexasInvasives.org) and a few especially problematic species are described below.

It is your responsibility to ensure that you and your team are not possessing, transporting, or introducing controlled AIS. However, it is not always necessary for you to know how to identify each species. By implementing a few general BMPs, you can achieve a high degree of confidence that you aren't accidentally doing so. In addition, implementing these BMPs will help to prevent transfer of non-prohibited, yet potentially harmful, AIS as well as harmful algae or pathogens that could negatively impact native species.

## General BMPs

- DURING surveys, introductions, and relocations, do not transfer water from one site to another unless specifically approved by the department; minimize water transfer whenever possible, using nets to transfer fish. For questions about treating hauling water, see the section on fish hauling units below.
- AFTER work in the water is complete:
  - **CLEAN:** Remove mud, plant fragments, and other debris from all equipment before leaving the site—this includes nets, mesh bags, buckets, boot tread, waders, snorkel/SCUBA gear, boats, trailers, vehicles, and ANY other equipment used in or adjacent to the water. Before leaving the site, you should also rinse equipment that may harbor plant fragments (e.g., boot tread)—a gallon jug of water and a scrub brush or scraper can help to get things clean. If a carwash is available, the high pressure spray can help to clean boats, trailers, vehicles, and equipment. Otherwise, you should use a spray nozzle and water hose to finish cleaning equipment before use in another water body.
  - **DRAIN:** Drain all water from boats, fish hauling units, buckets, or other receptacles at a location where the water will not drain into any water body.
  - Soaking equipment with 10% bleach solution (i.e., 1 part household bleach to 9 parts water) for 10 minutes followed by a thorough rinse before drying can help to prevent transfer of zebra mussel larvae, golden algae, and fish pathogens such as viruses and should neutralize any hidden snails or plant fragments. Milder disinfectants (e.g., 1% Virkon Aquatic® for 10 minutes) or a 20-30 minute soak in very hot tap water (at least 110°F) can help decontaminate nets or equipment that bleach could damage.
  - **DRY:** Allow all equipment to dry completely before use in another water body.

## **Special Rules and Recommendations**

### ***Boats***

Regulations require that all water be drained from vessels traveling to and from any public water body, except for travel between access points on the same water body within the same day [31 TAC §57.1001]. Texas law also specifically requires that all controlled aquatic plants be immediately removed from boats, trailers, and vehicles used to transport or launch them, and disposed lawfully [TPW 66.0071].

### ***Vehicles***

Vehicles used to launch boats or driven in the water or in mud adjacent to the water can easily harbor and transport AIS. It is especially important to check them thoroughly, remove all vegetation, rinse well with a spray nozzle, and allow them to dry completely before you visit another water body. Check the wheels, axle, bumper, and undercarriage carefully and be sure to rinse well everything well. Texas law specifically requires that all controlled aquatic plants be immediately removed from vehicles used to transport or launch boats and disposed lawfully.

### ***Fish Hauling Units***

For specific recommendations for decontaminating fish hauling units and treating hauling water to prevent transferring controlled AIS, golden alga, or fish pathogens, please see “A Biosecurity Manual for Inland Fisheries Division Hatcheries,” online at:

[http://tpwd.texas.gov/publications/pwdpubs/media/pwd\\_rp\\_t3200\\_1776.pdf](http://tpwd.texas.gov/publications/pwdpubs/media/pwd_rp_t3200_1776.pdf)

### ***Zebra Mussel Infested Water Bodies***

A current map and list of infested lakes can be found on the department website at: <http://tpwd.texas.gov/huntwild/wild/species/exotic/zebramusselmap.phtml>. Zebra mussels are spread via both transfer of adults and microscopic larvae in water. When working at a site on a water body where zebra mussels or their larvae have been found, it is critical to ensure that no water is transferred and all equipment is allowed to dry thoroughly. You will also need to be very thorough in checking equipment for mud or debris that could harbor dislodged adults. For these projects, your methods should specify where decontamination will take place and identify and address any special equipment that could transfer zebra mussel larvae (e.g., bladder dam) and how it will be cleaned. **If zebra mussels are found at the site, you must report the finding to TPWD immediately by calling Monica McGarrity (512-552-3465), Brian Van Zee (254-495-8341), or your department contact. Native mussels with zebra mussels attached should never be relocated to another water body; if zebra mussels are attached, consult the department before proceeding.**

## ***Non-native Species and Aquatic Resource Relocations***

The aquatic resource relocation plan (ARRP) should stipulate that non-native species will not be relocated or specifically describe which species will (or will not be) relocated. In most cases, the department will not issue a permit for relocation of non-native species (i.e., not native at the watershed or sub-watershed level), regardless of whether or not they are designated as controlled AIS by TPWD regulations, because permitting their introduction would be inconsistent with department management goals. For example, suckermouth catfishes (genera *Hypostomus* and *Pterygoplichthys*) are highly invasive and their relocation will not be permitted, even though they are not controlled AIS. Asian Clams (*Corbicula fluminea*) are not native to Texas and should not be relocated. Rio Grande Cichlids (*Herichthys cyanoguttatum*) are native only to the lower Rio Grande drainage in Texas but may be found in other water bodies and can impact some native species; although they should not typically be relocated outside their native range, in some cases it may be permitted (e.g., park ponds or reservoirs). In some cases, relocation of Common Carp (*Cyprinus carpio*) may be permitted at the discretion of the Inland Fisheries district supervisor.

**Controlled fish AIS, such as tilapia, that are removed from a water body cannot be relocated and also must be promptly beheaded or gutted prior to disposal or transport for disposal.** A complete list of controlled AIS can be found on the department website at: [http://tpwd.texas.gov/huntwild/wild/species/exotic/prohibited\\_aquatic.phtml](http://tpwd.texas.gov/huntwild/wild/species/exotic/prohibited_aquatic.phtml)

Grass Carp (*Ctenopharyngodon idella*) is a controlled AIS for which relocation could be approved, but only if the relocation site is in the same water body. If triploid (sterile) Grass Carp were stocked for nuisance aquatic vegetation control, they must be relocated within the same water body unless otherwise approved by the department. The department website provides a current list of all public water bodies where triploid grass carp have been stocked:

[http://www.tpwd.state.tx.us/landwater/water/enviroconcerns/nuisance\\_plants/public\\_tgc\\_permits.phtml](http://www.tpwd.state.tx.us/landwater/water/enviroconcerns/nuisance_plants/public_tgc_permits.phtml)

If grass carp are encountered in other water bodies not on this list, they must be beheaded or gutted and disposed unless otherwise directed by the Inland Fisheries district supervisor.

### ***Disposal of Fish (Non-native or Native)***

Dead animals, including fish, are classified as municipal solid waste [30 TAC §330.3]. Although they are considered special waste [30 TAC §330.171], no special authorization is required for disposal at any Type I or Type IAE landfill. For government roadway maintenance projects by TxDOT or county or municipal agencies, fish may be disposed by burial on the highway right away as long as the disposal does not cause a nuisance or endanger public health or the environment and the carcasses are covered with at least two feet of soil [30 TAC §330.13; TCEQ communication]. Other individuals or entities should dispose of fish in a landfill.

## Some Controlled AIS to Know – Easily Transported by Accident

### ***Giant Salvinia (Salvinia molesta)***

[http://www.texasinvasives.org/plant\\_database/detail.php?symbol=SAMO5](http://www.texasinvasives.org/plant_database/detail.php?symbol=SAMO5)

### ***Common Salvinia (S. minima)***

[http://www.texasinvasives.org/plant\\_database/detail.php?symbol=SAMI7](http://www.texasinvasives.org/plant_database/detail.php?symbol=SAMI7)

### ***Hydrilla (Hydrilla verticillata)***

[http://www.texasinvasives.org/plant\\_database/detail.php?symbol=HYVE3](http://www.texasinvasives.org/plant_database/detail.php?symbol=HYVE3)

<http://aquaplant.tamu.edu/plant-identification/alphabetical-index/hydrilla/>

<http://plants.ifas.ufl.edu/node/183>

### ***Eurasian Watermilfoil (Myriophyllum spicatum)***

[http://www.texasinvasives.org/plant\\_database/detail.php?symbol=MYSP2](http://www.texasinvasives.org/plant_database/detail.php?symbol=MYSP2)

<http://aquaplant.tamu.edu/plant-identification/alphabetical-index/eurasian-watermilfoil/>

<http://plants.ifas.ufl.edu/node/278>

### ***Alligatorweed (Alternanthera philoxeroides)***

[http://www.texasinvasives.org/plant\\_database/detail.php?symbol=ALPH](http://www.texasinvasives.org/plant_database/detail.php?symbol=ALPH)

<http://aquaplant.tamu.edu/plant-identification/alphabetical-index/alligator-weed/>

### ***Torpedograss (Panicum repens)***

<http://aquaplant.tamu.edu/plant-identification/alphabetical-index/torpedograss/>

<http://plants.ifas.ufl.edu/node/308>

### ***Zebra mussels (Dreissena polymorpha)***

[http://texasinvasives.org/animal\\_database/detail.php?symbol=10](http://texasinvasives.org/animal_database/detail.php?symbol=10)

### ***Island applesnail (Pomacea insularum)***

[http://texasinvasives.org/animal\\_database/detail.php?symbol=15](http://texasinvasives.org/animal_database/detail.php?symbol=15)

A complete list of controlled AIS can be found on the department website at:

[http://tpwd.texas.gov/huntwild/wild/species/exotic/prohibited\\_aquatic.phtml](http://tpwd.texas.gov/huntwild/wild/species/exotic/prohibited_aquatic.phtml)

To learn more about other AIS, how to identify them, and where they've been found in Texas, visit: [http://www.texasinvasives.org/invasives\\_database/](http://www.texasinvasives.org/invasives_database/)



# Texas Parks and Wildlife Department Kills and Spills Team

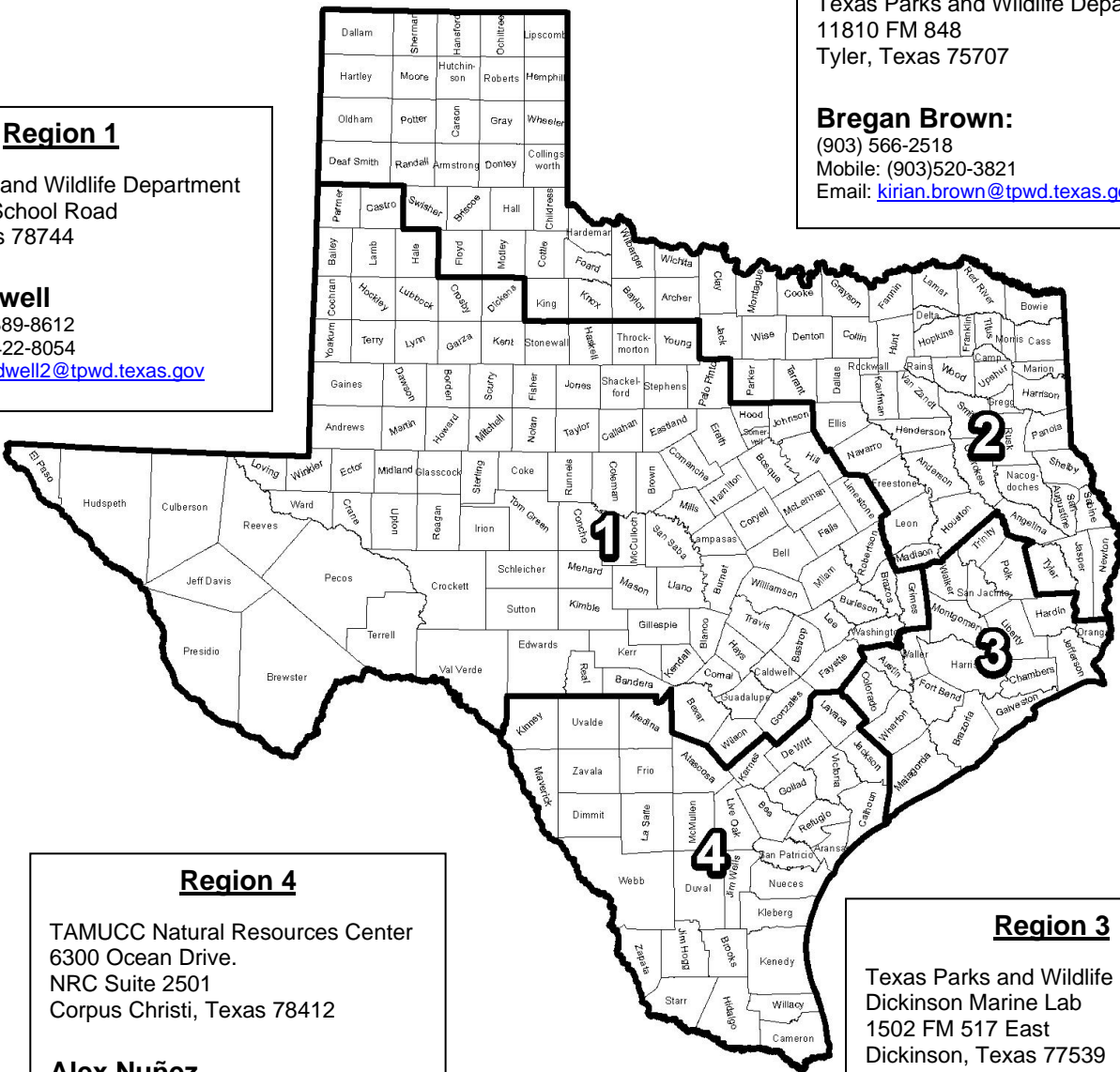


If you see dead or dying fish and wildlife, or pollution threatening fish and wildlife, please call our 24-hour Communications Center below immediately. Or call your local Game Warden.

**24-Hour Communications Center**  
(512) 389-4848  
4200 Smith School Road  
Austin, Texas 78744

**Region 1**  
Texas Parks and Wildlife Department  
4200 Smith School Road  
Austin, Texas 78744  
  
**Travis Tidwell**  
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# Texas Freshwater Mussel Survey Protocol

Prepared by

United States Fish and Wildlife Service  
Texas Ecological Services Field Offices

and

Texas Parks and Wildlife Department



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## Table of Contents

Introduction.....	1
Stream Groupings and Site Delineation.....	1
Stream Groupings.....	2
Reservoirs.....	2
Impoundment Spillways.....	3
Project Survey Areas and Salvage Zone .....	3
Survey Qualifications and Required Permits.....	4
Surveyor Qualifications.....	4
Permits and Approvals .....	5
Aquatic Resource Relocation Plan (ARRP).....	6
Survey Condition Requirements.....	6
Mussel Survey Period and Time of Year Restrictions .....	6
Visibility.....	6
Substrate .....	7
Workable Flow .....	7
Documenting Safety Concerns.....	7
Mussel Handling and Processing .....	7
Survey Methodologies .....	8
Desktop and/or Land-based Assessment.....	8
Phase 1 Survey Types .....	9
Cells.....	9
Transects.....	9
Qualitative Timed Searches.....	9
Rio Grande Basin Requirements .....	10
Phase 2 Survey Methods .....	10
Quantitative Sampling.....	10
Cells.....	11
Transects .....	11
Survey and Coordination Decision Making.....	11
Stream Group-Specific Survey Requirements .....	13
Group 1 Streams.....	13
Group 2 Streams.....	13



Group 3 Streams.....	14
Group 4 Streams.....	14
Group 5 Streams.....	14
Relocation Methods .....	15
Reporting Requirements .....	16
Data Validity Period .....	17

**Appendix A:** Freshwater Mussel Recommended Survey Area and Salvage Zone Summary  
Tables

**Appendix B:** Recognized Freshwater Mussel Species List

**Appendix C:** Diverse Freshwater Mussel Assemblage Exceptions

**Appendix D:** Freshwater Mussel Reconnaissance Survey Form

**Appendix E:** Freshwater Mussel Survey Report Checklist

**Appendix F:** Texas Parks and Wildlife Department and U.S. Fish and Wildlife Service Contacts

**Appendix G:** Survey Examples

**Appendix H:** Non-invasive Genetic Sample Collection Methods

## **Table of Acronyms**

ADI – Area of Direct Impact

ARRP – Aquatic Resource Relocation Plan

B-to-B – Bank to Bank

DSB – Downstream Buffer

ESA – Endangered Species Act

LB – Lateral Buffer

MZ – Mixing Zone

Protocol – Texas Freshwater Mussel Survey Protocol

Service – United States Fish and Wildlife Service

TPWD – Texas Parks and Wildlife Department

USB – Upstream Buffer

USGS – U.S. Geological Survey

## Introduction

The Texas Freshwater Mussel Survey Protocol (Protocol) is designed by the Texas Parks and Wildlife Department (TPWD) and the U.S. Fish and Wildlife Service (Service) to determine the presence or probable absence of freshwater mussels inside the footprint of or within the immediate vicinity of proposed construction projects with instream impacts. The methods and requirements described herein were developed to provide standardized procedures for a variety of frequently encountered projects and were intended to be applicable to all streams and impoundments that may be occupied by freshwater mussels in Texas. For projects or stream segments that are not adequately described or covered by the Protocol, additional coordination with TPWD and/or the Service may be necessary to determine project-specific methods and/or requirements. The goals of this document is to provide an efficient and standardized approach for conducting surveys, minimize the impacts of projects, and protect native mussel populations that inhabit waterbodies across Texas. By implementing standardized protocols, TPWD and the Service believe that these goals are achieved by increasing project review efficiency by decreasing the frequency of developing project-specific sampling methodologies, minimizing project impacts by ensuring that proposed sampling methods are adequate to identify and avoid at-risk freshwater mussels within the vicinity of proposed projects, and protecting native mussel populations by reducing unintentional take of at-risk mussels resulting from inadequate mussel survey scope or coverage.

These Protocols are adapted from several previously published survey methods, including the “Ohio Mussel Survey Protocol” prepared by the Ohio Department of Natural Resources, “West Virginia Mussel Survey Protocols” prepared by the West Virginia Division of Natural Resources, “Virginia Freshwater Mussel Guidelines” prepared by the Virginia Department of Game and Inland Fisheries, and “Freshwater Mussel Survey Protocol for the Southeastern Atlantic Slope and Northeast Gulf Drainages in Florida and Georgia” prepared by the Service and Georgia Department of Transportation.

## Stream Groupings and Site Delineation

Recommended survey methods will be determined by Stream Grouping and the stream dimensions at the proposed project location. Stream Groups have been defined based upon known or probable species occurrence within a stream segment, stream size, and dominant substrate type. These determinations were made using a combination of the TPWD Texas Natural Diversity Database (TXNDD), Mussels of Texas Database (MoTX), U.S. Fish and Wildlife Service Environmental Conservation Online System (ECOS), and best professional judgement of Service and TPWD staff. The Stream Group classification of individual streams will be updated on an annual basis as additional species distribution data become available. These updates could include upgrading or downgrading stream groups due to change in regulatory status of federally- or state-listed species, upgrading in stream grouping due to observation of a previously unknown population of federally- or state-listed species, downgrading of stream grouping due to consensus among regulatory agencies that a federally- or state-listed species is unlikely to currently occupy a historically occupied stream, or the removal of a stream from the stream groupings list due to sufficient evidence that no freshwater mussels occupy a stream reach. The most current Stream Group information can be found in .kml and .shp format here:

The provided .kml and .shp files are to serve as guides and may not include all perennial streams in the state.

### Stream Groupings

- Group 1 – Small/medium stream reaches that include designated or proposed Critical Habitat for federally-listed or federally-proposed mussel species, or reaches known to or may be inhabited by federally-listed species.
- Group 2 – Large stream reaches that include designated or proposed Critical Habitat for federally-listed or federally-proposed mussel species, or reaches known to or may be inhabited by federally-listed species.
- Group 3 – Small/medium stream reaches that are known to, or may be inhabited by state-listed freshwater mussel species, but presence of federally-listed freshwater mussel species is not anticipated.
- Group 4 – Large stream reaches that are known to or may be inhabited by state-listed freshwater mussel species, but presence of federally-listed freshwater mussel species is not anticipated.
- Group 5 – Streams where no federally- or state-listed freshwater mussels occur, but mussels are known to occur; or, perennial streams where it is anticipated that live freshwater mussels may occur, but presence or diversity have not been confirmed.

### Reservoirs

Reservoirs will be included using the above referenced stream groupings as appropriate, based upon expected freshwater mussel occupancy and diversity. If the reservoir in which the project is proposed to be completed maintains riverine flow (i.e. run of the river impoundments, such as Lady Bird Lake in Austin, TX) the upstream, downstream, and lateral buffers prescribed in [Appendix A](#) should be followed for the project. If the reservoir in which the project is proposed to be completed does not maintain riverine flow and is more lentic (e.g., large, lake-like impoundments such as the main body of Lake Livingston) the lateral buffers prescribed in [Appendix A](#) for the proposed Project Type and Stream Group shall be used on all sides of the project's proposed area of direct impact.

For large-scale reservoir de-watering projects, project proponents are requested to coordinate individually on a project-by-project basis with TPWD and/or the Service to determine project-specific sampling and relocation methods and requirements.

## Reservoir Spillways

Due to the potentially unsafe conditions that can exist in reservoir spillways a mussel survey may not be recommended prior to instream construction activities in these areas. In instances where it is deemed unsafe to sample, the surveyor may skip a full Phase 1 survey and reduce the survey to the footprint of the proposed cofferdam. Mussels observed within the proposed footprint and those within dewatered areas following the construction of cofferdam will be salvaged and relocated. The suitability of a site for surveying will be discussed with TPWD and/or the Service prior to initiating any instream work.

## Project Survey Areas and Salvage Zone

Four distinct survey areas can be defined for each project. These include the area of direct impact (ADI), upstream buffer (USB), lateral buffer (LB), and downstream buffer (DSB).

Where the proposed project footprint spans the entire wetted width of the stream, no LB project survey areas will be included in the survey.

Where the proposed project footprint does not span the entire wetted width of the stream, the survey widths of the USB and the DSB shall be equal to the width of the ADI and any associated LB applied to the ADI.

The size of the recommended survey buffers will correspond to those prescribed by the project type and stream grouping that are included in Table 1 in [Appendix A](#).

The Salvage Zone is a predetermined area from which live mussels must be relocated prior to initiating instream construction for a project. A project's Salvage Zone will consist of the proposed ADI as well as prescribed USB, LB, and DSBs described in Appendix A, Table 2. Like the prescribed survey buffers, the Salvage Zone dimensions will vary depending upon the project type and stream group. Additionally, the boundaries of the Salvage Zone will be dependent upon the dominant substrate type observed during the survey. It is anticipated that streams with fine, loose substrates (Habitat A) are more likely to be mobilized and at risk of scour and sediment deposition due to temporary, localized changes in hydrology caused by the placement of a temporary instream structure (e.g., cofferdam, causeway, flume, etc.), therefore the extent of the recommended Salvage Zone boundaries for sites with this habitat will be greater than those of streams with larger, substrate particle compositions that typically require greater changes in stream velocity and/or sheer stress in order to mobilize substrate particles (Habitat B).

Habitat A – predominant substrate types within the survey area are homogenous, consisting of sand, silt, small gravel, and/or bedrock sheets.

Habitat B – predominant substrate types within the survey area are heterogenous mixtures of sand, silt, gravel, cobble, large rock, boulder, bedrock, and/or clay.

Based upon the habitat observed and documented located within the ADI during the Phase 1 Survey described below, the permitted mussel surveyor on-site will make a determination on the

generalized substrate composition and stability of the site (greater than 50% of Habitat A vs Habitat B or vice versa located within the ADI) and, if necessary, will apply the appropriate Salvage Zone buffers to any relocation activities at the site. Prescribed Salvage Zone buffers for common project types for Habitat A and Habitat B substrates are included in Table 2 in [Appendix A](#).

Example site diagrams demonstrating various potential survey area types and associated Salvage Zones are included in [Appendix G](#).

## Surveyor Qualifications and Required Permits

### Surveyor Qualifications

Personnel who will be conducting in-stream field surveys must have adequate knowledge of the instream habitats and fauna within the basin they propose to survey. This includes species-specific biology and ecological requirements, and the demonstrated ability to identify freshwater mussel species from the basin. This requirement may be met by successful completion and passing of a freshwater mussel identification test provided by TPWD and/or the Service. A mussel surveyor must have adequate technical ability and experience leading surveys utilizing sampling methods and protocols comparable to those presented in the Protocol. These skills include the ability to independently execute the proposed survey methods, locate, identify, and handle state-listed, federally-listed, Federal candidate, and/or federally-petitioned freshwater mussel species, and record and present survey data in an appropriate format. It will be incumbent upon the mussel surveyor to complete the activities in a safe manner. Individuals familiar with freshwater mussels, but not with state-listed, federally-listed, Federal candidate, and/or federally-petitioned species found within Texas, must work with a permitted mussel surveyor who has experience with these species, and cannot complete the requested survey without a permitted mussel surveyor on site. The permits required to complete the surveys outlined in this Protocol are described below.

Additional requirements and supporting documentation demonstrating surveyor ability are required by TPWD and/or the Service species leads when reviewing scientific collection permit applications. These additional requirements may include, but are not limited to:

- Documentation of field-time and/or a letter of recommendation regarding the surveyor's in-basin experience.
- Demonstrated knowledge in surveying, handling, and identifying freshwater mussels (including state- and federally-listed threatened, endangered, candidate, and/or petitioned species).

In some instances, personnel may not have to collect detailed knowledge of the instream habitats and fauna within the basin when completing a land-based assessment. These instances could include instances in which non-perennial streams have been designated as requiring freshwater mussel surveys or stream reaches that clearly include water quality factors that would prevent occupation by freshwater mussels.

## Permits and Approvals

Permission for stream access on private property must be granted by the appropriate landowners prior to sampling, TPWD and/or Service permits **do not authorize trespassing** on private property. Obtaining access to TPWD or federally-owned lands may require additional authorizations and the surveyor will coordinate with TPWD and/or the Service as appropriate.

- Groups 1 and 2 Streams

For Group 1 and Group 2 streams, the lead surveyor must obtain a Section 10(a)(1)(A) recovery permit from the Service prior to surveying. Section 10(a)(1)(A) permit application procedures and guidance can be found at:

<https://www.fws.gov/forms/3-200-59.pdf>

Under the ESA, a section 10(a)(1)(A) permit allows the permittee to handle federally-threatened and/or endangered species for scientific purposes.

Contact Information: U.S. Fish and Wildlife Service, Endangered Species Permit Office, 500 Gold Avenue S.W. P.O. Box 1306 Albuquerque, New Mexico 87103-1306. Questions regarding permits or the application process can be sent to [permitsR2ES@fws.gov](mailto:permitsR2ES@fws.gov).

In addition to holding a current Service Section 10(a)(1)(A) recovery permit, the surveyor must currently hold a Wildlife Diversity Permit: Scientific Permit for Research (SPR) obtained from the Texas Parks and Wildlife Department before conducting surveys. Texas Parks and Wildlife Department Scientific collection permit information can be found at:

<https://tpwd.texas.gov/business/permits/land/wildlife/research/>

Contact Information: Texas Parks and Wildlife Department, Wildlife Diversity Permits Section, 4200 Smith School Road, Austin TX 78744. Questions regarding permits or the application process can be sent to [wpoffice@tpwd.texas.gov](mailto:wpoffice@tpwd.texas.gov).

- Group 3, 4, and 5 Streams

Only a TPWD issued SPR is required to complete instream surveys for Group 3, 4, and 5 streams. No Section 10(a)(1)(A) recovery permit from the Service would be required to complete surveys these streams, unless a previously undocumented population of federally-listed species is observed during a survey. If this event occurs, all work must immediately cease, and the Service must be contacted. Then an approved Section 10(a)(1)(A) permit would be required to complete the survey, or the surveys must be completed by a permitted 10(a)(1)(A) mussel surveyor.

## Aquatic Resource Relocation Plan (ARRP)

Prior to initiating any freshwater mussel survey, regardless of stream group, an applicant must develop a written Aquatic Resource Relocation Plan (ARRP) to control and limit the impacts of dewatering, maintenance, or construction related impacts on aquatic resources and submit it to the appropriate TPWD representative. The plan should be submitted no less than four weeks prior to beginning the project. The applicant must receive approval of the ARRP and issuance of a stocking permit by TPWD prior to initiating dewatering, maintenance, or construction related activities. For additional information regarding the specific requirements of preparing an ARRP and identifying the TPWD point of contact for the project location, review the ARRP guidance at:

[https://tpwd.texas.gov/publications/pwdpubs/media/pwd\\_if\\_t3200\\_1958\\_arrp\\_guidelines\\_packet.pdf](https://tpwd.texas.gov/publications/pwdpubs/media/pwd_if_t3200_1958_arrp_guidelines_packet.pdf)

For Group 1 and Group 2 streams, the Service requests to receive a concurrent submission of the completed ARRP for review. Following a review to ensure that appropriate sampling methods and surveyor qualifications are proposed for the project, the Service will provide either written concurrence with TPWD's approval of the survey, via email, or a request to discuss and/or edit portions of the proposed methodology that appear to be deficient for project needs. The Service does not request to review ARRPs for Group 3, 4 or 5 streams, but will gladly provide technical guidance for surveys in these streams at the request of the ARRP applicant.

## Survey Condition Requirements

### Mussel Survey Period and Time of Year Restrictions

Mussel sampling is to be conducted during the months of April through November, or when water temperatures are greater than or equal to 50°F (10°C) in order to maximize probability of detection prior to mussels retreating into the streambed for winter months. When sampling outside of the date window, but adhering to the minimum temperature threshold, documentation of stream conditions at the time of survey should be included in the submitted survey report. Sampling outside of these approved temperature and calendar conditions will require prior authorization from TPWD and/or the Service as appropriate based upon the grouping of the stream in question.

### Visibility

Surveys where visibility meets or exceeds one-half meter (50 cm, or approx. 20 in), with or without lights at depth of survey, may have amended sampling effort requirements. If suitable visibility is present at the intended time of the survey, survey effort may be reduced from 0.5 min/m<sup>2</sup> or 0.75 min/m<sup>2</sup> to 0.2 min/m<sup>2</sup> if no mussels are observed within a search cell or transect segment. If mussels are observed with a cell or transect segment, the standard 0.5 min/m<sup>2</sup> or 0.75 min/m<sup>2</sup> sampling effort will be requested for that cell or segment. In order to apply the reduced sampling effort exception, documentation of the improved visibility must be photo-documented (i.e., secchi disc, depth rod, etc.) and presented within the survey report. If previously waived visibility requirements deteriorate during completion of the survey, the standard sampling effort



will be recommended for areas searched following the visibility reduction. If the reduced sampling effort is applied and visibility is not formally documented, the survey results may be considered invalid and the survey will need to be repeated.

### Substrate

Search efficiency when conducting qualitative tactile surveys varies with substrate types. Softer, less consolidated substrate types, such as sand and silt, are more efficiently surveyed using tactile searches than are more consolidated substrates, such as gravel and cobble. Given these differences, additional search effort may be necessary for certain substrate types. Stream groups within type A consist of sites with homogenous, finer substrates, small gravel and/or bedrock sheets in which the standard survey effort of 0.5 min/m<sup>2</sup> applies (unless visibility requirements are met). Stream groups within type B consist of sites with complex, heterogeneous mixtures of sand, gravel, cobble, boulder, bedrock, etc. in which additional survey effort would be requested. Substrates consisting of a heterogeneous mixture of medium/large gravel, cobble, large rock, or boulder will require 0.75 min/m<sup>2</sup> of survey effort. Cells or transects in Type B stream groups with clay and bedrock substrates can follow the standard 0.5 min/m<sup>2</sup> survey effort. As stated in the Visibility requirements above, only 0.2 min/m<sup>2</sup> of survey effort is requested, regardless of substrate type, if visibility requirements are met and no mussels are observed.

### Workable Flow

Surveys should be conducted at base flow or lower flow conditions. If the area cannot be safely and effectively surveyed under existing flow conditions, the survey must be re-scheduled. If sampling must be conducted above base flow, a variance must be approved by TPWD and/or the Service, based upon the grouping of the stream in question, to initiation of the survey.

### Documenting Safety Concerns

While determining the safety of a site for conducting this mussel sampling protocol is at the discretion of those performing the sampling, if the site is deemed unsafe, or a portion of the recommended survey area is deemed unsafe by those performing the work, approval is required from TPWD and the Service in writing (e.g., email) prior to any deviations from the methodologies outlined in this protocol. To approve any variance requests due to safety concerns, documentation may be necessary. Examples of documentation that may be required could include photographs of the site, depth and current velocity measurements, and recreational grade side-scan imagery of the site.

### Mussel Handling and Processing

All mussels collected during the survey are to be brought to the surface for processing and positive identification by the permitted mussel surveyor. Mussels shall always be kept in water, except for short periods for measurement and photographing that should last no longer than 5 minutes. It is imperative that surveyors adhere to this handling requirement as being exposed to high ambient temperatures can quickly become lethal for freshwater mussels, especially when compounded with the inherent stress from handling.

Individual mussel data are to be recorded in discrete units according to the cells or transect segments from which they were collected. The requested data to be collected during mussel processing can be found in [Appendix E](#).

Any difficult-to-identify individuals that resemble Federal candidate or federally-listed species will be assumed to be the Candidate or listed species. If the surveyor chooses to use genetic confirmation to determine if the individual is not a federally-listed species, they will do so at their own discretion. If a surveyor chooses to pursue genetic confirmation, the mussel in question must be photo vouchered and non-lethal genetic sample collection methods must be used (i.e., foot swab). **If an individual is lethally vouchered without authorization associated with a study previously approved by the Service and proven to be a listed species through genetic confirmation, the voucher will be considered unauthorized take of a listed species associated with the project requiring the survey.** Non-invasive sample collection methods based on those prepared by the USFWS Warm Springs Conservation Genetics Lab can be found in [Appendix H](#).

All mussels are to be hand-placed into the substrate in a proper, filtering position at the identified relocation site. Federally-listed mussel species are to be returned to substrates within the immediate vicinity from which they were collected and hand-placed in a proper, filtering position. Consultation with and approval from the Service are required prior to initiating the salvage and relocation of federally-listed mussels.

## Survey Methodologies

Unless alternative methods are agreed to by appropriate TPWD and/or the Service **PRIOR** to initiation of fieldwork, the following appropriate survey methodologies are recommended to be followed for all freshwater mussel surveys. The survey methodologies below are considered adequate to identify species presence/absence within or within the vicinity of proposed project footprints and will allow TPWD or the Service to determine if proposed projects will have impacts on protected resources. If alternate methods are not agreed to prior to fieldwork initiation, the survey will be considered invalid.

### Desktop and/or Land-based Assessment

A desktop or land-based assessment can be used to determine whether a water-based survey is warranted for a proposed project. During this assessment, the surveyor should investigate indicators that would negate the need for a freshwater mussel survey. These indicators may include, but are not limited to, clear evidence that the stream in question is not perennial, there is not adequate instream habitat to support any mussel species, or other clear indicators of site unsuitability for occupation by freshwater mussels (e.g., anoxic conditions). These indicators must be supported by data that could include but is not limited to water quality sonde readings, mapping, site photographs, etc... If the appropriate agency, TPWD and/or Service, **concur in writing** that the site does not contain suitable mussel habitat, no further survey will be necessary. If it is determined that suitable habitat is potentially present for freshwater mussels, the appropriate survey should be completed.

## Phase 1 Survey Types

### Cells

Cells are the preferred sampling method for surveying Group 1, 3, and most Group 5 streams. A cell survey is conducted by dividing each survey area into a series of cells in which each cell is surveyed, and data recorded by cell. Maximum acceptable cell size is 100 m<sup>2</sup>. Minimum search effort shall be 0.5 min/m<sup>2</sup> for cells containing homogenous substrates consisting of bedrock, silt, and/or sand, or 0.75 min/m<sup>2</sup> for cells containing heterogenous substrates consisting of mixtures of silt, sand, gravel, cobble, boulder, and/or bedrock as prescribed by cell substrates, except for streams that meet the visibility requirement or substrate type requirement described above.

### Transects

Transects are more appropriate for surveying Group 2, 4, and some larger Group 5 streams, but may be applied to Group 1 and 3 streams on a project-by-project basis after consultation with TPWD and the Service. Transect surveys are conducted by placing evenly spaced lines perpendicular to flow which are subdivided into 5 m segments. Spacing distances between transects will follow the limits presented in Table 1 in [Appendix A](#), based upon the type of project requiring the survey. At minimum, a transect survey should contain at least 250 m of combined transect length and consist of at least five transects. When utilizing the minimum number of transects due to a small proposed ADI, three transects must be placed within the ADI, one must be placed in the DSB, and one must be placed in the USB. The minimum level of transect search area may be reduced on a case-by-case basis following approval by TPWD and/or the Service. Along each transect, surveyors shall visually/tactility search an area 1 m wide for mussels and record all data separately for each segment. The entire segment must be covered and search efforts per m<sup>2</sup> will adhere to search rates described above.

### Qualitative Timed Searches

When conducting transect surveys to cover the ADI and prescribed buffer areas, qualitative timed searches will also be completed in areas with suitable habitat (as evidenced by the presence of live mussels along completed transects) following completion of the transect surveys to increase the probability of documenting all species that are present within the survey area. Qualitative timed searches will be structured to develop species richness accumulation curves for each qualitative timed search area. An example of a qualitative timed search conducted in conjuncture with a transect survey is provided in [Appendix G](#), Example 3.

Within each qualitative timed search area, qualitative sampling will be conducted in 10-person/minute (min) increments documenting species collection. Additional 10-person/min increments will be completed throughout the entire qualitative timed search area until at least 6 10-person/min increments are completed without the collection of a new species. Species observed during the qualitative timed search increment will be compiled and presented as a species accumulation curve in the survey report presented to the appropriate agency personnel. The additional time increments requested to be completed after finding no new species will

demonstrate that sampling efforts have likely led to the observation of most species that occupy the qualitative timed search area.

### Rio Grande Basin Requirements

Due to the unique habitat utilized by the Texas Hornshell (*Popenaias popeii*), in addition to the Phase 1 sampling referenced above additional bedrock, boulder, and bank searches will be recommended for all surveys of streams in the Rio Grande basin. These searches will include a focus on tactile searches beneath boulders and bedrock shelves that are encountered when conducting cell or transect surveys, and an additional search of boulders, bedrock overhangs, and root wad habitats within 2 m of each bank. The additional bank searches will be completed at a rate of 0.5 min/m<sup>2</sup> and will be documented as cells or transect segments independent from the remainder of the survey. Example site diagrams demonstrating survey areas including the Rio Grande Basin requirements are included in [Appendix G](#).

### Phase 2 Survey Methods

If approved as part of the ARRP process, Phase 2 sampling may be, but is not required to be, completed immediately following the conclusion of Phase 1 sampling. If the Phase 1 survey results for the proposed project require additional coordination with TPWD and/or Service, such as a mussel surveyor does not hold a Section 10(a)1(A) collection permit finding a federally-listed species in a Group 3, 4, or 5 stream, the Phase 2 survey may be requested to be completed at a later date. If the proposed project is occurring in locations known to be occupied by state-listed, federally-listed, Federal candidate, and/or federally-petitioned species, as documented by survey records completed within 10 years, the surveyor may coordinate with TPWD and/or the Service, depending upon the stream grouping in question, and proceed directly to completing a Phase 2 survey. In these instances, sampling methods may need to be altered depending upon the quality and recency of the previously collected survey data and will be amended on a case-by-case basis.

### Quantitative Sampling

In order to search for additional species not observed during Phase 1 sampling and calculate species densities. These data could be used to identify at-risk mussel species that were not observed during Phase 1 sampling or provide species-specific density data that could be used to calculate Direct or Indirect Impacts if formal consultation with the Service is required. Quantitative sampling will be requested in cells or transect segments that meet one of the three following triggers:

- State-listed, federally-listed, Federal candidate, and/or federally-petitioned species are observed,
- Mussel density of equal to, or greater than, 0.5 mussels/m<sup>2</sup> are observed within a 5 m segment along a transect, or,
- Observation of at least two species, live or fresh dead (still containing soft tissue), that are not listed in the Diverse Freshwater Mussel Assemblage Exceptions ([Appendix C](#)).

At sites where state-listed, federally-listed, Federal candidate, and/or federally-petitioned mussel species are not observed during Phase 1 sampling, quantitative sampling will only be requested within the prescribed Salvage Zone (Appendix A) in cells or along transect segments where one of the triggers are met. At sites where federally-listed, Federal candidate, and/or federally-petitioned mussel species are observed, quantitative sampling will be requested within the Salvage Zone (Appendix A) where any of the 3 triggers are met and within buffer area cells or transects where the protected species were observed. Quantitative data collected for federally-listed, Federal candidate, and/or federally-petitioned species could then be used to calculate direct and/or indirect impacts during formal consultation with the Service, if necessary.

### Cells

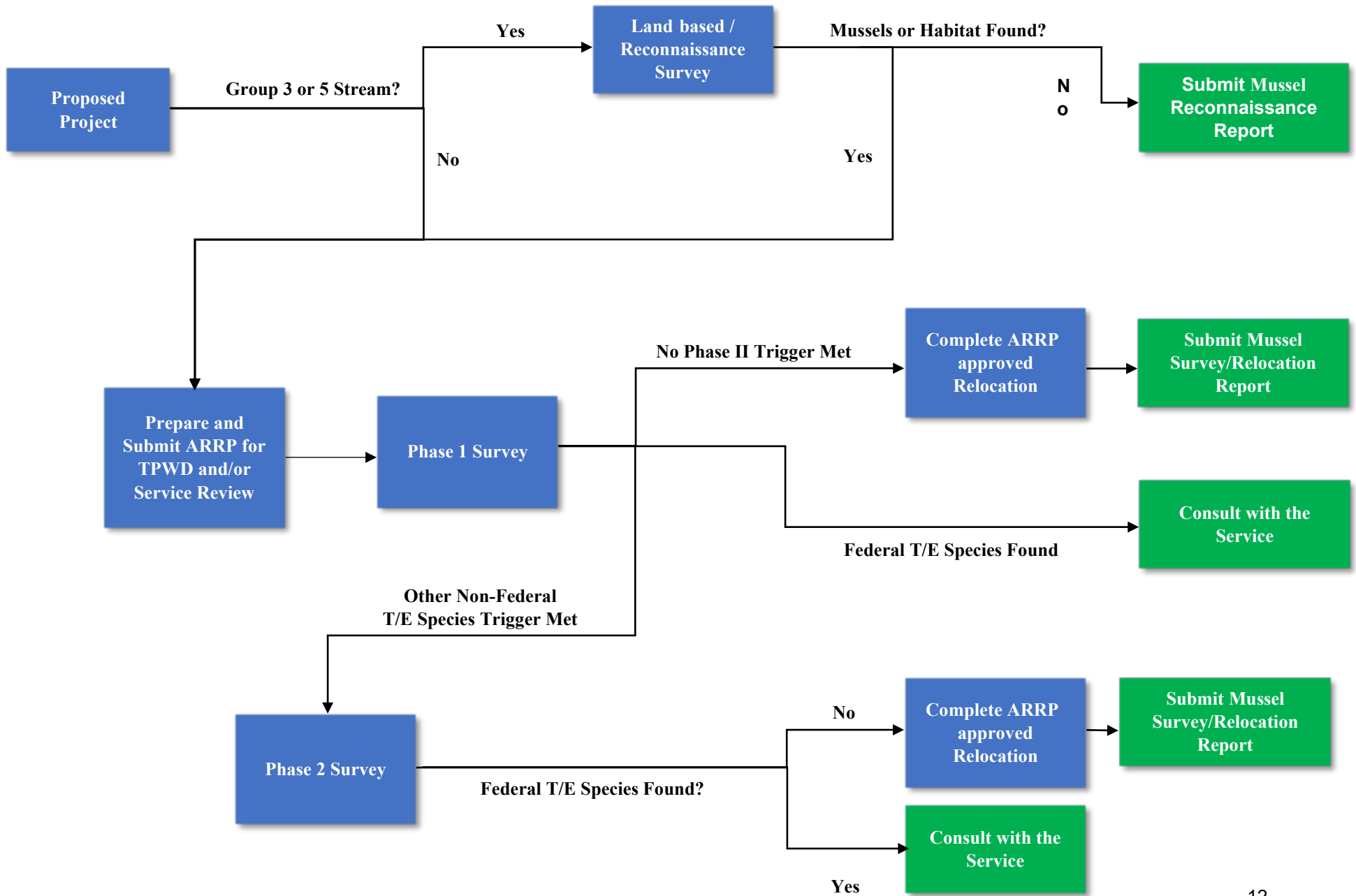
At sites where the Phase 1 survey is completed using cells, this sampling shall consist of excavating 0.25 m<sup>2</sup> quadrats using a systematic, three random start methodology like that described in the Guide to Sampling Freshwater Mussel Populations (Strayer and Smith, 2003). Substrate shall be excavated to a depth of 15 cm (6in) or hardpan. The material shall be collected and taken to the surface and sorted, removing all live and dead shell material. The minimum number of samples requested is based on the rate of 1 quadrat per 5 m<sup>2</sup> of delineated area of the cells that met the above triggers. For sites that meet diversity or density triggers, but not the protected species trigger, the boundary of the Phase 2 quantitative sampling should not extend beyond the boundary of the Salvage Zone. A minimum of 50 quadrat samples shall be collected regardless of size of the area within the Salvage Zone that met the above triggers.

### Transects

For streams where transect sampling was selected for the Phase 1 survey, the Phase 2 survey shall consist of excavating one quadrat sample within each 5 m transect segment of the Phase 1 transect as well as additional transects placed between the original surveyed transects that met the Phase 2 trigger in order to adequately distribute quantitative sampling efforts across instream habitats that met the trigger. The excavated areas of these additional transects, in addition to that surveyed during the Phase 1 survey, will be used to compile a species list for the project area and calculate densities of species observed with the observed mussel aggregations.

## Survey and Coordination Decision Making

A flow chart outlining the coordination and freshwater mussel survey phase steps for proposed projects where recent or all species presence data is lacking is attached below. In instances where sufficient species presence and density or abundance data exists, project proponents may coordinate with TPWD and/or the Service and proceed to an ARRP-approved relocation or initiate formal consultation, whichever is appropriate in the given scenario.



## Stream Group-Specific Survey Requirements

### Group 1 Streams

**Phase 1:** The preferred survey method for Group 1 streams is the cell methodology described above. If the proposed survey area is wider than 40 m at its widest point, the Transect and Qualitative Timed Search methods may be used. If the surveyor prefers to use the Transect and Qualitative Timed Search method for a stream with a width less than 40 m, the deviation from the preferred methodology will need to be approved by TPWD and the Service in writing prior to initiation of the survey.

If state-listed, federally-listed, Federal candidate, and/or federally-petitioned mussel species are observed during Phase 1 sampling, the project proponent will need to conduct a Phase 2 quantitative survey in the area(s) where Phase 2 triggers were met in order to detect additional species and calculate densities of listed species.

**Phase 2:** Quantitative sampling, as described above, shall be completed within Salvage Zone cells where all mussels were observed at a density of greater than 0.5 mussels/m<sup>2</sup>, a diverse mussel assemblage, as defined above, was observed, and/or state-listed, federally-listed, Federal candidate, and/or federally-petitioned species were observed. The objective of Phase 2 sampling is to increase the probability of detecting all present species and, in some cases, determine the density of federally-listed species within the proposed project area that will be directly and/or indirectly impacted by the proposed project.

### Group 2 Streams

**Phase 1:** The preferred survey methods for projects that span Group 2 streams are the Transect and Qualitative Timed Search methods. If the proposed project does not span the width of the stream and will only impact a portion of the stream, the Cell method may be appropriate. Instances in which the Cell method shall be utilized are listed in [Appendix A](#).

If state-listed, federally-listed, Federal candidate, and/or federally-petitioned mussel species are observed during Phase 1 sampling, and the project proponent/surveyor cannot avoid the proposed impact to the study area, the surveyor will need to conduct a Phase 2 quantitative survey in the area(s) where Phase 2 triggers were met in order to detect additional species and calculate densities of listed species that were observed.

**Phase 2:** Quantitative or additional transect sampling, as described above based upon stream width and methods used during the Phase 1 survey, shall be completed within Salvage Zone cells where all mussels were observed at a density of greater than 0.5 mussels/m<sup>2</sup>, a diverse mussel assemblage, as defined above, was observed, and/or state-listed, federally-listed, Federal candidate, and/or federally-petitioned species were observed. The objective of Phase 2 sampling is to increase the probability of detecting all present species and, in some cases, determine the density of federally-listed species within the proposed project area that will be directly and/or indirectly impacted by the proposed project.



### Group 3 Streams

**Land-Based and Reconnaissance Survey:** Surveyors may determine if a water-based survey is necessary based on the Desktop/Land-based Assessment methodology described above. If a water-based survey is deemed necessary, a Phase 1 mussel survey will be recommended.

**Phase 1:** The preferred survey method for Group 3 streams is the Cell methodology described above. If the proposed survey area is wider than 40 m at its widest point, the Transect and Qualitative Timed Search methods may be used. If the surveyor prefers to use the Transect and Qualitative Timed Search method for a stream with a width less than 40 m, the deviation from the preferred methodology will need to be approved in writing by TPWD prior to initiation of the survey.

If federally-listed mussel species are observed during Phase 1 sampling, the project proponent/surveyor will cease the survey and immediately contact the Service to determine next steps if they do not currently possess a Service 10(a)(1)(A) permit with species specific authorizations. The Service will be notified immediately upon finding a federally-listed species, as based upon the proposed project's timeline, the Service may require additional survey work is delayed in order to avoid disturbing resident mussels until a plan forward is agreed upon. This coordination may allow involved parties to avoid additional, unnecessary disturbance.

### Group 4 Streams

**Land-Based and Reconnaissance Survey:** These forms of surveys are not permitted for Group 4 streams due to stream size and unlikely ability for a site assessment to adequately determine absence of suitable mussel habitat.

**Phase 1:** The preferred survey methods for projects that span Group 4 streams are the Transect and Qualitative Timed Search methods. If the proposed project does not span the width of the stream and will only impact a portion of the stream, the Cell method may be appropriate. Instances in which the Cell method shall be utilized are listed in Appendix A.

If federally-listed mussel species are observed during Phase 1 sampling, the project proponent/surveyor will immediately suspend all survey activities and contact the Service within 24 hours to determine next steps if they do not currently possess a Federal collection permit. The Service will be notified immediately upon finding a federally-listed species, as based upon the proposed project's timeline, and the Service may prefer that additional survey work is delayed in order to avoid disturbing resident mussels until a plan forward is agreed upon. This coordination may allow involved parties to avoid additional, unnecessary disturbance.

### Group 5 Streams

Group 5 streams may be assessed using the Desktop and/or Mussel Reconnaissance Survey (Appendix D) methodology to determine if suitable habitat is present. The results of this assessment, along with site photos, and aerial/satellite imagery with associated annotations and delineations will be sent to TPWD. Following review by TPWD, the agency may concur in writing that the site is unlikely to be inhabited by live freshwater mussels and no further survey will be necessary. If it is determined that live mussels are potentially present, a qualitative timed survey



of a minimum of five person-hours will be requested within the survey area as described in [Appendix A](#). The timed searches should be broken up into one person-hour search periods. If no new species are encountered after the 5<sup>th</sup> person-hour, the survey is complete. If a new species is encountered in the 5<sup>th</sup> person-hour, additional one-person hour searches would be necessary until no new species are encountered. If at any point during the timed searches a state-listed species is encountered, move immediately to Group 3 survey methods. If federally-listed mussel species are observed during sampling, the project proponent will need to cease the survey and immediately contact the Service to determine next steps.

## Relocation Methodology

**For any stream where federally-listed mussel species were collected during the Phase 1 survey, coordination with the Service must occur prior to the initiation of any relocation efforts.** Guidance on initiating the formal consultation process can be found online at:

[Section 7 Consultations | U.S. Fish & Wildlife Service \(fws.gov\)](#)

If no federally-listed mussels were observed during Phase 1 sampling and avoidance options are exhausted, mussels must be relocated from the Area of Direct Impact and appropriate buffer areas (Salvage Zone). The recommended Salvage Zone boundaries for each project and habitat type can be found in Table 2 in [Appendix A](#). **No mussels are to be moved without prior authorization from TPWD and/or the Service.**

In streams where freshwater mussels are observed, coordination with TPWD and the approval of an ARRPs **must occur** prior to any relocation efforts. In some instances, an ARRPs may be issued to approve the salvage and relocation of live mussels during completion of the Phase 1 survey. TPWD Guidelines for Aquatic Resource Relocation Plans can be found here:

[https://tpwd.texas.gov/publications/pwdpubs/media/pwd\\_Lf\\_t3200\\_1958\\_arrp\\_guidelines\\_packet.pdf](https://tpwd.texas.gov/publications/pwdpubs/media/pwd_Lf_t3200_1958_arrp_guidelines_packet.pdf)

If no state- or federally-listed mussels are collected during the Phase 1 survey, the surveyor will relocate the live common mussels collected within the Salvage Zone without the requirement to complete the multiple-pass depletion method to be described below. In this scenario, mussels collected from the Salvage Zone during the Phase 1 mussel survey may be immediately relocated to the selected relocation site. The relocation of these mussels must adhere to the relocation site selection methods, processing and data-entry procedures, and reporting requirements presented within this Protocol and agreed to in the project's approved ARRPs.

Relocation procedures to be followed when relocating state-listed and common live freshwater mussels:

- Relocations must be conducted within the same year as the start of instream construction. If instream activities have not commenced prior to July 1<sup>st</sup> of the next year, additional relocation efforts may be requested just prior to construction activities depending on the results of earlier relocation efforts.

- The salvage effort shall be systematically conducted by a “moving transect” or establishing cells not to exceed 100 m<sup>2</sup>. The minimum effort is 1 min/m<sup>2</sup> (0.5 min/m<sup>2</sup> first pass, 0.5 minutes/m<sup>2</sup> second pass).
- A multiple pass depletion effort, with a minimum of two passes, shall be made through each cell or moving transect until less than 10 percent of the original number of live common mussels collected are recovered on the final pass. If a state-listed species is found during the final pass of a cell or transect, an additional pass will be completed in that cell or transect regardless of the percentage threshold achieved during that pass. Additional passes will only be requested in cells or transects that have not reached the necessary threshold or where state-listed species were collected. Other cells that have met the threshold will require no further effort.
- Salvage efforts shall meet the same Survey Requirements as Phase 1 surveys (i.e. visibility requirement, workable streamflow conditions, and mussel survey period).
- Relocation sites shall be preferably located at least 100 m upstream of the proposed impact area in a location of equal or better habitat that is inhabited by a comparable mussel assemblage. If it is known during the relocation planning stages that a suitable site is not within the vicinity of the proposed project due to upstream or downstream habitat disturbances, TPWD and/or Service can provide technical assistance and recommend a relocation site in another area. When identifying a potential relocation site, one 15 min qualitative survey shall be completed to identify habitats occupied in similar or greater densities by similar species to those collected during the salvage. If the initially surveyed area does not contain comparable habitat or a similar resident mussel fauna, additional 15 min qualitative surveys will be completed until an appropriate relocation site is found. The relocation area shall be equal or larger in size to the salvage area in order to avoid overcrowding of the relocation site. All observations of resident mussels are to be reported in the survey report. If no sites with comparable mussel assemblages can be located within the vicinity of the salvage site, identification of a suitable relocation site will be left to the best professional judgement of the permitted mussel surveyor on-site and the situation should be explained in the final relocation report.
- If federally-listed species are found during relocation efforts and no incidental take authorization from the Service has been received, all relocation efforts will be suspended and the Service and TPWD must immediately be informed of the presence of federally-listed species. Additional coordination with the Service will be needed under the ESA.

## Reporting Requirements

A report documenting the project location and justification, completed survey methods, results, and discussion must be submitted to TPWD and/or the Service for review and acceptance within 30 days or prior to initiation of the proposed in-stream work, whichever comes first. A list of the items requested in each section of the submitted survey report are presented in [Appendix E](#).

## Data Validity Period

Surveys that result in a collection of no live or fresh-dead mussels are valid for a period not to exceed 3 years. After 3 years, the survey report will no longer be considered valid and a new mussel survey must be completed prior to initiating a project at the site.

Surveys that result in the collection of live or fresh-dead mussels will result in an indefinite assumption that mussel habitat is present at the site and that a relocation would need to be completed prior to any instream disturbance. If a project is delayed and scheduled to begin outside of the 3-year time period, contractors may pursue 1 of 2 options: 1) assume that freshwater mussels are still considered present at the site and proceed directly to completing the appropriate Phase 2 or salvage and relocation activities, or 2) complete a new Phase 1 survey to demonstrate the freshwater mussels are no longer present at the site and that no salvage and relocation activities are necessary prior to instream construction. Under the first scenario, coordination and relocation activities would still be requested at the site based upon the data collected during the previously completed Phase 1 survey. Under the second scenario, the necessity for additional coordination and relocation activities would be determined based upon the data collected during the new Phase 1 survey.

## Appendix A.

### Freshwater Mussel Recommended Survey Area Summary Table

**Table 1. Freshwater Mussel Recommended Survey Areas**

Stream Tier and Project Classification	Upstream Buffer (USB, m)	Downstream Buffer (DSB, m)	Lateral Buffer (LB, m)	Cells or Maximum Transect Spacing (m)
<b>Group 1 Stream</b>				
Scoping Projects	Project Specific			
Linear (Pipeline corridors, waterlines, roads, bridges, etc.)	50	100	B to B	Cells
Bank Disturbance/Shoreline Protection	50	100	10	Cells
Water Intakes (at shoreline)	25	50	10	Cells
Outfalls	10	MZ + 100	10	Project Specific
<b>Group 2 Stream</b>				
Scoping Projects	Project Specific			
Linear (Pipeline corridors, waterlines, roads, bridges, etc.)	50	100	B to B	Cells / 10 / 25*
Bank Disturbance/Shoreline Protection	50	100	10	Cells / 10 / 25*
Water Intakes (at shoreline)	25	50	10	Cells / 10 / 25*
Outfalls	10	MZ + 100	10	Project Specific
<b>Group 3 Stream</b>				
All Projects	25	50	10 / B to B	Cells
<b>Group 4 Stream</b>				
All Projects	50	100	10 / B to B	Cells / 10 / 25*
<b>Group 5 Stream</b>				
All Projects	25	50	10 / B to B	N/A

MZ = Mixing Zone; B to B = Bank-to-Bank.

\*Transects for projects with ADI's <100 m in length will require spacing of no greater than 10 m. Transects for projects with ADI's >100 m in length will require spacing of no greater than 25 m

## Appendix A.

### Freshwater Mussel Recommended Salvage Zone Summary Table

**Table 2. Freshwater Mussel Recommended Survey Areas for Salvage Zones**

Stream Tier and Project Classification	Salvage Zone (m)			Salvage Zone (m)		
	USB*	DSB*	LB*	USB	DSB	LB
<b>Group 1 and 3 Streams</b>	<b>Habitat A</b>			<b>Habitat B</b>		
Scoping Projects	Project Specific			Project Specific		
Linear (Pipeline corridors, waterlines, roads, bridges, etc.)	25	50	10*	10	25	10 <sup>+</sup>
Bank Disturbance/Shoreline Protection	25	50	10	10	25	10
Water Intakes (at shoreline)	25	50	10	10	25	10
Outfalls	Project Specific			Project Specific		
<b>Group 2 and 4 Streams</b>	<b>Habitat A</b>			<b>Habitat B</b>		
Scoping Projects	Project Specific			Project Specific		
Linear (Pipeline corridors, waterlines, roads, bridges, etc.)	25	50	10*	25	50	10*
Bank Disturbance/Shoreline Protection	25	50	10	25	50	10
Water Intakes (at shoreline)	25	50	10	25	50	10
Outfalls	Project Specific			Project Specific		

\* USB = Upstream Buffer; DSB = Downstream Buffer; LB = Lateral Buffer

<sup>+</sup> LB = 10 m if stand-alone pier. Complete bank to bank projects will not have a lateral buffer

## Appendix B.

### Recognized Texas Freshwater Mussel Species List

Scientific Name	Common Name
<i>Amblema plicata</i>	Threeridge
<i>Arcidens confragosus</i>	Rock pocketbook
<i>Arcidens wheeleri</i>	Ouachita rock pocketbook
<i>Cyclonaias necki</i>	Guadalupe orb
<i>Cyclonaias nodulata</i>	Wartyback
<i>Cyclonaias petrina</i>	Texas pimpleback
<i>Cyclonaias pustulosa</i>	Pimpleback
<i>Cyrtonaias tampicoensis</i>	Tampico pearlymussel
<i>Fusconaia askewi</i>	Texas pigtoe
<i>Fusconaia chunii</i>	Trinity pigtoe
<i>Fusconaia flava</i>	Wabash pigtoe
<i>Fusconaia mitchelli</i>	False spike
<i>Glebula rotundata</i>	Round pearlshell
<i>Lampsilis bergmanni</i>	Guadalupe fatmucket
<i>Lampsilis bracteata</i>	Texas fatmucket
<i>Lampsilis cardium</i>	Plain pocketbook
<i>Lampsilis hydiana</i>	Louisiana fatmucket
<i>Lampsilis satura</i>	Sandbank pocketbook
<i>Lampsilis teres</i>	Yellow sandshell
<i>Lasmigona complanata</i>	White heelsplitter
<i>Leptodea fragilis</i>	Fragile papershell
<i>Ligumia subrostrata</i>	Pondmussel
<i>Megalonaias nervosa</i>	Washboard
<i>Obliquaria reflexa</i>	Threehorn wartyback
<i>Obovaria arkansasensis</i>	Southern hickorynut

Scientific Name	Common Name
<i>Plectomerus dombeyanus</i>	Bankclimber
<i>Pleurobema riddellii</i>	Louisiana pigtoe
<i>Popenaias popeii</i>	Texas hornshell
<i>Potamilus amphichaenus</i>	Texas heelsplitter
<i>Potamilus metnecktayi</i>	Salina mucket
<i>Potamilus ohioensis</i>	Pink papershell
<i>Potamilus purpuratus</i>	Bleufer
<i>Potamilus streckersoni</i>	Brazos heelsplitter
<i>Pyganodon grandis</i>	Giant floater
<i>Quadrula apiculata</i>	Southern mapleleaf
<i>Quadrula nobilis</i>	Gulf mapleleaf
<i>Quadrula quadrula</i>	Mapleleaf
<i>Strophitus undulatus</i>	Creeper
<i>Toxolasma parvum</i>	Lilliput
<i>Toxolasma texasiense</i>	Texas lilliput
<i>Tritigonia verrucosa</i>	Pistolgrip
<i>Truncilla cognata</i>	Mexican fawnsfoot
<i>Truncilla donaciformis</i>	Fawnsfoot
<i>Truncilla macrodon</i>	Texas fawnsfoot
<i>Truncilla truncata</i>	Deertoe
<i>Unio merus declivis</i>	Tapered pondhorn
<i>Unio merus tetralasmus</i>	Pondhorn
<i>Utterbackia imbecillis</i>	Paper pondshell
<i>Utterbackiana suborbiculata</i>	Flat floater
<i>Villosa lienosa</i>	Little spectaclecase

## Appendix C.

### Diverse Freshwater Mussel Assemblage Exceptions

Species	Common Name	Stream Group 1	Stream Group 2
<i>Leptodea fragilis</i>	Fragile papershell		X
<i>Pyganodon grandis</i>	Giant floater	X	X
<i>Unio merus declivis</i>	Tapered pondhorn	X	X
<i>Unio merus tetralasmus</i>	Pondhorn	X	X
<i>Utterbackia imbecillis</i>	Paper pondshell	X	X
<i>Utterbackiana suborbiculata</i>	Flat floater		X

## Appendix D. Freshwater Mussel Reconnaissance Survey Form

### Project Information

<b>Project Name</b>	<b>Date</b>
<b>Survey Leader</b>	<b>Scientific Collection Permit Numbers (TPWD and/or Service)</b>
<b>County</b>	<b>City or Nearest City/Town</b>
<b>Upstream Latitude, Longitude (Decimal Degree)</b>	
<b>Downstream Latitude, Longitude (Decimal Degree)</b>	
<b>Stream and Site Name</b>	

### Habitat Description

<b>Water Temp (C)</b>	<b>Air Temp (C)</b>
<b>Are typical stream conditions present? Describe general water quantity and quality. Are stream levels High? Normal? Low? Dry? These can be identified by USGS stream gauge, stream levels in comparison to bankfull levels, or other methods. Any evidence of environmental stressors, pollution, discharges, odors, etc.?</b>	

<b>Substrate Types (%)</b>				
<b>Bedrock</b>	<b>Boulder</b>	<b>Cobble</b>	<b>Gravel</b>	<b>Pea Gravel</b>
<b>Sand</b>	<b>Silt</b>	<b>Clay</b>	<b>Detritus</b>	<b>Woody Debris</b>
<b>Visibility (cm)</b>	<b>Average Depth (m)</b>		<b>Max Depth (m)</b>	
<b>Evidence of current or historic freshwater mussel presence (shell material)?</b>				
<b>Other habitat variables precluding mussel presence?</b>				



## **Appendix E.**

### **Freshwater Mussel Survey Report Checklist**

#### **INTRODUCTION**

- Description of the proposed project, including:
  - Project Client/Proponent
  - Project type
  - Proposed construction methodology
  - Proposed impacts
- Description of the stream and watershed including:
  - Name (if stream is named)
  - Receiving waters of surveyed stream
  - Location, including:
    - Nearest Town or City
    - County
  - Observational notes of any obvious point or non-point source pollution discharges in the project area that may be causing impacts to resident mussels or adjacent habitats

#### **METHODS**

- Area surveyed, including:
  - Description of survey buffer areas (e.g., length, bank-to-bank)
  - Coordinates of survey AID and buffer areas (US, DS, and LB) (NAD83, Decimal Degrees)
- Survey method, including:
  - Type of mussel survey completed (e.g. Phase I, Phase II, Relocation)
  - Length and spacing of transects or size of the cells
  - Time searched (Was a visibility condition applied, provide photo proof if so)
  - Sampling methods used (e.g. SCUBA, mask and snorkel, view bucket, quadrats)
  - Whether banks were searched for shells (Was additional Rio Grande Basin effort necessary?)
- Mussel handling and processing procedures
- Quality control procedures

#### **RESULTS**

- Personnel (Identify permitted mussel surveyor on-site)
- Date(s) of survey
- Habitat Assessment Results, including:

- Substrate composition (include information about the stability of the substrates)
- In-stream features (e.g. channel alterations, impoundments)
- Average stream depth
- Visibility (document on-site visibility if using Visibility Condition)
- Water temperature
- Suitable habitats within the area of the survey
- Photos of stream and substrate
- Mussel Survey Results, including:
  - Documentation of observed species
  - Total number of individuals found and relative abundance of observed species
  - Any notable species found
  - If observed, report any qualitative indications of stressed individuals during handling, including unintentional mortality

## **MUSSEL RELOCATION**

- Relocation site, including:
  - Location (coordinates for project center, upstream boundary, and downstream boundary in decimal degrees)
  - Results of requested 15 minute qualitative survey (provide coordinates in decimal degrees)
  - Method of salvaging mussels from survey area
- Additional information as required by the issued Biological Opinion for Group 1 and 2 streams.

## **CONCLUSION**

- Summary of findings, and conclusions

## **REFERENCES**

- Citations for any literature cited within the text of the report

## **FIGURES & TABLES**

- Figures
  - Vicinity Map and Aerial Imagery of Project Area and Relocation Site (If necessary)
  - Map of survey extents (ADI and Buffers)
    - Visual depiction of locations/distribution of utilized cells, transects, and/or qualitative searches
  - Spatial depiction of mussel survey data (including overall totals and species abundance)
    - Highlighting locations of state-listed, federally-listed, Federal candidate, and/or federally-petitioned species
  - Map of Relocation Site extents

- Tables
  - Habitat Assessment data for each transect and/or cell (if requested)
  - Species data for each transect and/or cell
    - Relative abundance of live individuals
    - Condition of observed individuals (living/fresh dead/weathered/subfossil)
    - Sex of live individuals (if determinable)
    - Size distribution of observed, live individuals (Min., Mean, Max. (mm))

## **APPENDICES**

- Photos of stream and substrates observed in the ADI and Buffers
- Representative photos of each mussel species found
- Copy of site-specific ARRP authorization from TPWD (if necessary)
- Copy of site-specific authorization from Service (if necessary)
- TXNDD Mussel Data Reporting Form – **Separate Excel File**

**Appendix F.**  
**Texas Parks and Wildlife Department and**  
**U.S. Fish and Wildlife Service Contacts**

# Texas Parks and Wildlife Department

## Kills and Spills Team

### Region 1

Contact	District Office Address	Office Phone	Mobile Phone
Travis Tidwell	4200 Smith School Road Austin, TX 78744	(512) 389-8612	(512) 422-8054

### Region 2

Contact	District Office Address	Office Phone	Mobile Phone
Bregan Brown	11810 FM 848 Tyler, TX 75707	(903) 566-2518	(903) 520-3821

### Region 3

Contact	District Office Address	Office Phone	Mobile Phone
Claire Iseton	1502 FM 517 East, Dickinson, TX 77539	(281)-534-0137	(281) 299-8027

### Region 4

Contact	District Office Address	Office Phone	Mobile Phone
Alex Nuñez	TAMUCC Natural Resources Center 6300 Ocean Drive, NRC Suite 2501 Corpus Christi, TX 78412	(361) 825-3246	(361) 658-3181

**24-Hour Phone : 215-389-4848**

## U.S. Fish and Wildlife Service

### Arlington Texas Ecological Services Field Office

Contact	Office	Office Phone	Email
Jennifer Khan	2005 Northeast Green Oaks Boulevard, Suite 140 Arlington, Texas 76006	(817) 277-1100 ext. 22105	Jennifer_Khan@fws.gov

### Austin Texas Ecological Services Field Office

Contact	Office	Office Phone	Email
Matthew Johnson	10711 Burnet Road, Suite 200 Austin, TX 78757	(512) 490-0057 ext. 253	Matthew_S_Johnson@fws.gov
Christina Williams		(512) 490-0057 ext. 235	Christina_Williams@fws.gov

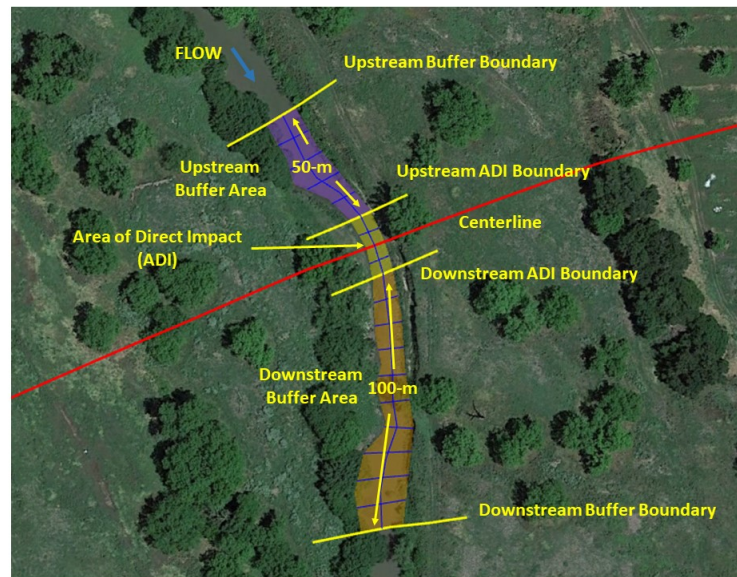
### Texas Coastal Ecological Services Houston Field Office

Contact	Office	Office Phone	Email
Sheena Waters	17629 El Camino Real, Suite 211 Houston, TX 77058	(281) 212-1504	Sheena_Waters@fws.gov

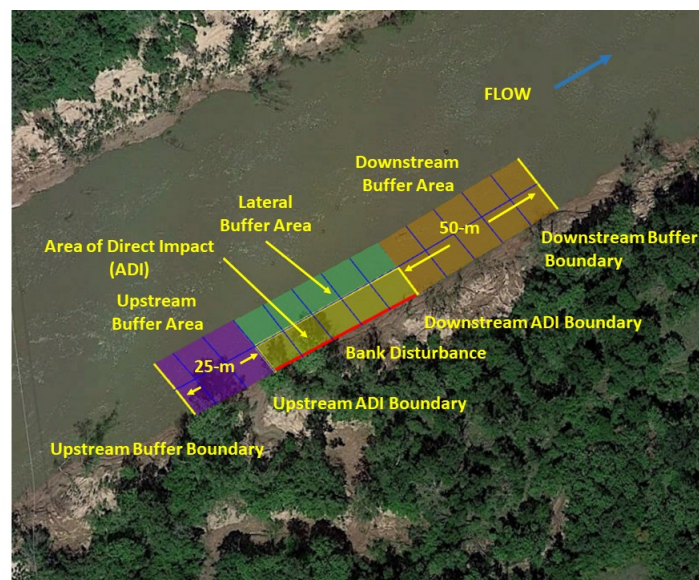
## Appendix G.

### Survey Examples

**Example 1.** A linear, cut and cover pipeline crossing of a Group 1 stream is proposed (red line). The proposed Right-of-Way (ROW) is 26 meters (m) wide, extending 13 m upstream and downstream of the proposed center line of the crossing. In order to allow the contract flexibility in laying the proposed pipeline's centerline and avoiding additional surveys in the future due to realignment, the surveyor has decided to treat the entire proposed ROW as the ADI. As the survey is being conducted within a Group 1 stream, the prescribed sampling protocol includes a Cell Survey with buffer areas extending from 50 m upstream of the proposed ADI to 100 m downstream of the proposed, ADI. Mussel, habitat, and survey effort data will be recorded independently within each cell and included within the survey report.



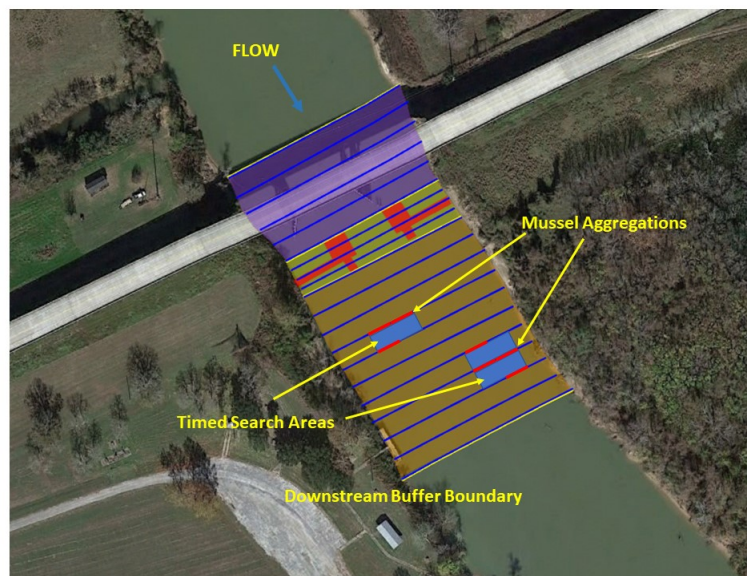
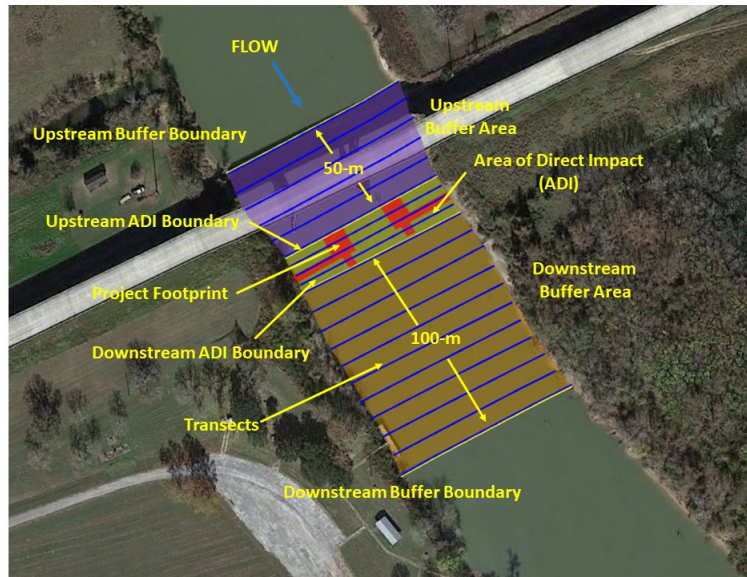
**Example 2.** A bank stabilization project on a Group 2 stream is proposed. The proposed ADI will occur along 50 m of streambank and extend 10 m into the river. As the survey is being conducted for a streambank disturbance project in a Group 2 stream, the prescribed sampling protocol includes a Cell Survey with buffer areas extending from 25 m upstream of the proposed ADI to 50 m downstream of the proposed ADI and includes a 10 m riverward lateral buffer. Mussel, habitat, and survey effort data will be recorded independently within each cell and included within the survey report.





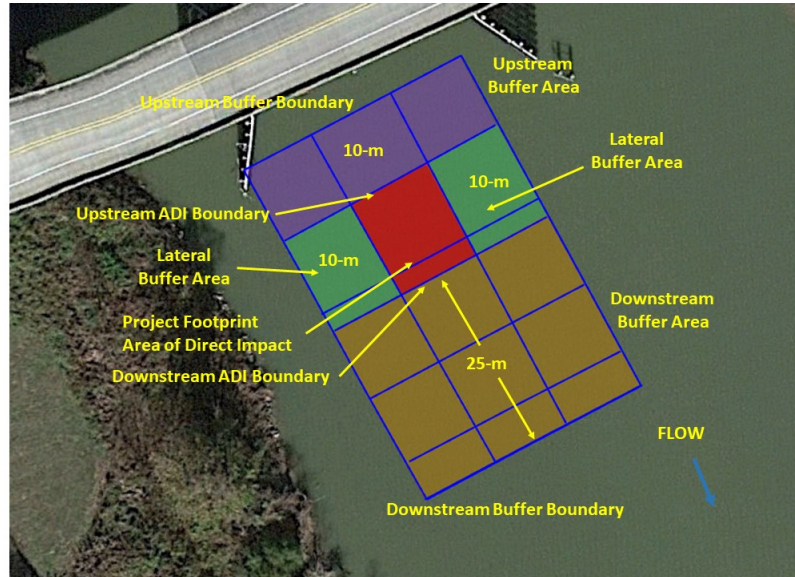
**Example 3.** A bridge widening project, assuming no work is to be completed on the existing bridge, adding a new bridge structure over a Group 2 stream is proposed. The proposed project will include the creation of two causeways extending from each bank which will serve as work areas for the construction of two instream support structures for the bridge. As the survey is being conducted for a bridge project in a Group 2 stream that is wider than 40 m, the prescribed sampling protocol includes a Transect Survey with buffer areas extending from 50 m upstream of the proposed ADI to 100 m downstream of the proposed ADI. The required minimum of 3 transects are evenly spaced within the proposed ADI and the transects in the Upstream and Downstream Buffer areas are spaced no more than 10 m apart. Because mussel aggregations were found during the transect survey, an additional qualitative timed search was required between transects that met the appropriate triggers. Mussel, habitat, and survey effort data will be recorded independently within each 5 m segment along each transect and within each timed search area and will be included within the survey report.

\*Please note that the ADI would extend upstream to include the footprint of the upstream bridge if the project anticipated to remove that existing bridge or complete repairs that could intentionally or inadvertently lead to instream impacts.

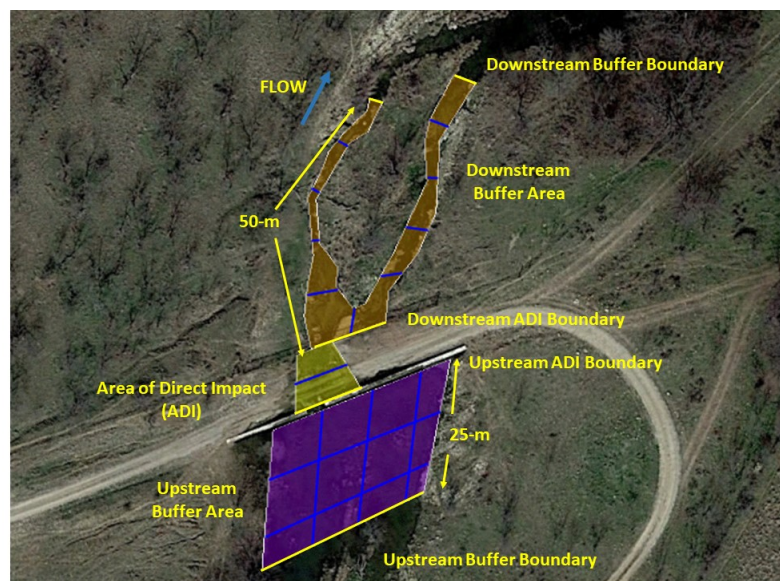




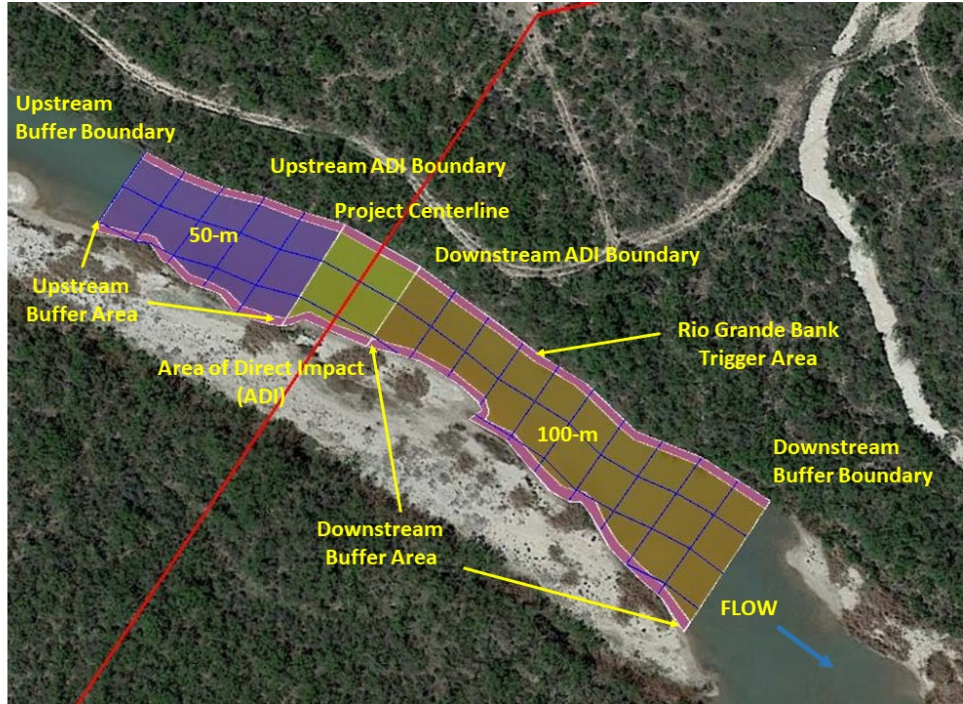
**Example 4.** A bridge project over a Group 4 stream is proposed. The proposed construction plan utilizes a barge platform to reduce the instream footprint and avoids the need for causeways. The proposed construction footprint is 13 m x 10 m in size. As the survey is being conducted for a bridge construction project in a Group 4 stream, the prescribed sampling protocol includes a Cell survey with buffer areas extending from 10 m upstream of the proposed ADI to 25 m downstream of the proposed ADI and include a 10 m riverward lateral buffers on each side of the ADI. Mussel, habitat, and survey effort data will be recorded independently within each cell and included within the survey report.



**Example 5.** An existing ford crossing is proposed to be replaced with a box-culvert bridge across a Group 5 stream. The proposed ROW for the new road is 12 m wide and it is anticipated that grading and bank work will extend throughout the entire ROW; therefore, the ADI will extend along the entire ROW. As the survey is being conducted for a linear crossing project in a Group 5 stream, the prescribed sampling protocol includes a Cell survey with buffer areas extending from 25 m upstream of the proposed ADI to 50 m downstream of the proposed ADI. Mussel, habitat, and survey effort data will be recorded independently within each cell and included within the survey report.



**Example 6.** A linear, cut and cover pipeline crossing of a Group 1 stream in the Rio Grande basin is proposed. The proposed ROW is 20 m wide, extending 10 m upstream and downstream of the proposed center line of the crossing. As the survey is being conducted within a Group 1 stream that is less than 40 m wide, the prescribed sampling protocol includes a Cell survey with buffer areas extending from 50 m upstream of the proposed ADI to 100 m downstream of the proposed, ADI. Because the survey is being completed in the Rio Grande Basin, the Rio Grande trigger is met and additional sampling effort along the bank is required to ensure that bank overhang habitat frequently utilized by Texas Hornshell has been adequately surveyed. Mussel, habitat, and survey effort data will be recorded independently within each cell and included within the survey report.



**Appendix H.**  
**Non-invasive Genetic Sample Collection Methods**

## Standard Operating Procedure

as adapted from the Warm Springs Conservation Genetic Lab

### Collecting tissue samples for freshwater mussels using swabs

#### **Field notes**

Collections that lack precise documentation are almost worthless, and the genetics labs may reserve the right to refuse specimens that are not properly documented or preserved. Therefore, proper field documentation is required for each collection submitted. Such documentation is best achieved when the researcher responsible for each collection maintains a field notebook. Field notes are a part of the specimen collection and may kept for reference by the genetics lab; therefore, they are an invaluable reference source about the collection. Important information that should be recorded in field notes are *field number, state and locality data, sampling site, drainage, latitude and longitude (UTM data preferred), date, names of people and agencies who collected samples, genus and species, length (metric preferred), weight (metric preferred), tag number (when appropriate) and preservation type.*

#### **Species identity and vouchers**

Taxonomic certainty is required when identifying specimens from which tissues are taken. Voucher specimens, either photo or physical animals when explicitly permitted to do so, should be associated with each tissue sample if doubts exist about species identification.

*Note on voucher specimens.*

A photograph or a shell serves as a suitable method to voucher species including larger specimens or specimens of species that are endangered or threatened. Each specimen should be anesthetized prior to being photographed (see below). Documenting references to size is very important. Size can be estimated from photographs if a tape measure, meter stick, or other calibrated item is placed next to the specimen in photo. In addition, physical documentation of the species name, field number, locality information, and date should accompany each photograph. For example, digital cameras or photographic processing software can be used to electronically add numbers and letters to digital images.

Voucher photographs will be used for specimen identification; therefore, the captured image should be as large as possible. Fill as much of the field of view as possible with the subject, and when using a digital camera, always choose the highest resolution setting. Natural history museums are typically equipped to handle digital or shell voucher material.

#### **Sampling Protocol**

- Carefully open mussel
- Gently swab mussel making several passes with the swab tip over the foot if possible (3-4 passes are sufficient – try to avoid any dirt if possible).
- Deposit swab head in the collection tube by breaking the plastic applicator (at indentation point) on side of tube.

- Secure tube with one of the caps (make sure it is secure), label tube with individual identifier (we recommend that researchers preprint labels with waterproof ink or on laser printer using write-in-rain paper prior to field collection and either place tag inside tube or tape to outside of tube).
- The tube containing the swab can be stored at room temperature for at least 5 months prior to extracting the DNA.
- How does it work? The capsule contains silica that dries the swab (including tissue); thus, preserving the tissue and DNA.

### **Tissue sample contamination**

Care should be taken to prevent cross-contamination among tissue samples. Ideally, sterile surgical gloves should be worn to prevent contamination with human tissue, and instruments should be cleaned and sterilized after each use. However, such precautions are often inconvenient for field researchers. Contamination of mussel tissue with human tissue is of little concern because the PCR primers, which have a sequence that is different from that of humans, will only work on closely related DNA sequences. Wiping the instruments after every use and ensuring that remnants of tissue or blood are not present before handling the next specimen will eliminate most sample contamination issues.