This protocol is a living document and may change over time and will be updated as relevant data become available. These methods represent the minimum amount of effort needed to conduct a mussel survey. These protocols are typically used for, but not limited to, construction and maintenance type projects that may impact aquatic life.

A. Desired Surveyor Qualifications
Personnel who will be conducting surveys for freshwater mussels should have sufficient knowledge within the river basin they propose to survey. This includes familiarity with species-specific biology and ecological requirements, and the ability to identify mussel species from the basin, particularly state and federally listed species. A potential mussel surveyor should have field experience and possess demonstrable skills in the independent execution of standard freshwater mussel survey methods. Surveyors should be able to demonstrate their experience in the safe-care and handling of freshwater mussels. Individuals familiar with freshwater mussels, but lacking experience with rare species, should work with a malacologist who is familiar with those species.

B. Review of Existing Information
Prior to any fieldwork, surveyors should conduct a thorough review of available resources to determine if historical freshwater mussel data exists for a particular site or river basin. This data should include historical distribution information, life history information such as spawning and brooding behavior, and general ecology information such as burrowing behavior and habitat preferences. Potential information resources include peer-reviewed journal articles, published and unpublished reports, TPWD’s Natural Diversity Database, museum collections, and malacologists who have experience with the relevant species or water body. This information can be used to develop a preliminary list of mussel species historically known from an area and help determine appropriate sampling. Absence of available data or other information does not imply that a mussel species is absent from a particular area. Historical data can only be used to indicate that mussels have been found in past surveys but cannot be used as a definitive statement to presence/absence of a mussel species within a particular area. If a survey has been conducted in the area of interest within the last two years, and no mussels were found at that time, TPWD will review the previous survey methodology and results to determine if an additional survey is necessary.

C. Survey Time Frame and Proper Handling of Specimens
Mussel sampling is to be conducted during the months of April through November (Carlson et al. 2008) or when water temperatures are greater than or equal to 60°F (Mackie et. al. 2008) and
avoiding spawning and glochidia release timeframes for state-listed mussel species (when known). Sampling outside of this time frame will need prior authorization from TPWD. Sampling during warm water periods when mussels are more active will allow individuals disturbed during sampling to re-establish themselves in the substrate. Disturbing mussels during cold temperatures could potentially cause adverse impacts to mussels by increasing their vulnerability to predation, or causing individuals to be swept downstream due to slower re-anchoring capabilities. Sampling could also be impacted by cold temperatures from decreased detection rates as the result of seasonal vertical mussel migrations (Amyot and Downing 1997, Schwalb and Pusch 2007, Block et.al. 2013), and/or decreased surveyor efficiency.

To minimize stress on mussels, live or suspected-live native mussels should be handled gently at all times and be placed back to the original location where they were found. An exception to returning native mussels to their original location is if mussels are being handled under an approved Aquatic Resource Relocation Plan (ARRP) which states that mussels will have to be removed from harm’s way due to potential impacts from any construction and maintenance type project (Section F). Mussels should not be exposed to air any longer than necessary (less than 10 minutes) for identification, measurement, and photographic documentation. Mussels should be kept in mesh bags submerged in the water at all times when not being processed. Once identified, native mussels should be returned to their original point of collection except under an ARRP as described above. All native mussels should be carefully placed partially into the sediment ensuring that the posterior side is facing upwards above the sediment. If there is uncertainty as to the posterior side, then the mussel should be placed on the substrate surface and left to burrow into the sediment. Typically, the surveyor should only retain shells or dead mussels, but live mussels may be kept if species verification is necessary.

D. Survey Area Delineation

The survey area for mussels in lotic (flowing water) habitats shall include areas of the wetted stream bed that would be directly impacted by the project footprint (e.g., bridge support structure, in-channel coffer dam, dewatered area, etc.) as well as areas that could potentially be impacted from project activities (e.g. sedimentation). As such, the survey area should include the entire project footprint, as well as buffers for areas of potential impact that include a minimum of 50 m upstream of the project footprint, and a minimum of 100 m downstream of the project footprint. As these are minimum buffer lengths, projects with larger instream project footprints and/or potential for larger downstream sediment plumes (e.g. dredging), longer downstream buffer lengths may be required.

The survey area for mussels in lentic (still water) habitats (e.g. bridges over reservoirs) shall include the wetted area that would be directly impacted by the project footprint (e.g., bridge support structure, in-channel coffer dam, dewatered area, etc.) as well as areas that could potentially be impacted from project activities (e.g. sedimentation). As such, the survey area should include the entire project footprint area, as well as buffers for areas of potential impact.
that include a minimum of 50 m upstream of the project footprint, and a minimum of 50m downstream of the project footprint.

The survey area for mussels along reservoir shorelines (not to include a bridge) shall include the wetted area that would be directly impacted by the project footprint as well the areas that could be potentially impacted from project activities (e.g. sedimentation). As such, the survey area should include the entire project footprint area, as well as a buffer for areas of potential impact of 20 m out from the project footprint.

E. Survey Procedures
Survey protocols for wadeable and non-wadeable waterbodies, as well as reservoir shorelines, are presented below. The level of effort for the survey procedure is generally based on whether or not the location is wadeable or non-wadeable and whether state listed species are present.

If state listed mussel species have previously been found in the vicinity of the project area during previous mussel surveys, then bypass the initial survey method and proceed with the comprehensive survey methods (referred to in this document as a quantitative survey) described below, for either wadeable or non-wadeable locations.

Wadeable

Initial Survey
A stream or river is considered wadeable if ≥50% of the channel is accessible by wading during normal flow conditions. Generally these streams are third order or less. Pool areas or high-flow conditions may make the stream inaccessible to wading in certain places or at certain times (but does not preclude these areas from being searched); however, the stream will still be considered wadeable when determining which survey procedures to employ.

The initial survey for wadeable locations is a qualitative survey (Strayer and Smith 2003) during which sampling is used to establish the presence/absence of state-listed mussel species. This method allows the surveyor to also develop a species list of mussels present within the survey area using timed searches. Because the amount of survey effort (person-hours) varies by water body type to obtain an adequate representation of the mussel species present (Huang et al. 2011), a minimum search time of five person-hours (divided into five one person-hour searches) is required within the delineated search area as described in Section D above. At the end of each search period (e.g. a one person-hour search), mussels should be identified and retained in mesh bags submerged in the stream. If no new species of mussels are collected during the fifth search period, the survey is complete. If at least one new mussel species is collected in the fifth search period, additional one person-hour search periods are required until no new species are collected. If at any time during the timed search periods a live state-listed mussel species is encountered, terminate the initial survey process and begin the quantitative survey.
Visual, combined with tactile searching (hand-grubbing into the top 1-4 inches of substrate to increase detection of more-deeply buried mussels) should be used. Searchers should select a shoreline and begin searching from downstream to upstream moving back and forth across the stream, ensuring that all of the delineated search area is sufficiently covered. For areas in the delineated search area that are non-wadeable (depths greater than 3 ft), SCUBA or surface supplied air (i.e. hookah) equipment should be utilized to efficiently sample those areas.

Searchers should stop occasionally in areas of loose or fine substrate and hand grub to detect mussels not visible at the substrate surface. Areas of mixed loose gravel should be fanned occasionally to detect mussels hidden between the substrate. Searchers need to explore all habitat types within the search area including riffles, banks, pools, and backwater areas to locate species that prefer these habitats. Additional habitat types that should be searched if present within the site include crevices in bedrock, root-wads, undercut banks, and woody-debris.

All live mussels collected during the timed searches shall be identified, enumerated, and one color photograph should be taken of each live mussel species and of the total mussels collected (for quality assurance purposes).

**Quantitative Survey**

When state-listed mussel species are present within the delineated search area, quantitative sampling methods are necessary to remove mussels from the delineated survey area to minimize take of these species due to construction and maintenance type projects.

A multiple-pass depletion survey method is required within the survey area to ensure that the majority of state-listed mussel species present (and all other subsequently collected mussel species) at the site are removed for relocation. The delineated survey area would be divided into 1 m wide lanes parallel to flow for the entire wetted width of the survey area and for the entire length of the delineated survey area which includes the project footprint and the upstream and downstream buffer. Each 1 m wide lane is divided into 1 m x 5 m cells (or any cell arrangement providing full coverage of the delineated search area with cells no greater than 5m2) (Figure 1) and each cell will be searched for a minimum of two 10 minute search periods with all live mussels collected in each search period identified and enumerated separately. At the end of the second 10 minute search period if the catch rate (number of individual mussels) is less than 20% of the first 10 minute search period, then that cell is complete and the surveyors may move on to the next cell.

If the catch rate during the second 10 minute search period is greater than 20% of the first 10 minute search period then additional 10 minute search periods are required until the catch rate is less than 20% of the first search period for that cell.
All live mussels collected during the multiple-pass depletion surveys shall be identified, enumerated, and one color photograph should be taken of each live mussel species and of the total mussels collected (for quality assurance purposes).

**Figure 1.** Example cell layout for multiple-pass depletion type comprehensive surveys for wadeable locations (not drawn to scale, for illustrative purposes only).

**Non-Wadeable**

**Initial Survey**

In non-wadeable locations, difficulty exists in efficiently using timed searches to determine if mussels are present and their species composition. Therefore, semi-quantitative survey methods are required to adequately determine the mussel species present in the delineated survey area. Due to water depths that are prohibitive of snorkel surveys (generally greater than 3 ft) in non-
wadeable sites, SCUBA or surface supplied air (i.e. hookah) equipment may be required to perform the survey.

In order to ensure adequate coverage of the site, transects oriented parallel with flow (or the bank in lentic habitats) should be placed on both left bank and right bank of the delineated survey area and additional transects equally spaced between these at intervals no greater than 5 m. Each transect is then divided into 10 m segments, where a minimum of 10 minutes of search time is conducted for each segment using tactile searches along the transect and a meter on both sides of the transect centerline until all segments of each transect have been surveyed. At the end of each search period within each 10 m segment, all mussels are identified, enumerated, and placed in mesh bags submerged in flowing water.

When all transects have been surveyed, the area around segments with the highest mussel abundances will be resurveyed for an additional one person-hour and will include the area between the adjacent transects on both sides of the segment. If no additional species are collected, the survey is complete for that area. Additional one person-hour searches will be conducted in the area until no new species are collected.

All live mussels collected during the timed searches shall be identified, enumerated, and one color photograph should be taken of each live mussel species and of the total mussels collected (for quality assurance purposes).

Quantitative Survey
When state-listed mussel species are present within the delineated search area, quantitative sampling methods are necessary to remove mussels from the delineated survey area to minimize take of these species due to construction and maintenance type projects.

A multiple-pass depletion survey method is required within the survey area to ensure that the majority of state-listed mussel species present (and all other subsequently collected mussel species) at the site are removed for relocation. Utilizing the mussel density information obtained from the previously established transects during the initial survey, the segments that contain 75% of the total mussel abundance can be identified. Additional transects (along the entire length of the survey area for convenience) will be placed at 2.5 m (parallel to the existing transect) on each side of the previously established transect identified with a segment with high mussel abundance. The search area for the multiple-pass depletion survey will then be each identified segment with high mussel abundance, and each adjacent segment (up, down, left and right) surrounding it. Each segment will be searched for a minimum of two 20 minute search periods with all live mussels collected in each search period identified and enumerated separately.

At the end of the second 20 minute search period, if the catch rate (number of individual mussels) is less than 20% of the first 20 minute search period, then that cell is complete and the survey may move on to the next cell. If the catch rate during the second 20 minute search period
is greater than 20% of the first 20 minute search period then additional 20 minute search periods are required until the catch rate is less than 20% of the first search period for that cell.

All live mussels should be handled gently and remain in mesh bags submerged in flowing water while not being processed. All live mussels collected during the multiple-pass depletion surveys shall be identified, enumerated, and one color photograph should be taken of each live mussel species and of the total mussels collected (for quality assurance purposes).

**Reservoir Shorelines**

**Initial Survey**
Mussel surveys for reservoir shoreline projects that do not include a bridge component (reservoir bridge surveys should follow the wadeable/non-wadeable guidelines described above) may include wadeable and non-wadeable areas within the delineated search area (see Section D) and may require the use of SCUBA or surface supplied air (e.g. hookah) equipment. The initial survey will comprise of a qualitative survey utilizing timed searches. A minimum search time of five person-hours (divided into five one person-hour searches) is required within the delineated search area as described in Section D above. At the end of each search period (e.g. a one person-hour search), mussels should be identified and retained in mesh bags submerged in water. If no new species of mussels are collected during the fifth search period, the survey is complete. If at least one new mussel species is collected in the fifth search period, additional one person-hour search periods are required until no new species are collected. If at any time during the timed search periods a live state-listed mussel species is encountered, terminate the initial survey process and begin the comprehensive survey.

Visual, combined with tactile searching (hand-grubbing into the top 1-4 inches of substrate to increase detection of more-deeply buried mussels) should be used. Searchers should spread throughout the delineated search area, and search moving back and forth across the area ensuring that all of the delineated search area is sufficiently covered. For areas in the delineated search area that are non-wadeable (depths greater than 3ft), SCUBA or surface supplied air (i.e. hookah) equipment should be utilized to efficiently sample those areas.

All live mussels collected during the timed searches shall be identified, enumerated, and one color photograph should be taken of each live mussel species and of the total mussels collected (for quality assurance purposes).

**Quantitative Survey**
When state-listed mussel species are present within the delineated search area, quantitative sampling methods are necessary to remove mussels from the delineated survey area. A multiple-pass depletion survey method is required within the survey area to ensure that the majority of state-listed mussel species present (and all other subsequently collected mussel species) at the site are removed for relocation.
A minimum of 6 transects parallel to the shoreline will be established within the delineated survey area. One transect will be placed along the shoreline edge (this should be at average pool elevation if sampling is occurring at above average pool elevation) with the 5 remaining transects to be placed at equally spaced intervals of 20% of the width of the delineated survey area. Each transect shall be divided into a maximum of 10 m segments. Each segment will be searched for a minimum of two 20 minute search periods, to include a meter on each side of the transect, with all live mussels collected in each search period identified and enumerated separately. At the end of the second 20 minute search period, if the catch rate (number of individual mussels) is less than 20% of the first 20 minute search period, then that segment is complete and the survey may move on to the next cell.

If the catch rate during the second 20 minute search period is greater than 20% of the first 20 minute search period then additional 20 minute search periods are required until the catch rate is less than 20% of the first search period for that cell.

All live mussels collected during the multiple-pass depletion surveys shall be identified, enumerated, and one color photograph should be taken of each live mussel species and of the total mussels collected (for quality assurance purposes).

F. Mussel Relocation Protocols
Prior to any mussel relocation activity, it will be necessary to have an approved ARRP, along with a stocking/relocation permit. All mussels collected during survey efforts need to be relocated as a best management practice in order to minimize impacts to mussels from construction and maintenance projects. At a minimum, all state-listed mussels should be tagged, preferably with a passive integrated transponder (PIT) tag, to aid in the recovery of relocated individuals during post-relocation monitoring. Mussel relocation protocols should follow those outlined in Tsakiris and Randklev (2014).

Mussel relocation sites should be located well outside of the project’s area of direct and indirect impact (preferably upstream), and ideally nearby the survey site (relocation sites identified that are greater than 5 river miles from the survey sites will need prior TPWD approval). Mussel relocation sites should be of similar or better quality to that of the survey area and should be of the same habitat type (e.g. run, pool, backwater, etc.), comparable area of habitat, and comparable depths and velocities from which the mussels were initially collected. Efforts should be made to relocate mussels to a site that already has established mussels of the same species, which should ensure that hydraulic habitat conditions are suitable for relocated mussels, as well as evaluate the site for any potential future threats (e.g. unstable banks, land-use changes, etc…) which would preclude the site from being acceptable.

Mussels should be transported in an ice chest with a layer of ice to keep them cool and moist. Mussels should be wrapped in a wet towel and a piece of cardboard or similar material should be
placed over the ice to protect the mussels from coming in direct contact with the ice and meltwater.

At the relocation site, the mussels should be carefully placed partially into the sediment ensuring that the posterior side is facing upwards above the sediment. If there is uncertainty as to the posterior side, then the mussel should be placed on the substrate surface and left to burrow into the sediment.

Post-relocation monitoring is an integral component to mussel relocation and for assessing the efficacy of relocation efforts for the conservation of these species. Post-relocation surveys should be conducted around one month post-relocation to assess short-term survival of relocated individuals. Observed mortality rates should be less than 10% one month post-relocation. If observed mortality rates are greater than 10% one month post-relocation, TPWD shall be notified and alternative relocation sites shall be discussed. A one year post-relocation survey should also be conducted to assess long-term survival of relocated individuals. Observed mortality rates should be less than 20% one year post-relocation. If observed mortality rates are greater than 20% on year post-relocation, TPWD shall be notified and alternative relocation sites shall be discussed.
References


