Texas
Freshwater Mussel Survey Protocol

Prepared by

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and

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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 are 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.

This Protocol is 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 Northeastern 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:

Texas Freshwater Mussel Sampling Protocol | U.S. Fish & Wildlife Service

The provided .kml and .shp files are to serve as guides and may not include all perennial streams in the state. If your proposed project is located on a perennial stream that is not included within the provided files, please coordinate with the appropriate TPWD KAST office to determine if freshwater mussel reconnaissance or survey efforts are necessary.

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, our 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 our 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 (e.g. run of the river impoundments, such as Lady Bird Lake in Austin, Texas) 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 Instream 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 Instream 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 F.

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://fwsepermits.servicenowservices.com/fws

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.

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 Texas 78744. Questions regarding permits or the application process can be sent to 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_lf_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 0.5min/m^2 or 0.75 min/m^2 to 0.2 min/m^2 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^2 or 0.75 min/m^2 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^2 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^2 of survey effort. Cells or transects in Type B stream groups with clay and bedrock substrates can follow the standard 0.5 min/m^2 survey effort. As stated in the Visibility requirements above, only 0.2 min/m^2 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 TWPD 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 D.

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 G.

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 or the site has been dewatered within the past 3 years, and there is not adequate instream habitat to support any mussel species such as lined channels (e.g. concrete canals) or 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, **concurs 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.

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^2. Minimum search effort shall be 0.5 min/m^2 for cells containing homogenous substrates consisting of bedrock, silt, and/or sand, or 0.75 min/m^2 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^2 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 F, Example 3.

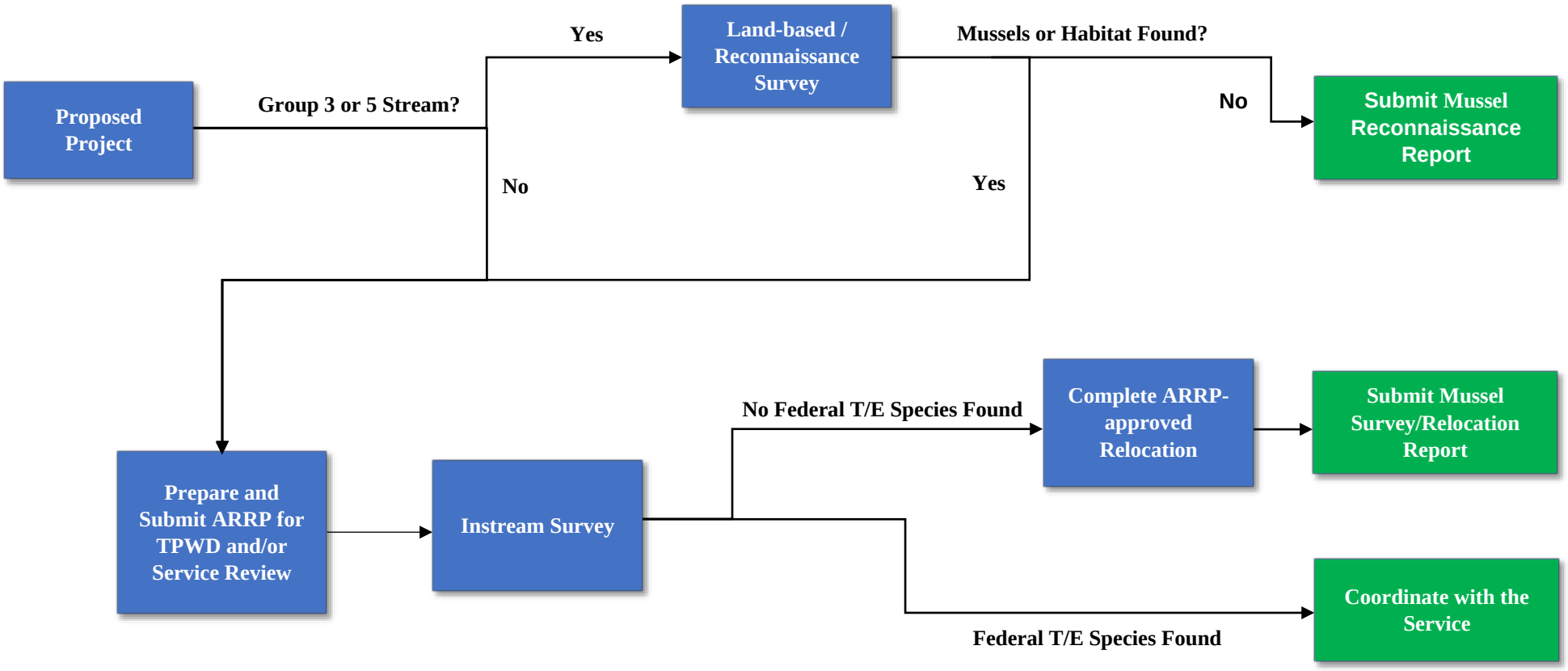
Within each qualitative timed search area, qualitative sampling will be conducted in 10-person/minute (min) increments documenting species collection. Additional 10-person/min increments will be completed throughout the entire qualitative timed search area until at least 6 10-person/min increments are 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 Instream Survey 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^2 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 F.

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.


Proposed Project
Group 3 or 5 Stream?
Yes
Land-based / Reconnaissance Survey
Mussels or Habitat Found?
No
Submit Mussel Reconnaissance Report
No
Yes
Prepare and Submit ARRP for TPWD and/or Service Review
Instream Survey
No Federal T/E Species Found
Complete ARRP-approved Relocation
Submit Mussel Survey/Relocation Report
Federal T/E Species Found
Coordinate with the Service

Stream Group-Specific Survey Requirements

Group 1 Streams

Instream Survey: 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 federal candidate, and/or federally petitioned mussel species are observed during Instream Survey sampling, the project proponent may wish to coordinate with the Service and potentially conference on the project. If federally listed species are observed during Instream Survey sampling, the project proponent must coordinate with the Service for possible consultation on the project.

Group 2 Streams

Instream Survey : The preferred survey methods for projects that span Group 2 streams wider than 40m 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 federal candidate, and/or federally petitioned mussel species are observed during Instream Survey sampling, the project proponent may wish to coordinate with the Service and potentially conference on the project. If federally listed species are observed during Instream Survey sampling, the project proponent must coordinate with the Service for possible consultation on the 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, an Instream Survey will be recommended.

Instream Survey : 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 Instream Survey sampling, the project proponent/surveyor will cease the survey and immediately contact the Service and TPWD 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.

Instream Survey : The preferred survey methods for projects that span Group 4 streams wider than 40 m 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 Instream Survey sampling, the project proponent/surveyor will immediately suspend all survey activities and contact the Service and TPWD within 24 hours to determine next steps if they do not currently possess a Section 10 (a)(1)(A) permit from the Service. 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 C) 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 department 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 5th person-hour, the survey is complete. If a new species is encountered in the 5th 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 and TPWD to determine next steps.

Relocation Methodology

For any stream where federally listed mussel species were collected during the Instream 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:

https://www.fws.gov/service/esa-section-7-consultation

If no federally listed mussels were observed during the Instream Survey 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 ARRP **must occur** prior to any relocation efforts. In some instances, an ARRP may be issued to approve the salvage and relocation of live mussels during completion of the Instream 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

If no state or federally listed mussels are collected during the Instream 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 Instream 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 ARRP.

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

- Relocations should be conducted within the same field season as the start of instream construction for a proposed project. If instream activities have not commenced during that field season or prior to the following field season, additional relocation efforts may be requested prior to construction activities commence at the discretion of the Service and/or TPWD.

- The salvage effort shall be systematically conducted by a "moving transect[1]" or establishing cells not to exceed 100 m^2. The minimum effort is 1 min/m^2 (0.5 min/m^2 first pass, 0.5 minutes/m^2 second pass).

[1] Moving Transect is a mussel salvage methodology where an area is defined by the presence of a transect line, boundaries on either side of the transect line are set and cleared of mussels, and then the transect is repositioned upstream or downstream of the boundaries set for the initial transect line to define a new area for clearing.

- 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 Instream 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 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 D.

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, if no federally listed species were observed during the original Instream Survey and proceed directly to completing the appropriate salvage and relocation activities following appropriate coordination with TPWD, or 2) complete a new Instream 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 Instream Survey. Under the second scenario, the necessity for additional coordination and relocation activities would be determined based upon the data collected during the new Instream 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)
Group 1 Stream				
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
Group 2 Stream				
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
Group 3 Stream				
All Projects	25	50	10 / B to B	Cells
Group 4 Stream				
All Projects	50	100	10 / B to B	Cells / 10 / 25*
Group 5 Stream				
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
Group 1 and 3 Streams	**Habitat A**			**Habitat B**		
Scoping Projects	Project Specific			Project Specific		
Linear (Pipeline corridors, waterlines, roads, bridges, etc.)	25	50	10*	10	25	5+
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		
Group 2 and 4 Streams	**Habitat A**			**Habitat B**		
Scoping Projects	Project Specific			Project Specific		
Linear (Pipeline corridors, waterlines, roads, bridges, etc.)	25	50	10*	10	25	5+
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		

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

+ 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
Amblema plicata	Threeridge
Arcidens confragosus	Rock Pocketbook
Arcidens wheeleri	Ouachita Rock Pocketbook
Cyclonaias necki	Guadalupe Orb
Cyclonaias nodulata	Wartyback
Cyclonaias petrina	Texas Pimpleback
Cyclonaias pustulosa	Pimpleback
Cyrtonaias tampicoensis	Tampico Pearlymussel
Fusconaia askewi	Texas Pigtoe
Fusconaia chunii	Trinity Pigtoe
Fusconaia flava	Wabash Pigtoe
Fusconaia iheringi	Balcones Spike
Fusconaia mitchelli	False Spike
Glebula rotundata	Round Pearlshell
Lampsilis bergmanni	Guadalupe Fatmucket
Lampsilis bracteata	Texas Fatmucket
Lampsilis cardium	Plain Pocketbook
Lampsilis hydiana	Louisiana Fatmucket
Lampsilis satura	Sandbank Pocketbook
Lampsilis Sietmani	Canary Kingshell
Lampsilis teres	Yellow Sandshell
Lasmigona complanata	White Heelsplitter
Leptodea fragilis	Fragile Papershell
Ligumia subrostrata	Pondmussel
Megalonaias nervosa	Washboard
Obliquaria reflexa	Threehorn Wartyback

Scientific Name	Common Name
Obovaria arkansasensis	Southern Hickorynut/ Ouachita Creekshell
Plectomerus dombeyanus	Bankclimber
Pleurobema riddellii	Louisiana Pigtoe
Popenaias popeii	Texas Hornshell
Potamilus amphichaenus	Texas Heelsplitter
Potamilus metnecktayi	Salina Mucket
Potamilus ohiensis	Pink Papershell
Potamilus purpuratus	Bleufer
Potamilus streckersoni	Brazos Heelsplitter
Pyganodon grandis	Giant Floater
Quadrula nobilis	Gulf Mapleleaf
Quadrula quadrula	Mapleleaf
Strophitus howellsi	Hill Country Creeper
Strophitus undulatus	Creeper
Toxolasma parvum	Lilliput
Toxolasma texasiense	Texas Lilliput
Tritigonia verrucosa	Pistolgrip
Truncilla cognata	Mexican Fawnsfoot
Truncilla donaciformis	Fawnsfoot
Truncilla macrodon	Texas Fawnsfoot
Truncilla truncata	Deertoe
Uniomerus declivis	Tapered Pondhorn
Uniomerus tetralasmus	Pondhorn
Utterbackia imbecillis	Paper Pondshell
Utterbackiana suborbiculata	Flat Floater
Villosa lienosa	Little Spectaclecase

Appendix C. Freshwater Mussel Reconnaissance Survey Form

Project Information

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

Habitat Description

Water Temp ©	**Air Te©(C)**
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.?	

Substrate Types (%)				
Bedrock	**Boulder**	**Cobble**	**Gravel**	**Pea Gravel**
Sand	**Silt**	**Clay**	**Detritus**	**Woody Debris**

Visibility (cm)	**Average Depth (m)**	**Max Depth (m)**
Evidence of current or historic freshwater mussel presence (shell material)?		
Other habitat variables precluding mussel presence?		

Appendix D.

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., Instream Survey, 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 E.
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	505 Staples Rd. Bldg. 2 San Marcos, Texas 78666	(512) 389-8612	(512) 422-8054

Region 2

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

Region 3

Contact	District Office Address	Office Phone	Mobile Phone
Nicole Plowman	1502 FM 517 East, Dickinson, Texas 77539	(281)-534-0114	(281) 705-5592

Region 4

Contact	District Office Address	Office Phone	Mobile Phone
Alex Nuñez	1409 Waldron Rd Corpus Christi, Texas 78418	(361) 658-3181	(361) 658-3181

24-Hour Phone : 512-389-4848

U.S. Fish and Wildlife Service

Arlington Texas Ecological Services Field Office

Contact	Office	Office Phone	Email
Jennifer Khan	3233 Curtis Drive	(682) 432-6281	Jennifer_Khan@fws.gov

Austin Texas Ecological Services Field Office

Contact	Office	Office Phone	Email
Matthew Johnson	1505 Ferguson Lane Austin, Texas 78754	(512) 577-0989	Matthew_S_Johnson@fws.gov

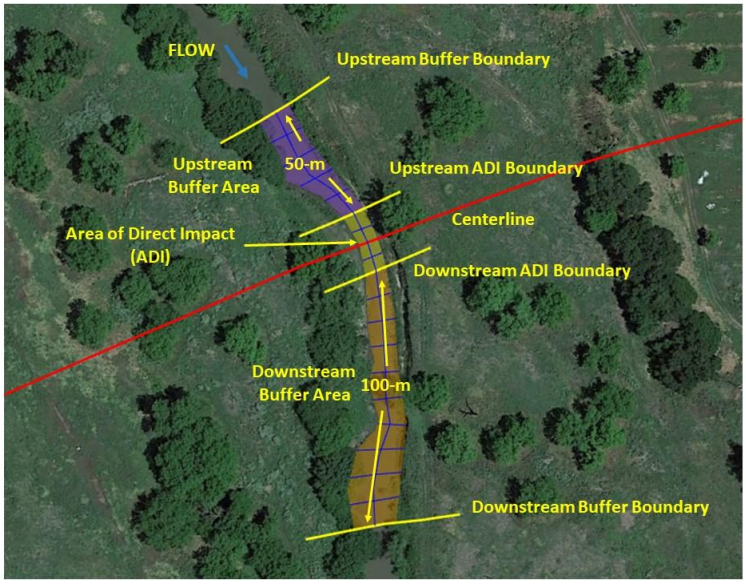
Texas Coastal Ecological Services Houston Field Office

Contact	Office	Office Phone	Email
Courtney Dvorsky	17629 El Camino Real, Suite 211 Houston, Texas 77058	(281) 212-1504	Courtney_Dvorsky@fws.gov

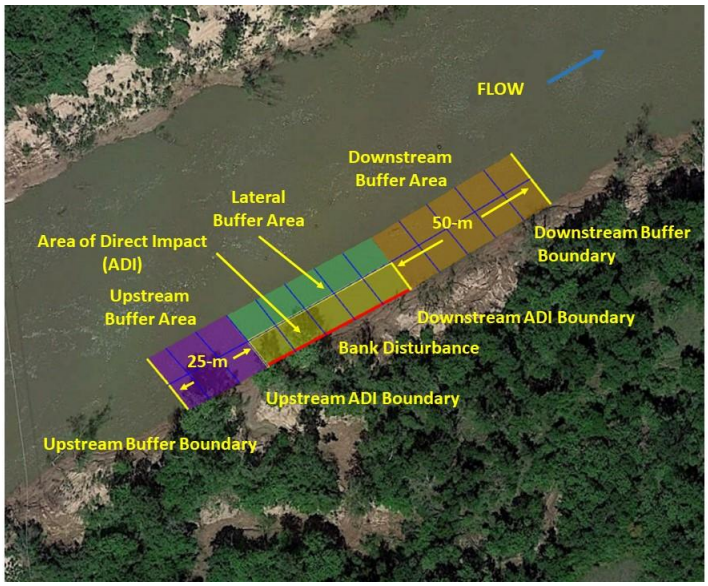
Appendix F.

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.

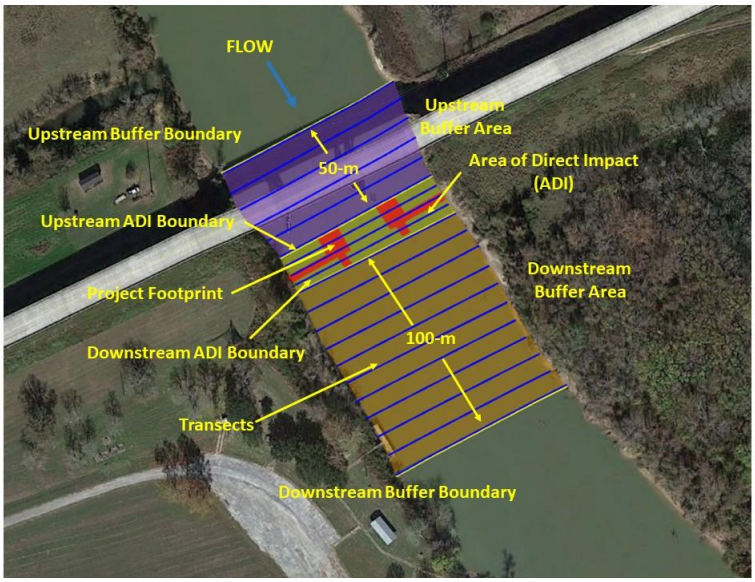


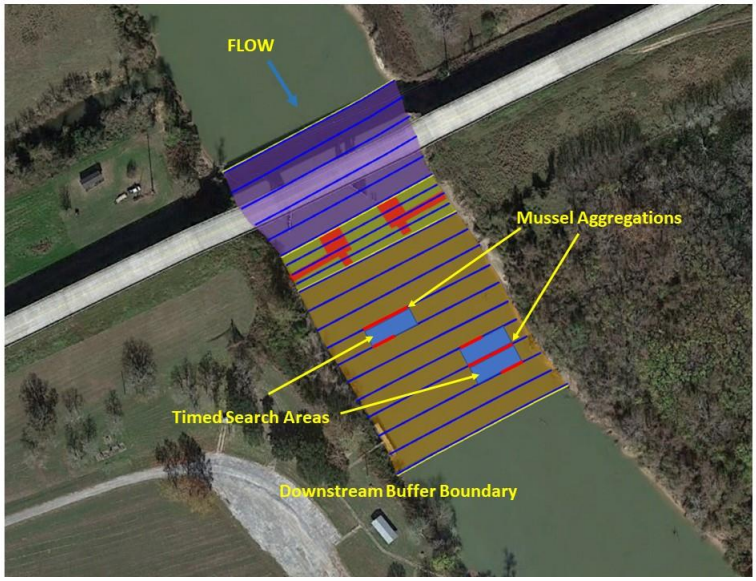
Example 2. A bank stabilization project on a Group 2 stream is proposed. The proposed ADI will occur along 50 m of streambank and extend 10 m into the river. As the survey is being conducted for a streambank disturbance project in a Group 2 stream, the prescribed sampling protocol includes a Cell Survey 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.



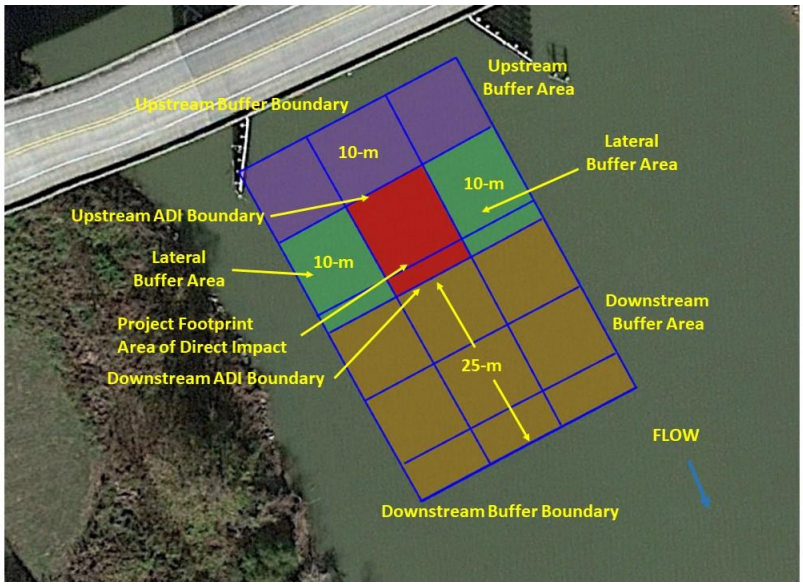
Example 3. A bridge widening project, assuming no work is to be completed on the existing bridge, adding a new bridge structure over a Group 2 stream is proposed. The proposed project will include the creation of two causeways extending from each bank which will serve as work areas for the construction 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.

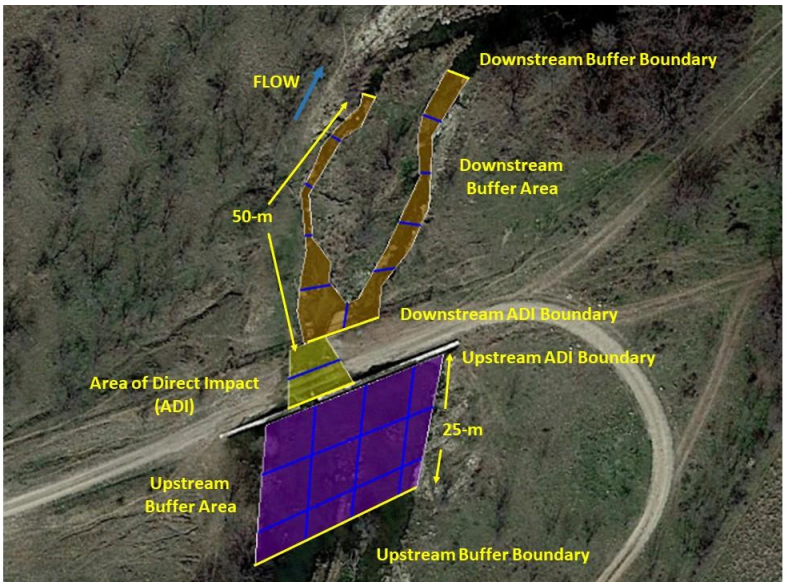




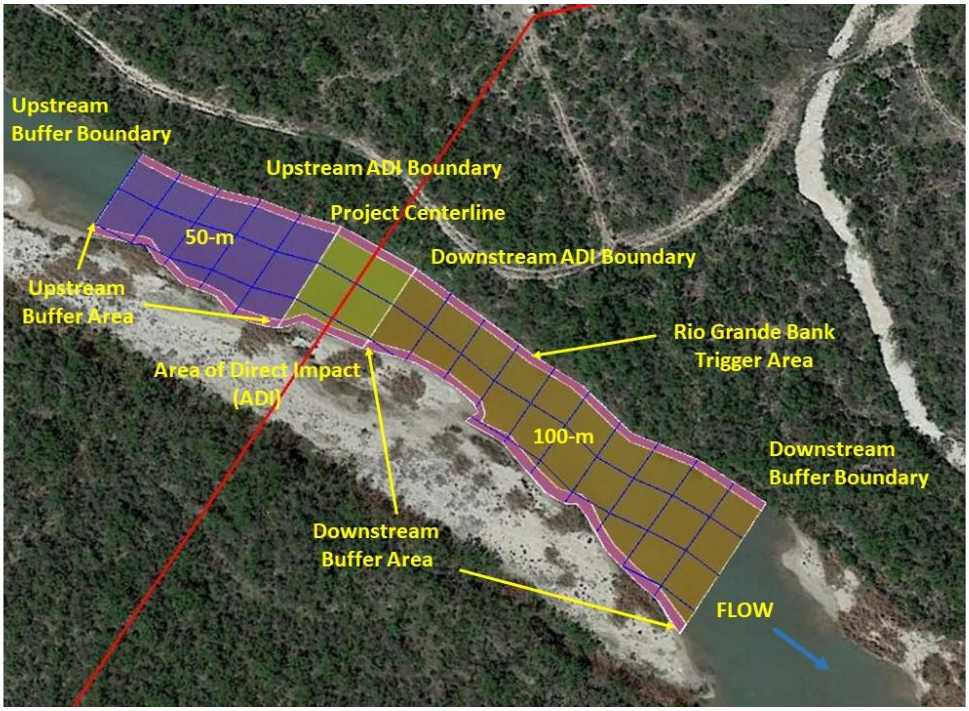
Example 4. A bridge project over a Group 4 stream is proposed. The proposed construction plan utilizes a barge platform to reduce the instream footprint and avoids the need for causeways. The proposed construction footprint is 13 m x 10 m in size. As the survey is being conducted for a bridge construction 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 G.

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.