A literature review of Freshwater Mussel Survey and Relocation Guidelines

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Executive Summary

This report summarizes the results of a literature review on sampling guidelines and methodologies for freshwater mussels (Family: Unionidae), identifies research needs based on that review and provides recommendations for when and how to conduct mussel surveys and relocations. This report pertains to persons planning bridge construction projects, but can be adapted for other instream activities. We examined peer-reviewed articles and grey literature (e.g., technical reports) using online database search engines. Only studies that offered detailed descriptions of sampling and relocation methods were considered, and each study was categorized by the following regions: Southeastern United States, North Eastern United States, Canada, and Europe. In total, we reviewed 98 published articles and technical reports, including 84 from the United States (mostly from the southeast) and 14 from Canada and Europe. Based on this review, we recommend that mussel surveys associated with instream construction projects follow a tiered dichotomized (wadeable vs. nonwadeable) sampling strategy. First, surveys should begin with a historical record search followed by a land-based review to evaluate if mussels may occur near the project location. Second, if surveys are necessary and the waterbody is considered wadeable, qualitative sampling within a fixed area should be performed to determine mussel presence at the project location. If the waterbody is nonwadeable and the goal is to determine presence then a combination of qualitative and semi-quantitative transect sampling should be considered. If mussels are found during a qualitative survey then alternative construction activities should be considered in coordination with regulatory agencies as appropriate. In cases where construction activities cannot be avoided, then mussels should be removed and relocated to suitable habitat nearby and monitored yearly for a period of two years. Finally, if information on population size and structure are needed, then quantitative sampling using 0.25 m^2 quadrats and excavating substrate should be performed.

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Introduction

Freshwater mussels (Bivalvia: Unionidae) are among the most imperiled groups of aquatic organisms in North America (Strayer et al. 2004). Destruction of habitat, loss of host fishes, introduction of invasive species (e.g., zebra mussels) and river impoundments have contributed to population declines and extinctions (Bogan 1993; Williams et al. 1993; Lydeard et al. 2004; Strayer et al. 2004; Downing et al. 2010). Freshwater mussels are filter feeders and through their excretion, deposition of feces and pseudo-feces and physical presence contribute to nutrient dynamics, trophic interactions and habitat availability. As such, the loss of mussels from streams and rivers has likely had negative consequences to ecosystem function and other aquatic communities (Vaughn and Hakenkamp 2001; Allen and Vaughn 2011).

As sedentary bottom-dwelling filter feeders mussels are susceptible to numerous environmental impacts such as sedimentation, pollutants and habitat destabilization (Bogan 1993; Mehlhop and Vaughn 1994). Additional threats to mussels include sand and gravel mining, dredging and channelization, impoundments and flow alterations (Neves et al. 1997; Vaughn and Taylor 1999). Construction activities that can potentially change sediment deposition, erosion patterns, substrate composition, and local hydrology can negatively affect mussel population performance (i.e., growth and reproduction) in addition to causing direct mortality (Ellis 1936; Marking and Bills 1979). Because of this it is important to implement mussel surveys for projects where there is a risk of these types of impacts to ensure that threatened species are not harmed as a result of construction activities.

Transportation agencies continue to promote economic growth through infrastructure development; however, with this development comes the responsibility of preserving important natural resources. When road or bridge construction is proposed, an environmental assessment is conducted to determine the potential consequences to sensitive species or critical habitat. For freshwater mussels, surveys are often performed to determine their presence and distribution within a project area. If mussels are found, rare or common, a relocation plan is often implemented wherein mussels are moved to minimize impacts from construction activities.

In Texas, 15 of the 52 mussel species are listed as state-threatened (Texas Parks and Wildlife [TPWD] 2010) and 12 of these species are now being reviewed for protection under the Endangered Species Act (USFWS 2001, 2011). To date, there are no established guidelines for the survey or relocation of state-threatened or federally listed or candidate species in Texas. Currently, methods for collecting and relocating mussels have been left to the discretion of individual biologists or the TPWD Aquatic Resource Relocation Plan process and as a result methods are inconsistent throughout the state. Standardized survey and relocation guidelines are an important component of any mussel mitigation strategy because they reduce collector bias and sampling error and facilitate statewide comparison of mussel data. From a project planning perspective, having standardized protocols can support effective project scoping and planning, which can improve the efficiency of project delivery, ensuring consistent consideration of mussel conservation into the project planning process.

Given the importance of minimizing impacts to state- and potentially federally listed mussel species along with the lack of established mussel survey guidelines in Texas the objectives of this project were the following: 1) summarize the literature on sampling guidelines and methodologies; 2) identify research needs; and 3) provide recommendations for when and how to conduct surveys and relocations for freshwater mussels.

Methods

To evaluate sampling guidelines and methodologies for freshwater mussels in the literature, we reviewed peer-reviewed papers and grey literature. We focused our efforts on examining the current knowledge of 1) procedures used to review historical mussel data within the project area (i.e., historical searches), 2) assessment of suitable habitat (i.e., land-base reviews), 3) sampling guidelines, 4) surveyor experience and permitting, 5) reporting guidelines, 6) stress to mussels from sampling and handling, and 7) translocations. These categories were chosen following USFWS and VDGIF (2008). The resulting information was then used to provide recommendations and identify research and implementation needs for sampling and translocating mussels at project locations. Literature searches were conducted using online database search engines and focused primarily on the following journals: *Freshwater Science* (formerly the *Journal of North American Benthological Society*), *American Midland Naturalist*, *Southeastern Naturalist*, and *Journal of Shellfish Research*. Search key words included: freshwater mussel translocation, freshwater mussel survey methods, Unionids, and sampling protocol. We selected these journals because of their long standing in mussel related research. Published grey literature was obtained from state and federal agency sources (web pages, bulletins etc.). For inclusion in the review, studies had to meet the following criteria: 1) must be work (research, sampling protocols etc.) related to freshwater mussels; and/or 2) contained information or offered descriptions of methods, which were deemed useful for designing a standardized sampling protocol. After selecting the articles, we organized them into the following regions: Southeastern United States, North Eastern United States, Canada, and Europe.

Analysis and Discussion

Historical searches

Historical searches refers to actions such as consulting with experts, examining range maps and reviewing the literature for habitat requirements, life-history and ecological information for target species (Piette 2005; Carlson et al. 2008; Mackie et al. 2008). This information is important because it can be used to determine the likelihood of a species occurring within a project survey area, whether a proposed activity may impact the target species and is invaluable for sampling program design [e.g., selecting appropriate sampling methods, level of effort, timing of sampling, and target habitats] (Mackie et al. 2008). This information is also useful for identifying potential translocation sites (Mackie et al. 2008; Luzier and Miller 2009; Butler et al. 2012; MacCallum 2013; Tiemann 2014).

The percentage of literature that provided specific recommendations on how to conduct historical searches was low (~8%; 8/98). Of the 8 that provided guidelines, the following unranked list summarizes the steps that were recommended: (1) range maps showing the historical and current distribution of the species in question; (2) descriptions of optimal vs. suboptimal habitat; (3) life history information pertaining to spawning season and fish hosts; (4) consulting with regional malacologists and state and federal biologists; (5) reviewing published journal articles and grey literature; (6) assessing federal register documents; and (7) visiting local, state and university museums (Piette 2005; Carlson et al 2008; Grabarkiewicz 2008; Luzier and Miller 2009; Huang et al. 2011; Butler et al. 2012). For example, Huang et al. (2011) used museum records to guide site selection for a study assessing sampling adequacy in Illinois Streams. In Canada, surveyors use the Aquatic Species at Risk (SAR) mapping tool developed by the Department of Fisheries and Oceans (DFO), which displays the likelihood of occurrence for threatened species, to determine whether surveys are needed at a given project site (Mackie et al. 2008). Similar mapping applications are provided by the Wisconsin Department of Transportation (Piette 2005), Texas Parks and Wildlife Department (http://tpwd.texas.gov/gis/rtest/), Georgia Department of Natural Resources (http://www.georgiawildlife.com/conservationstatus_assessment_maps) and USFWS, though the latter two are primarily used to support state and federal status assessments for threatened species.

Recommendation

In summary, our review revealed that there was some guidance on how to perform historical searches and which types of data and literature should be used. Based on this guidance and our own survey experience, we recommend surveyors should consult current and historical range maps to determine what species may be encountered at a particular site. Ideally, such range maps should be made available by governmental agencies or universities using a program like the DFO, the Conservation Status Assessment Maps provide by the GDNR, the Wisconsin Department of Transportation's Mussel Atlas, or the Texas County Species Lists provided by TPWD and USFWS.

However, a challenge when using the latter two resources is the information provided is often at a scale too coarse for determining whether a survey should be performed for a given project. Moreover, for collection information near a project it is often difficult to access the primary data to determine the level of effort for that record. Because of this, we recommend that agencies responsible for maintaining these tools provide the following: 1) maps at a scale relevant for identifying survey gaps and potential environmental impacts; and 2) collection data to determine the exact location and level of effort for a particular record. Surveyors should consult multiple sources when trying to determine the most current distribution of mussels as state and federal agencies may not share or use the same inventory data. Surveyors should also review life history and ecological information, such as spawning season and habitat associations, to determine timing, habitat and appropriate methods of collection for the target species. This information can be found in published journal articles or technical reports, museums, state and federal wildlife agencies, NGO's, and malacologists who have experience in the specific area of interest.

Research needs:

1. Centralize existing mussel data to develop a mussel database containing up-to-date information on the location of mussels in Texas that can be easily queried. Centralized database could also be used to update mussel inventory data maintained by state and federal agencies.

2. Mussel Atlas showing distribution for all mussel species in Texas and a fact sheet for each, though emphasis should initially be placed on threatened species.

Implementation needs:

1. A list of individuals maintained by regulatory agencies that have been determined to be experts based on species-specific expertise or experience with their regional or river basin mussel fauna. This list should include only individuals that have successfully passed a mussel identification test and have at least 3 years of field experience in their region or basin.

2. Increased coordination between federal and state agencies to share species inventory data to ensure consistency in range maps showing the historical and current distribution of threatened mussel species.

Land-based review/site assessment

Land-based reviews/site assessments are performed to determine if mussel surveys are warranted at or near a project area and to provide guidance on selecting appropriate translocation and reintroduction sites. These assessments often entail determining whether or not the water body within the project area is perennial (USFWS and VDGIF 2008), contains suitable mussel habitat (Havlik 1997; Levine et al. 2003; Christian et al. 2005; Miller and Mosher 2008; Peck et al. 2014), or if mussels are present (based on live individuals or shell material). Assessments can include a combination of site visits and desktop reconnaissance efforts utilizing USGS stream gage data and aerial imagery (Carlson et al. 2008). The former is used to determine when stream levels are near base flows and temperatures are above predefined thermal criteria and the latter is used to determine whether a stream is perennial or intermittent, though gage data will also show this (Piette 2005).

The results of the literature review revealed that for the U.S., Canada and some European countries there is some guidance for conducting land-based reviews/site assessments. Of the 84 articles reviewed from the United States 22 (26%) performed a land-based review/site assessment and also provided guidelines on how to do so. For Canada and Europe, four papers or 28% of the 14 articles reviewed performed land-based reviews/site assessments while only two specifically provided guidelines for conducting these reviews. The purpose of the land-based reviews/site assessments varied but all used these surveys, in part, to determine whether the project survey area was publicly accessible, had environmental conditions conducive for sampling or contained suitable mussel habitat. Environmental factors assessed during these reviews included stream flow (e.g., using nearby USGS gaging stations), aerial imagery, weather conditions and water quality parameters [e.g., chemical, temperature, turbidity] (Carlson et al. 2008; Huang et al. 2011). For studies using these assessments to identify suitable habitat the following criteria were used: waterbody type (perennial vs. intermittent: USFWS and VDGIF 2008; Carlson et al. 2008) stable substrate (Dunn et al. 2000; Cope et al. 2003; Mackie et al. 2008; MacCallum 2013), riparian features such as presence of natural vegetation (Carlson et al. 2008), channel stability (Carlson et al. 2008), and overall site accessibility (Piette 2005; Carlson et al. 2008; Huang et al. 2011; MacCallum 2013). For several of the reviewed studies if the project area did not meet these criteria then the site was determined to be unsuitable for mussels and surveys were not performed (Levine et al. 2003; Mackie et al. 2008; USFWS and VDGIF 2008).

Recommendation

The results of this review suggest that although there is some guidance for how to conduct a land-based review/site assessment, the majority of the articles reviewed did not consider these types of assessments prior to implementing a survey within a project area. For the few articles that did provide guidance, the objectives of those assessments and the criteria used to either exclude potential project sites from sampling or to refine sampling methods varied considerably. This lack of consistency may be due to limited knowledge on habitat requirements and tolerance of many mussel species to potential project impacts, which is a key challenge for all applications. However, there are general environmental factors for defining mussel habitat broadly regardless of project type, geographic location and focal species. For example, Strayer (2008) and Newton et al. (2008) argued that the lack of scientific support for specific mussel-habitat associations was related to a mismatch in scales of observation between researchers and mussels. As a result, they proposed a mussel-centric approach where habitat is defined based on what each mussel species requires of its environment. This framework entails identifying key properties of habitat (i.e., functional attributes) that influence survival, growth and reproduction and then translating these processes to environmental variables. Using this approach, Strayer (2008) proposed the following key attributes of suitable mussel habitat: support, stability, food availability, temperature, protection against predators and toxicants, and presence of host fish (Table 1). Although this framework has not been rigorously tested, it is based on well-substantiated explanations of mussel habitat and so we believe it is useful for project planning because it allows non-specialists and resource managers to conceptualize and identify potential mussel habitat. In addition to using this frame work, we also recommend that surveyors spend time determining whether the site is: 1) publicly accessible, and if not, adjacent landowners should be contacted; and 2) if there are USGS gaging stations and TCEQ stations located nearby so that water quality data as well as current weather conditions can be evaluated prior to sampling to ensure conditions are safe for survey work and to assist with planning and logistics (Carlson et al. 2008; Huang et al. 2011).

Table 1. Proposed functional characteristics of suitable mussel habitat following Strayer (2008) and Newton et al. (2008).

Attribute	Description
Support	Substrate that is soft enough for burrowing but firm enough to support and anchor mussels
Stability	Substrate stays in place during floods, remains wetted during periods of low flow, stable during periods of juvenile settlement
Food availability	Water transport is adequate to deliver food and other essential materials and to remove wastes
Temperature	Favorable temperature for growth, reproduction, and survival
Protection against predators	Sediment composition, stream bed topography, and presence of woody debris may protect adults and juveniles from increased predation rates
Toxicants	Absence of toxic concentrations of pollutants
Presence of host fish	Primary host fish are available during spawning

Research needs:

1. Determine species-specific habitat requirements for adults and juveniles, to include information on threats like sediment loading, siltation, pesticides and other pollutants, and identify general water quality requirements (i.e., water temperature, pH, salinity, DO).

2. Development of modeling tools for state-threatened mussel species, which can be used for planning and prioritizing surveys.

Implementation needs:

1. Use framework presented by Strayer (2008) and Newton et al. (2008) as an assessment tool to help distinguish suitable vs. unsuitable mussel habitat at a given project site. This tool should be developed in coordination with TPWD and USFWS to ensure regulatory compliance at the state and federal level.

Sampling guidelines

Conducting surveys to determine whether or not mussels occur within a project area is an important component of mussel conservation and management programs. Good sampling designs should have well-articulated objectives, use standardized methods and account for incomplete detection (Yoccoz et al. 2001). Generally, well-articulated sampling designs should address three basic questions: 1) why survey; 2) what to survey; and 3) how the survey should be carried out (Yoccoz et al. 2001). Detectability refers to the probability that a species will be found if it is present (Martin et al. 2006) and is influenced by observer effects, a species' life history and environmental conditions, among others (Yoccoz et al. 2001, Martin et al. 2006). Standardization refers to using the same or similar sampling methods, which is important for reducing heterogeneity in detectability and for permitting comparisons of results between studies conducted by different researchers. Sampling programs should clearly define and state their goals, variables interested in (e.g., species richness) and methods that are chosen, which should address detection error. If this is not done, then these programs run the risk of providing data that at best is biased and at worst leads to inappropriate management decisions.

Of the 98 primary research papers and sampling guidelines reviewed from the U.S (84), and Canada and Europe (14) only 32 (28%) and 3 (21%) provided detailed survey guidelines, respectively. The guidance from these studies and reports included providing details on sampling design, timing of sampling, how to handle mussels (during and after sampling), determining the size of the search area, and assessing mussel habitat. The primary goals of the papers reviewed were to 1) qualitatively assess mussel presence/absence and then use the resulting data to describe the viability of surveyed populations and mussel assemblage structure (Piette 2005; Layzer and Scott 2006; Young and Isely 2008; Garner et al. 2009), 2) sampling protocol development for federally listed or candidate species (Smith et al. 2001; Carlson et al. 2008; Mackie et al. 2008) and 3) advancing sampling methods by comparing qualitative to quantitative methods (Miller and Payne 1993; Hornbach and Deneka 1996; Zieritz et al. 2014). In general, most of the papers reviewed advocated a tiered approach whereby informal survey techniques were used to first assess whether mussels were present at a site and then quantitative techniques were used second to further describe the population in more detail (Piette 2005; Christian et al. 2005; Garner et al. 2009; Mackie et al. 2008).

Qualitative sampling is used to determine mussel presence/absence and to provide assemblage structure data (such as a species list) at a given site (Miller and Payne 1993; Strayer et al. 1995; Vaughn et al. 1997; Dunn and Strayer 2010). Dunn and Strayer (2010) who summarized different mussel sampling techniques, noted that qualitative sampling includes the following main methods, though there are derivations of each; reconnaissance, timed-searches, and semi-quantitative. In general, qualitative methods tend to be the most cost-effective strategy if the goal of the project is to detect species presence within a sample site and to provide estimates of abundance (Vaughn et al. 1997). Qualitative methods that standardize search time and effort are the most useful because the likelihood of detecting a species can be determined (Metcalfe-Smith et al.

2000; Smith et al. 2001; Smith 2006). The qualitative methods used in the studies reviewed consisted of searching for shell (Smith et al. 2001; Carlson et al. 2008; Mackie et al. 2008); tactile and visual searching within fixed and unfixed areas (Piette 2005; Carlson et al. 2008; Crabtree and Smith 2009; Smith et al. 2010); and tactile and visual searches along transects (Levine et al. 2003; Christian and Harris 2005; Gangloff et al. 2011; Meador et al. 2011; Zieritz et al. 2014). The amount of time spent searching for mussels in these studies ranged from 0.25 to 15 hours per site and was determined using species accumulation curves (Garner et al. 2009), size of the search area (Piette 2005; Gangloff et al. 2009; Crabtree and Smith 2009; Smith et al. 2010) or arbitrarily chosen (Vaughn et al. 1997; Galbraith et al. 2008; Gangloff and Hartfield 2009; Huang et al. 2011). Search effort, the amount of time spent searching for mussels within a given area, varied from 0.2 to 0.5 min/m^2 and reasons for choosing a specific rate were never justified (Smith et al. 2001; Smith and Crabtree 2009)

Reconnaissance sampling involves haphazardly surveying for mussels to document presence, determine general mussel distribution, and help inform the design of more intensive qualitative or quantitative efforts (Dunn and Smith 2010). The main benefit of reconnaissance sampling is that it covers large areas with little effort. Data collected using this method are limited to assessing mussel presence because search area, effort and time are usually not recorded. Surveyor bias is also an issue because the search area is undefined. This is a common problem for all qualitative methods and why state and federal agencies are now moving toward prescribing search effort (i.e., time per area) (Smith et al. 2001; Smith 2006; Clayton et al. 2015). Search methods used in reconnaissance sampling include visual and tactile techniques. Surface supplied air or SCUBA may be used if water depths exceed 1 m (Obermayer 1998).

Timed-search sampling is a more robust method than reconnaissance sampling and is used to determine presence/absence, assemblage structure, abundance (i.e., catch-per-unit-effort), and population structure of mussels (Dunn and Strayer 2010). Unlike reconnaissance sampling qualitative surveys are restricted to a predefined area for a specific amount of time. The size of the search area used during qualitative surveys is based on the objective of the study while the amount of time spent searching for mussels is determined using either species accumulation curves (Garner et al. 2009) or a combination of search rate and size of the search area (Piette 2005; Smith 2006; Gangloff et al. 2009; Crabtree and Smith 2009; Smith et al. 2010). The benefits of this method are that its relatively inexpensive and large numbers of individuals can be collected in a short amount of time, thus increasing the chance of finding rare species. If species accumulation curves are estimated or search area and time are recorded, then detection rates can be inferred or calculated (Metcalfe-Smith et al. 2000; Smith 2006; Smith and Crabtree 2009). However, like other qualitative methods, comparisons among sites or years are limited to presence/absence data and a surveyors' ability to find mussels, which may be biased towards larger shallow-buried individuals/species.

Semi-quantitative transect sampling is a robust sampling method that entails the use of transects to determine mussel presence/absence, characterize mussel assemblage structure, and estimate abundance and age structure (Dunn and Strayer 2010)

Specifically, surveyors establish transects (i.e., lines of rope or cable) at specified intervals within a site and then visually and/or tactilely search for mussels along the length of those lines (Dunn and Strayer 2010). Search time and effort along each transect is usually standardized, though not always. Habitat information can be collected simultaneously with mussels resulting in a map of habitat within the search area, and when combined with mussel distribution information can provide insight on mussel-habitat associations (Ecological Specialists 2009). This survey method suffers from drawbacks similar to those of reconnaissance and timed-search surveys in that comparisons between sites and across time are limited to presence/absence. Data collected using this method may be biased by surveyor experience. Also, if interval distance is too large and mussels are aggregated then mussels may be overlooked.

Quantitative sampling methods are used if the goal of the survey is to assess population size and structure (Piette 2005; Dunn and Strayer 2010). Quantitative sampling encompasses the following three methods, though there are derivations of each (e.g., adaptive and double sampling): simple random sampling, systematic sampling with random starts and stratified random sampling (Strayer and Smith 2003). For the simple random sampling method, surveyors randomly select points within a specific area, usually containing mussels to sample. Quadrats (usually 0.25 m^2) are placed at each randomly selected point to excavate substrate to a depth of at least 15 cm. Sediment excavated from each quadrat is then passed through a sieve with a 0.25-inch mesh to separate mussels, including smaller individuals. Simple random designs provide unbiased estimates of mussel population densities and other attributes such as demography. However, this method is costly and density estimates can be imprecise if abundances are low or mussels are aggregated into clusters, which may leave many quadrats without mussels. This method is also ineffective at detecting rare species due to the limited spatial coverage of the sampling area and number of samples often required to locate species with low abundance (Strayer and Smith 2003; Dunn and Strayer 2010).

Systematic sampling with random starts begins by randomly selecting start points (usually three) and an interval distance from those points to other samples to ensure adequate spatial coverage within the survey area. Samples are collected from quadrats placed at each random point following the method described for simple random sampling. This method usually provides good spatial coverage of the sample area and thus density estimates typically have smaller variances compared to other methods, but spacing between points must be small enough to detect mussel aggregations. In addition to better precision, systematic sampling is easier to implement in the field compared to simple random sampling because only the start points have to be identified prior to sampling. However, this method is costly to implement, less so compared to simple random sampling, and does not detect rare species efficiently (Strayer and Smith 2003; Dunn and Strayer 2010).

The stratified random sampling design is a useful method when surveyors have an idea of where mussels may be concentrated in the sample area. For example, mussels may disproportionately occur near water's edge and so surveyors may want to focus more on those areas than other parts of a stream bank where mussels are less abundant. In this method surveyors first delineate the search area and then divide that area into different subgroups (i.e., strata) based on criteria such as mussel density, sampling costs and habitat type. The type of sampling method within these strata can vary (i.e. random, systematic, adaptive etc.), though it should be the same across all strata. Although stratified random sampling is useful for concentrating effort in areas where density is highest, it is costly to implement, is inefficient for locating rare species and requires *a priori* knowledge of mussel densities and habitat use (Strayer and Smith 2003; Dunn and Strayer 2010).

In addition to sampling design itself, other important technical considerations for quantitative sampling include sample size, size and type of quadrat sampler and depth of sediment excavation. For the studies reviewed, criteria used to determine sample size include: 1) size of the search area (Christian et al. 2005); 2) empirical models that integrate mussel density, variance in density and desired precision (Downing and Downing 1992; Smith et al. 2001; Strayer and Smith 2003) or 3) unreported (Vaughn et al. 1997). The size of quadrats used ranged from 0.15 m^2 to 1 m^2 (Christian et al. 2005; Crabtree and Smith 2009; Zieritz et al. 2014), though 0.25 m^2 was the most common (Vaughn et al. 1997; Crabtree and Smith 2009; Smith et al. 2010).

Sediment excavation influences search efficiency and therefore species detection (Smith et al. 2001). For the studies reviewed, most reported excavating sediment to a depth of 10 to 15 cm (Miller and Payne 1993; Mackie et al. 2008; Crabtree and Smith 2009; Smith et al. 2010). Although, there were several studies that excavated ≤ 5 cm of sediment (Piette 2005; Christian et al. 2005; Galbraith et al. 2008; Zieritz et al. 2014), while another recommended excavating sediment until bedrock is reached (Garner et al. 2009). Sediment excavated during quantitative sampling is typically passed through a single or series of sieves to retain small individuals and juveniles (Hornbach and Deneka 1996), and mesh sizes ranged from 6.1 to 7.0 mm (Miller and Payne 1993; Mackie et al. 2008; Crabtree and Smith 2009; Gangloff and Hartfield 2009; Smith et al. 2010), though sizes as small as 0.5 mm have been used (Hornbach and Deneka 1996; Grabarkiewicz 2008).

The physical environment, to a large extent, controls the distribution of mussels within a given waterbody, though the occurrence and distribution of a mussel's host fishes is also important (Haag and Warren 1998). In our review, we found that whether a site was considered suitable for mussels, and therefore appropriate to sample, was primarily based on physical habitat (Carlson et al. 2008; USFWS and VDGIF 2008; Young and Isely 2008; Gangloff and Hartfield 2009). The criteria used to define suitable habitat was unreported in some cases, despite claiming to use a method (Christian et al. 2005; Layzer and Scott 2006; Pilarczyk et al. 2006), in others it was developed in coordination with federal agencies (USFWS and VDGIF 2008) and for several studies it was based on qualitative descriptions of substrate (gravel and cobble), cover types (e.g., tree roots) and mesohabitats (e.g., pools, riffles, and runs). Interestingly, there were several cases where suitable habitat was not based on physical parameters but instead on the presence of existing mussel populations (Dunn et al. 2000; Cope et al. 2003; Pilarczyk et al. 2006; Kurth et al. 2008), though these studies were primarily focused on translocations.

Recommendation

Our review of the literature on mussel survey methods revealed that despite the availability of resources like Strayer and Smith (2003) or guidelines like Piette (2005) there continues to be a need for guidance on how to properly perform mussel surveys. Moreover, the general lack of standardization, use of untested sampling techniques, poorly defined objectives and lack of consideration of detection and surveyor error in many of these studies is troubling. Most of the papers reviewed were the product of projects focused on state or federally threatened or endangered mussel species. We recommend the following framework for conducting freshwater mussel surveys at construction projects to address this knowledge gap. It is important to note that sampling designs for scientific studies are somewhat project specific, but the goal of our recommendations is to help surveyors develop sound monitoring programs. Thus, many of these scientific studies, particularly status assessment surveys, could benefit by following or at least considering our recommendations.

To that end, a reasonable sampling design at a construction project site should include a dichotomized sampling strategy based on whether the site is wadeable vs. nonwadeable. For a wadeable stream (< 1m depth) the search area should include the project footprint and applicable buffers upstream, downstream and laterally, which should be developed based on the potential impact of the project and input from regulatory agencies. Table 2 provides a summary of buffer lengths for bridge projects. Search areas in nonwadeable streams (>1m depth) should include the area of immediate impact plus buffers (similar to those described for wadeable streams), which should be developed in coordination with regional malacologists and/or biologists with state and federal resource agencies. When determining whether or not a given site has suitable mussel habitat surveyors should use Strayer's (2008) functional habitat framework (see Table 1). Surveys should be conducted during base or subsistence flows (Mackie et al. 2008), when water velocity is low and turbidity is minimal, though some streams in Texas are naturally turbid.

If the stream is wadeable and presence/absence surveys are necessary, then we recommend qualitative sampling within a fixed area using visual and tactile techniques (Figure 1). Search time should be determined using species accumulation curves, which is used to determine the minimum effort required for adequate completeness of a survey (Metcalfe-Smith et al. 2000; ODNR and USFWS 2015; Clayton et al. 2015). This method can also be applied to the semi-quantitaive transect method. Collector curves should be developed by surveying the specified search area over multiple 1 p-h intervals; a person-hour is defined as one hour divided by the number of surveyors. Effort should be made to ensure that surveyors are distributed throughout the search area and are searching for mussels at a rate of ~ 0.6 minute/m^2. The overall search area should be divided into cells, and no cell should exceed 100 m^2. At the end of each interval the number of new species collected is recorded and sampling continues until at least 4 consecutive search intervals are sampled where no new species are found. Upon completion of each search interval, surveyors should place all live individuals into a mesh bag (or perforated bucket), which should be kept submerged in flowing water until the end of the survey. The resulting data should then be plotted showing cumulative time (x-axis) vs. cumulative number of species (y-axis) to demonstrate completeness of the survey.

If the stream is nonwadeable and presence/absence surveys are necessary, then we recommend qualitative sampling within a fixed area following the method described for qualitative sampling in wadeable streams, or double sampling can be used as described by Ecological Specialists (2009) (Figure 2). This procedure consists of using a combination of semi-quantitative and timed-search methods to survey for mussels. The objective of the semi-quantitative transect method is to determine mussel distribution in the project area. Transects are placed parallel to the current throughout the project area to ensure adequate coverage, surveyors should ensure that transect spacing is equidistant and that the distance isn't so wide that aggregated mussels are overlooked. Transects are demarcated every 10 m and each 10 m is considered a separate sample, though this interval can be adjusted. Surveyors then crawl along each transect collecting mussels encountered within a 1 m wide area at a minimum rate of 1 minute/m^2. At each 10 m mark mussels are brought to the surface for processing and habitat can also be characterized. Upon completion of the transect survey timed searches following the method described for wadeable streams are then used in areas with high mussel abundance (determined from the transect samples) to increase the likelihood of detecting state- or federally-listed mussel species. Search effort during qualitative sampling should be ~ 0.6 minute/m^2 depending on densities and substrate. Species accumulation curves are used to determine search time and completeness of the targeted sampling.

Surveys with objectives that require estimating mussel densities or assessing population demographics should use the three random start method as described by Strayer and Smith (2003) using 0.25 m^2 quadrats and excavating substrate to a depth of 15 cm (6 inches) or hardpan (whichever comes first). In reporting results for qualitative and quantitative sampling, surveyors at a minimum should explicitly define the size of the area searched, amount of time spent at each site (or number of quadrats excavated), and the number of people and their experience that participated in the sampling (see *Reporting guidelines*). Finally, all mussel surveys should be conducted within the same field season as the expected instream activities.

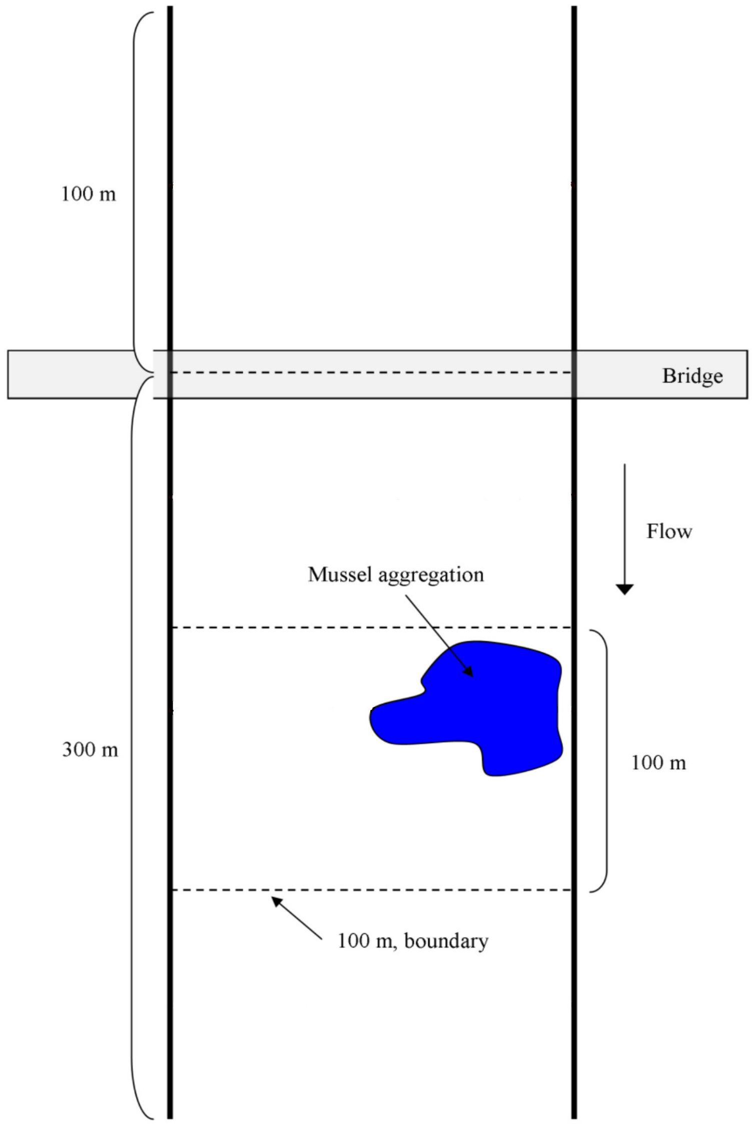

Figure 1. Diagram of sampling methodology for wadeable bridge crossings.

Table 2. Summary of survey area buffer distances for project crossings at wadeable and nonwadeable streams.

Source	Search Area Length				Notes
	Wadeable		Nonwadeable		
	Upstream	Downstream	Upstream	Downstream	
Carlson et al. 2008	100 m	300 m	Site specific	Site specific	Nonwadeable should be developed in conjunction with USFWS.
Levine et al. 2003	300 m	300 m	N/A	N/A	
USFWS and VDGIF 2008	100-200 m	400-800 m	100-200 m	400-800 m	
Battle et al. 2003	10-200 m	10-200 m	10-200 m	10-200 m	Sites were not differentiated between wadeable and not.
Pilarczyk et al. 2006	150 m	250 m	n/a	n/a	No differentiation between wadeable and not.
Ohio Mussel Survey Protocol	See notes	See notes	See notes	See notes	Buffers are in addition to the area of direct impact. These buffers are determined by project specific variables.
West Va. Mussel Survey Protocol	See notes	See notes	See notes	See notes	Buffers are in addition to the area of direct impact. These buffers are determined by project specific variables.
Smith et al. 2001	See notes	See notes	See notes	See notes	The direct effects area is 50 m up and 50 m downstream of centerline of bridge. The indirect effects area is 50-100 m upstream and 50-200 m downstream of the bridge.

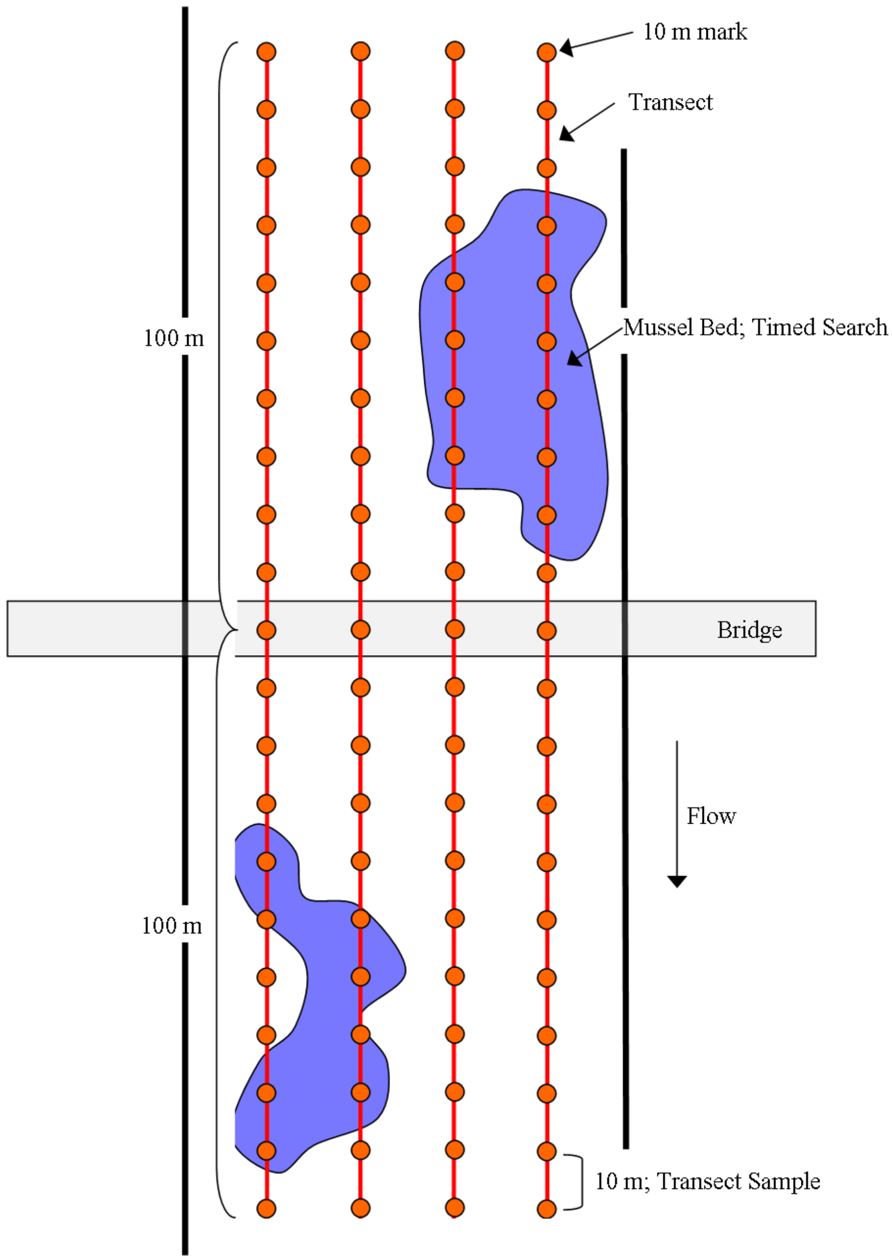

Figure 2. Diagram of sampling methodology for nonwadeable bridge crossings.

Research needs:

1. Evaluate whether search efforts (i.e., time/area) proposed by Smith et al. (2001) and Smith (2006) are sufficient for detecting focal species. Once this is determined test Smith's (2006) semi-quantitative sampling methodology in a real-world application to determine cost of implementation and accuracy in detecting rare species.

2. Examine population performance of mussels (i.e., growth, survivorship and reproduction) across multiple habitat types to better determine suitable vs. unsuitable habitat.

Implementation needs:

1. Clarification from regulatory agencies on whether to target just focal species or all species at a given project site.

2. Guidance from regulatory agencies regarding what it considered acceptable search effort, area and time, and the method surveyors should use to provide proof that their survey methodology was adequate to locate focal species.

3. If quantitative sampling is to be prescribed by regulatory agencies then guidance is needed on the method, size of sampler and number of quadrats to be used. For the latter, we recommend that both agencies refer surveyors to Smith and Strayer (2003) and Smith et al. (2001) for information on how to determine sample size. However, this will still require regulatory agencies to define either a probability of detection or level of precision (i.e., coefficient of variation), or both, beforehand.

4. Clarification is needed from stakeholders on the appropriate buffer lengths for various projects (see Table 2).

5. Consultation with regulatory agencies, with stakeholder input, to determine if the search area for nonwadeable streams can be defined in a manner similar to wadeable streams.

6. Clarification is needed from regulatory agencies on data longevity (i.e., how long is survey data considered valid).

7. Clarification is needed from regulatory agencies when agency review/approval of surveys is required, and if required on how many days in advance they should be notified before the actual survey will occur and numbers of days to review survey results prior to approving a project.

Surveyor experience and permitting

The accurate and precise identification of aquatic organisms is crucial to credible ecological studies and reliable bioassessment programs. For freshwater mussels, biologists often use morphological traits like shell size, color and texture to identify species during field surveys. Some species present a low risk of misidentification while others are routinely misidentified because they are either morphologically similar to other species or are highly variable among individuals. Consequently, untrained surveyors or those unfamiliar with the regional or local species pool are likely to misidentify mussel species. Misidentification of threatened or endangered mussel species is especially problematic because it can bias information (e.g., status and distribution) used to develop conservation and management strategies (Shea et al. 2011).

Of the 98 primary research papers and sampling guidelines reviewed from the U.S. (84), and Canada and Europe (14) only 4 (4.7%) and 1 (7%) addressed surveyors experience and skills, respectively. For example, Mackie et al. (2008) noted that applicants wishing to obtain a permit to collect threatened mussel species must demonstrate sufficient expertise to conduct the field survey as well as identify the species at risk. To demonstrate both, applicants are required to provide the number of years they have worked with mussels and their educational background. Additionally, documentation of field time and/or a letter of recommendation that evaluates the surveyor's experience in mussel surveying, handling and identification may be requested, though not required. MacCallum (2013) notes that the Natural heritage and Endangered Species Program (NHESP) requires, for any translocation project, the lead mussel biologist to provide a résumé detailing field experience with mussels and ability to identify threatened species as well as appropriate habitat for the species in question. Carlson et al. (2008), notes that surveyors working in the Southeastern Atlantic Slope and Northeastern Gulf drainages should have sufficient mussel knowledge within the basin of interest, which includes documented field-time, ability to successfully implement survey methods and ability to locate and identify federally protected and candidate freshwater mussel species. Similar to Mackie et al. (2008), a letter of recommendation regarding a surveyor's experience may be requested, though not required.

Currently, the gold standards for testing surveyor experience and skills come from the West Virginia Department of Natural Resources (Clayton et al. 2015) and Ohio Department of Natural Resource (ODNR and USFWS 2015). Surveyors in West Virginia working in streams where threatened species may occur are required to take a closed book and closed shell identification test. To successfully pass the test, surveyors must achieve an overall score of 85% for non-endangered species and a score of 100% on the endangered species. Prospective surveyors must also possess at least 3 years of field experience conducting surveys similar to those presented in the West Virginia Mussel Survey Protocol and submit two letters of reference (Clayton et al. 2015). In Ohio, prospective surveyors are required to take an open book identification test administered by the Ohio State University's Museum of Biological Diversity. To pass this test, surveyors must score 100% on federal threatened and endangered species, 80% on Ohio threatened and endangered species and an overall score of 80% of the entire test.

Surveyors must also possess at least 2 years of field experience in a position with direct responsibility for conducting mussel surveys. They must also have a Bachelor of Science in biology, environmental science, natural resources or related field with at least 9 credit hours from or related to aquatic ecology, fisheries, hydrology, aquatic entomology, limnology, ichthyology, and plant taxonomy (ODNR and USFWS 2015).

Recommendation

In Texas, individuals interested in collecting mussels as part of a research project, which includes environmental assessments, are required to obtain a TPWD scientific collection permit. Prospective surveyors are required to complete an application packet where they must demonstrate mussel expertise by providing details on their educational background and biological training as well as provide two letters of recommendation from individuals in an appropriate biological or professional field. Recently, the Texas Mollusk Society administered a pre- and post mussel identification test covering 60 specimens to participants of a 3-day workshop. Attendees at this workshop received training in mussel identification and survey methods and many already had TPWD collection permits. For the pre-test (which was closed book), participants were on average able to identify 22% of the specimens correctly. Scores ranged from 0 to 97% with a median score of 11%. The post-test (open book) results were higher, though still failing, with an average of 51% across all specimens and ranged from 13 to 100%; the median score was 48%.

The results of this test indicate that current requirements to obtain a collection permit are insufficient for assessing proficiency in mussel identification and sampling. To reduce the risk of pervasive misidentification in Texas by consulting firms, academics, and resource agency personnel, we recommend the following ideas for improving permitting requirements: 1) have applicants provide a curriculum vitae (CV) or résumé, a list of their own publications and other pertinent information that demonstrates proficiency in mussel identification and sampling; 2) require that applicants take a mussel identification test to demonstrate proficiency in mussel identification; 3) require a certain number of hours of continuing education for all permit holders. This would ensure that all persons holding a permit are maintaining or improving their identification and sampling skills as the ability to identify freshwater mussels correctly is a perishable skill. Attending mussel workshops and symposiums could count towards a required number of continuing education units. Permit holders could report this information to TPWD directly or as part of the "Research Annual Report" that is due each year to maintain the permit; 4) permits should not be issued to applicants that do not show appropriate qualifications or score poorly on an identification test; and 5) all biologists actively or desiring to work with mussels should be required to take and pass the mussel identification test. In addition to these recommendations, we suggest the collection of specimens (vouchers, and/or photos) during field surveys so that managers and biologists can account for potential misidentification biases.

Implementation needs:

1. Consultation with regulatory agencies to determine if requirements for obtaining a collection permit should include successfully passing a mussel identification test. Subpermittees on collection permits should be closely supervised by the collection permit holder and have successfully passed a mussel identification test.

Reporting guidelines

Technical reports are a means for researchers to communicate the results of a project and in the case of environmental assessments to provide data that will be used to anticipate the effects of a proposed project. Of the sampling guidelines reviewed most included criteria for reporting, though the level of detail and specific requirements varied among guidelines. For example, Carlson et al. (2008) noted that surveyors working in the Southeastern Atlantic Slope and North Eastern Gulf Drainages in Florida and Georgia are required to submit a final report upon completion of their survey to USFWS and the Georgia Department of Natural Resources or Florida Fish and Wildlife Commission depending on the geographic location of the survey. Carlson et al. (2008) notes that at a minimum survey reports should include the following components: "Results," "Discussion," and "References." For the Results section, surveyors are required to provide copies of all data forms as well as summaries of species found (including relict shells of federally listed species), locality data, shell length measurements, water quality parameters taken, discharge data from nearest USGS gage at time of sampling, and photographs of the project location. For the Discussion, surveyors are requested to describe the quality of habitats within the survey area, suitability of the habitat for supporting endangered or threatened species and if threatened species were expected to occur in the area possible reasons for not finding the species of interest. Deviations from the sampling protocol should also be explained, especially if it is suspected that those deviations aided or hindered detection of species of interest. Finally, for the Reference section, surveyors are required to include all scientific literature (published and unpublished) along with any additional personal communications related to the project.

Piette (2005), author of the *Wisconsin Guidelines for Sampling Freshwater Mussels in Wadeable Streams*, notes that a final report is required for all mussel surveys associated with transportation projects and that these reports should be filed with the WDNR regional project coordinator, the WDNR bureau of Endangered Resources, and the WDNR Mussel Atlas coordinator. Final reports should be submitted in a timely manner, though a timeframe is not specified, and contain names of surveyors, agency requesting the survey, reason for conducting the survey, project permit number, general site description, survey methods, copies of datasheets, and map of site and summary of results. Surveyors are also required to discuss any problems encountered during the survey that could affect the results and an assessment of impacts to mussels at the site.

Finally, the *Ohio Mussel Survey Protocol* is the most prescriptive of the reviewed sampling guidelines. Surveyors are required to complete both a standardized habitat form and a final report (ODNR and USFWS 2015). Termed the *Ohio Mussel Habitat Assessment Form*, surveyors are responsible for providing the total length of the survey area and buffers, a brief description of the methods used, habitat description, which includes substrate and mesohabitat types, average water depth, and any obvious pollution or stream stability issues. The final report should include the following components: "Introduction", "Methods," "Results," "Mussel Relocation," "Conclusion," "References," and "Appendices." For the Introduction, surveyors should provide the names of the stream surveyed and its receiving waters, location, drainage area, summary of water quality data, if available, summary of previous mussel surveys near the area of impact, if available, and description of surrounding land use. The Methods section should include personnel involved with the survey, date of the survey, geographic extent of the survey area, survey method, mussel handling and processing procedures, and quality control procedures. The Results section should include a habitat assessment of the search area, based off the habitat assessment form and a detailed overview of the survey results. The Mussel Relocation section, if performed, should include a description of the relocation site and the methods used to remove mussels from the search area. The Conclusion section should summarize findings and the Reference Section should include citations for any literature cited within the text.

Recommendation

Based on our review, we recommend that a final report summarizing mussel sampling for a given project should be mandatory and submitted in a timely manner to regulatory agencies. Reports should be standardized, regardless of project type or funding source, and include the following components: Introduction, Methods, Results, Mussel Relocation, Conclusion, References, and Appendices. Specific details that could be required for each component are listed below, which are taken from ODNR and USFWS (2015):

A. Introduction
    a. Description of the stream and watershed including:
        i. Name (if stream is named)
        ii. Receiving waters of surveyed stream
        iii. Location, including:
            1. Coordinates – at center of search area
            2. River mile (if available)
            3. City or town (if applicable)
            4. County
        iv. Drainage area at survey site
        v. Summary of any water quality data or previous mussel survey reports near the impact area
        vi. Surrounding land use
B. Methods
    a. Personnel
    b. Date(s) of survey
    c. Area surveyed, including:
        i. Description of survey/buffer areas (e.g., length, bank-to-bank)
        ii. Coordinates of survey/buffer areas
        iii. Map delineating survey/buffer areas
    d. Survey method, including:
        i. Type of sampling method
        ii. Time search
        iii. Number, size of quadrats and depth of excavation
        iv. Size of area searched

            v. Sampling method (SCUBA, tactile, view buckets, mask and snorkel)
            vi. Whether or not banks were searched for shells
    e. Mussel handling and processing procedures
    f. Quality Control Procedures (includes taking representative photos of each species and questionable specimens)

C. Results:
    a. Habitat assessment within search area:
        i. Substrate composition (include information about the stability of substrates)
        ii. In-stream features (e.g., channel alterations, impoundments)
        iii. Average stream depth
        iv. Discharge or water velocity (cubic feet per second or feet per second respectively)
        v. Visibility
        vi. Water temperature
        vii. Suitable habitats within the area of survey
        viii. Photos of stream and substrate
    b. An overview of the results, including:
        i. Number of individuals found
        ii. Number of species found
        iii. Any notable species found
    c. Table of results (include within the text and attached in appendix as a spreadsheet)
        i. Species data
            1. Raw numbers by species
            2. Condition of shell (living/fresh dead/weathered/subfossil
            3. Sex of individuals if determinable
            4. Shell length data

D. Mussel relocation:
    a. Relocation site, including
        i. Location (coordinates at center)
        ii. Map delineating area
    b. Method of removing mussels from the impact/survey area

E. Conclusion
    a. Summary of findings, and conclusions

F. References
    a. Include citations for any literature cited within the text of the report

G. Appendices
    a. Photos of stream and substrates
    b. Representative photos of each mussel species found
    c. Photos of questionable species
    d. Raw data sheets
        i. Copy of state and/or Federal Permits

Implementation needs:

1. Consultation with regulatory agencies on reporting requirements. Specific questions to address: 1) the purpose of the report and its components; 2) where will the reports be stored, for how long, and will they be made publicly available; and 3) who will manage it and how will it be made available in a timely manner.

Stress: sampling and handling

Stress induced during sampling can have a negative impact on mussels (Cope and Waller 1995; Dunn et al. 2000). Dunn et al. (2000) and Carlson et al. (2008) recommend that surveys should be conducted during times when water and air temperatures are similar. Other authors mention avoiding sampling for mussels during times with extreme temperatures (Reutter et al. 2001; Carlson et al. 2008; Mackie et al. 2008; Luzier et al. 2009). Piette (2005) notes that to minimize thermal stress, surveyors should not sample for mussels until water temperatures exceed 40°F. However, Block et al. (2013) demonstrated that mussels are less likely to burrow at temperatures less than 68°F, making them more susceptible to entrainment during high flows and predation. Mussel brooding or gravidity should also be considered when planning a survey as this is a critical time during a mussel's life history, which if interrupted can negatively impact the viability of a population (Havlik 1997; Layzer and Scott 2006; Carlson et al. 2008). As for processing, it is recommended that surveyors minimize the time mussels are out of the water (Cope et al. 1995; Patterson et al. 1999; Bolden and Brown 2002; Gatenby et al. 2006; Carlson et al. 2008). For example, Cope et al. (2003) stated that emersion times of 15-60 min have been shown not to impair mussel survival, however emersion times of < 20 minutes are recommended. Similarly, Bartsch et al. (2000) found that survival was high (93%) for individuals emersed for less than an hour at temperatures ranging from 15-35°C. Mackie et al. (2008) notes that during processing (i.e., measuring and enumerating individuals) mussels should be maintained in perforated buckets placed in the stream, care should be taken to ensure mussels are completely wetted and not exposed to direct sunlight. Piette (2005) and Tsakiris and Randklev (2014) recommend placing mussels in mesh bags submerged in flowing water; the number of mussels in each bag should be adjusted to avoid overcrowding. Upon completion of the survey it is recommend that mussels are returned back to the location from which they were collected and if possible rebedded into the substrate [anterior end down] (Carlson et al. 2008; Mackie et al. 2008; Luzier et al. 2009; USFWS and VDGIF 2008).

Recommendation

Proper handling procedures are key for ensuring mussel survival following a survey. Because of this, we recommend that surveys be performed between April to November, but during this time water temperatures should be greater than or equal to 68°F (~ 20 °C). Time restrictions should be applied once periods of gravidity are known for threatened mussel species. During sampling and processing, emersion times should not exceed 20 minutes and processed mussels need to be maintained in flow through containers such as mesh bags or perforated buckets placed in the stream. Upon completion of the survey, mussels should be hand-placed back to the location from which they were collected, and if possible rebedded in a natural position (anterior end down).

Research needs:

1. Determine reproductive biology of threatened species. Emphasis should be placed on determining period of spawning and brooding.

2. Determine temperature effects on burrowing behavior of threatened mussel species. Emphasis should be placed on testing species across their geographic range and using multiple substrate types that resemble the substrata from where they were collected.

3. Evaluate sensitivity to handling across short vs. long-term brooders.

Translocation

Translocation of mussels is used to move populations away from perceived threats (e.g., construction projects or colonization of zebra mussels), augment existing populations, establish additional populations, or hold populations in captivity until release is appropriate (Cope and Waller 1995; Newton et al. 2001; Cope et al. 2003; Haag and Williams 2014). Although, the most common use of translocation has been to remove mussels from bridge construction zones or river channels undergoing dredging (Haag 2012). This is because state and federal wildlife agencies typically require mitigation when threatened species are present at a construction site (Peck et al. 2007). Despite its frequent use, translocating mussels as both a conservation and management strategy has received criticism because of lack of guidance or consensus on: 1) methods for removing mussels from a site; 2) timing of the relocation; 3) handling and transporting individual mussels; 4) habitat criteria to identify suitable relocation sites; 5) methods for post-relocation monitoring and frequency of monitoring; and 6) biological endpoints (Cope and Waller 1995; Dunn et al. 2000; Chen et al. 2001; Newton et al. 2001; Cope et al. 2003; Peck et al. 2007). Moreover, the philosophical justification for translocating mussels, particularly in response to construction projects, has been drawn into question based on lack of post-relocation monitoring and short and long-term mortality (Haag 2012; Haag and Williams 2014).

The immediate goal of relocation is to remove as many mussels, common and threatened, as possible from the project survey area. For construction projects, the area where mussels are removed is typically based on the scale of the potential impact. For example, USFWS and VDGIF (2008) defines the construction project footprint (i.e., the area of impact) as: the area of potentially disturbed substrate, zone of heavy equipment operation, and the distance downstream that may experience sedimentation. For scientific studies, the size of the area and the number of individuals collected is primarily based on the objectives of the study (Bolden and Brown 2002; Battle et al. 2003; Layzer and Scott 2006; Young and Isely 2008). Of the guidelines and methodologies reviewed, sampling techniques used to remove mussels for the purpose of translocation can be broadly grouped into four categories: 1) multiple-pass-depletion; 2) quantitative; 3) semi-quantitative; and 4) qualitative. The latter three methods are similar to those described in the Sampling Guidelines of this report, though with some modifications (see below). Multiple-pass-depletion surveys refer to sampling methods that resample a known area (hereafter lanes) multiple times. The amount of time spent in each lane can be standardized or left to the discretion of the surveyor. In either case, the number of passes within each lane is based on whether or not the number of individuals collected exceeds a percentage (between 20 – 30%) of the total number of mussels collected during the initial pass (USFWS and VDGIF 2008). Quantitative sampling refers to using quadrats within a project footprint to remove mussels. To ensure that juveniles and subadults are collected sediment is excavated and passed through a sieve (Mackie et al. 2008). Semi-quantitative surveys refer to multiple-pass-depletion surveys along transects (Havlik 1997; Kesler et al. 2007; Grabarkiewicz et al. 2008). Finally, informal surveys refer to methods where surveyors removed mussels using visual/tactile searches for an undefined period of time and often over an undefined area (Jenkinson 1982; Hamilton et al.1997; Gatenby et al. 2000; Cope et al. 2003; Gatenby et al. 2006; Young and Isely 2008).

The most important factor ensuring high recovery of translocated mussels is the selection of a suitable translocation site (Dunn et al. 2000). We found in our review that the following criteria were used most often to identify translocation sites: 1) located within historical or current distribution of the focal species (Layzer and Scott 2006; Butler et al. 2012; Peck et al. 2014); 2) nearby the source site or within the watershed in which the source population occurred (Hamilton et al. 1997; Battle et al. 2003; Layzer and Scott 2006; Peck et al. 2014); 3) presence of live mussels, ideally of the same species being translocated (Layzer and Scott, 2006; USFWS and VDGIF 2008; Peck et al. 2014); 4) contains suitable habitat regardless of mussel presence (Jenkinson 1982; Hamilton et al. 1997; Battle et al. 2003; USFWS and VDGIF 2008; Peck et al. 2014); and 5) presence of host fish, seasonally appropriate, if known (Cope and Waller 1995; Peck et al. 2007; Luzier and Miller 2009; Mackie et al. 2008; MacCallum 2013; Tiemann 2014). Additionally, several studies recommended that potential translocation sites should be examined for imminent and future threats prior to moving mussels (Butler et al. 2012), carrying capacity of the receiving population, though in general data on this topic remains scant (USFWS 2008), the potential for disease transmission (USFWS 2008), and presence of and accessibility of the translocation site to predators (Luzier and Miller 2009; Peck et al. 2014).

Biological endpoints, such as growth and survival, are often used to evaluate the success of translocation projects (Cope and Waller 1995; Dunn et al. 2000; Cope et al. 2003; Layzer and Scott 2006). Unionid mussels are long-lived and changes in growth and survival in response to stress may be delayed; therefore, the effectiveness of these endpoints to judge translocation success has been questioned (Naimo et al. 1998; Newton et al. 2001; Peck et al. 2007). Researchers have now started testing macro-molecules like glycogen and lipids to infer physiological condition of mussels, though the results of these studies have been mixed (Naimo et al. 1998; Newton et al. 2001; Peck et al. 2007). Of the studies and guidelines that discussed translocations, the biological endpoints monitored include survivorship (Jenkinson 1982; Hinch et al. 1986; Alvarez et al. 2000; Battle et al. 2003; Gangloff et al. 2009; Zieritz et al. 2014), growth (Bolden and Brown 2002; Layzer and Scott 2006), reproduction/recruitment (Heinricher and Layzer 1999; Boyles 2004), and physiological condition (Naimo et al. 1998; Newton et al. 2001; Boyles 2004; Gatenby et al. 2006; Peck et al. 2007). In some cases, the endpoint used was left to the discretion of the agency performing the relocation (USFWS and VDGIF 2008). For all of the papers reviewed, there was no regulatory guidance for a particular endpoint, except that if survival of translocated mussels does not occur then a new recipient site should be chosen (Butler et al. 2012). This lack of criteria and guidance for not achieving an established baseline may explain why there is so much variability in the endpoints used to document the outcome of a translocation. Although, the lack of consensus for a particular suite of endpoints may also be a function of the fact that there are significant knowledge gaps as it relates to translating the onset of stress and the subsequent increases in mortality or decreases in reproduction and growth.

Handling, which includes collection, processing and emersion may impair mussel survival. As noted previously (see *Stress: sampling and handling*), extreme water temperatures, high or low, during sampling can impact mussel survival (Mackie et al. 2008), though few studies have looked specifically at the effects of transport and handling on survival and population performance. Tsakiris et al. (in review) examining the effect of translocation on survival, growth and gametogenesis found no association between these parameters and handling and processing of mussels at time of collection. Chen et al. (2001) observed similar results, though the authors of that study only examined survival. The results of both studies indicate that mussels can be collected and transported with little to no long-term effects, but only if done correctly using specific protocols, which are described below.

<u>*Recommendation*</u>

Based on our findings and those of other studies we recommend the following guidelines for future mussel relocations performed in Texas. These recommendations are largely adapted from Cope et al. (2003), Dunn et al. (2000) and Tsakiris and Randklev (2014):

A. *Habitat Selection*: Surveyors should perform mussel surveys near the targeted population to identify potential relocation sites. These sites should contain similar mussel species and habitat to that of the translocation site. Care should be taken to ensure that potential relocation sites remain wetted during low flows and stable during high flows and are not differentially affected by nearby anthropogenic activities. Sites that do not meet these criteria should not be used, as translocated mussels will likely experience high mortality at these locations.

B. *Removal*: Multiple-pass-depletion surveys should be used to remove mussels from the project site (Figure 3). These surveys should entail dividing the project area into lanes demarcated bank-to-bank and situated parallel to flow. The width of each lane should be ~ 1.0 m and the length should not exceed 50 m. To ensure consistent detection within and across lanes, each lane should be further demarcated into cells that do not exceed 5 m^2 (i.e., 5 m length x 1 m width). Within each cell, surveyors should qualitatively search for mussels at a minimum rate of 1 minute/m^2 using visual and tactile techniques. Upon completion, surveyors should move upstream to the next cell. This process will continue until all cells for that lane have been surveyed, at which time mussels will be tallied by lane. Surveyors will then resurvey each lane using the aforementioned sampling methodology, though it is advised that surveyors are rotated between lanes to reduce fatigue and sampling bias. At the completion of the second pass, surveyors should then tally all mussels and if the total number for any lane exceeds more than 20% of the original number of mussels observed in the first pass then surveys for that lane should continue. The relocation percentage (% of the total estimated population that remains at the site) may be adjusted depending on species and abundance of target population, though it is recommended that for sites with low mussel abundance a minimum of 3 passes should be performed. Species and abundance information by lane should be recorded to include in the final report. Multiple-pass-depletion surveys for nonwadeable sites should be developed in coordination with TWPD and USFWS.

Mussels collected during the multiple-pass-depletion surveys should be marked using passive integrated transponder (PIT) tags or vinyl tags, though mussels marked with PIT tags have a higher likelihood of being recovered compared to conventional tags. If PIT tags are used then a single tag should be secured to the posterior slope of the right valve using superglue. A non-toxic marine putty should then be used to completely cover the tag, which will help secure it in place and provide protection during scouring flows. If vinyl tags are used then two tags, both with the same alphanumeric sequence, should be secured to the posterior slope on the left and right valves using superglue or dental cement. At the relocation site a subset of the resident mussels should be tagged as a control population. The purpose of the control is twofold: 1) monitor the effects, if any, of translocated mussels on the resident population and 2) to compare population parameters (i.e., growth, survivorship, and reproduction) between the resident and translocated populations to separate impacts associated with the translocation itself, if any, from normal background variation for these parameters.

C. *Handling*: Only personnel that have experience with handling mussels should be involved with processing and transporting mussels. During sampling, mussels need to be maintained in mesh bags or perforated buckets placed in the stream and out of direct exposure to the sun. Mesh bags or buckets should be placed in areas of the stream with adequate flow to ensure individual mussels remain wetted and to provide food and prevent accumulation of waste; the number of mussels in each bag or bucket should be adjusted to avoid overcrowding. During processing, surveyors should ensure that mussels are not held out of water for more than 10 minutes and not exposed to direct sunlight. Transporting mussels should be conducted using the least amount of time possible. Mussels should be wrapped in moist paper towels or napkins, placed inside unsealed plastic bags and transported to the relocation site in an ice chest with ice. A piece of cardboard should be placed in between the mussels and bags of ice to ensure individuals do not come in direct contact with the ice or meltwater. If total emersion time is less than 20 minutes, then mussels can be transported without being wrapped individually or placed in an ice chest with ice. All translocations should be conducted within the same field season as the expected instream activities. The total time each individual is held out of the water and transportation time should be noted to include in the final report.

D. *Post-relocation monitoring*: All relocated mussels should be monitored annually for at least two years, though longer timeframes will allow for more accurate documentation of recruitment, which according to Cope and Waller (1995) is the true indicator of a successful translocation. Mortality should be assessed upon completion of the translocation, followed by a one-month monitoring and then two yearly monitoring efforts. If during these surveys mortality is disproportionate towards smaller individuals, particularly for the one-month monitoring, regulatory agencies should be contacted, as this could be an indication that the relocation site is unsuitable. Similarly, if survival does not exceed 80% after the first yearly monitoring event then regulatory agencies should be contacted. For all monitoring, surveyors should locate mussels using a portable underwater receiver if mussels were marked with PIT tags, or visual and tactile techniques if mussels were marked with vinyl tags. Surveyors should search the entire study area (inside and outside of the study plot) until all mussels are recovered. All mussels collected should be placed in mesh bags or perforated buckets in the stream during processing. Upon completion of the monitoring mussels should be returned back to the relocation area and if possible rebedded into the substrate.

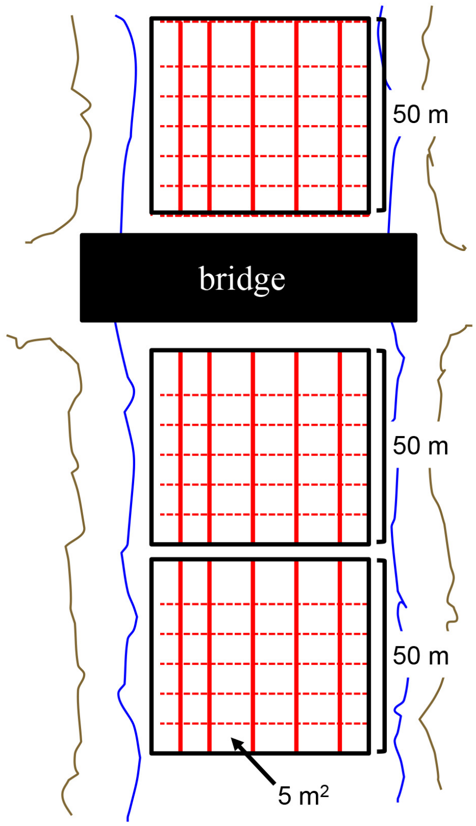

Figure 3. Diagram of multiple-pass-depletion survey, adapted from Tsakiris and Randklev (2014).

Research needs:

1. Continue evaluating the efficacy of mussel translocation with well-planned *in-situ* field studies that examine lethal and sublethal responses. Results by Tsakiris and Randklev (2014) demonstrate that relocation has very little impact to mussels, but the applicability of these results to other rivers and species is unknown.

2. Determine host fish for threatened mussel species. To date, host fish are only positively known for 13 of the 52 species native to Texas.

3. Determine if translocating mussels to an existing mussel population impacts population performance of the receiving population.

4. Evaluate different physiological methods (e.g., lipids and glycogen) for monitoring stress to mussels at or near bridge construction sites in relation to construction activities and translocated populations.

Implementation needs:

1. Discussion with regulatory agencies on: 1) whether species presently considered common should be translocated; 2) is there a minimum number of individuals in which translocations are considered unnecessary; and 3) for assemblages with threatened species, should the whole mussel community be translocated or just the threatened species.

2. Discussion with regulatory agencies on size of salvage area, to include upstream, downstream, and lateral buffers.

3. Discussion with regulatory agencies on the appropriate criteria for selecting suitable translocation sites.

4. Discussion with regulatory agencies on whether or not surveyors should check for brooding. Most surveyors have little to no mussel experience and so are there any concerns of incidental take.

5. Discussion with regulatory agencies on the relocation percentage, values for this measure range from 5 to 20%. Also, guidance is needed on the number of relocation surveys per site. Some protocols prescribe two while others only one.

6. Discussion with regulatory agencies on whether post-relocation monitoring is needed, if so, for how long, and what endpoints should be used to measure success.

7. Discussion with regulatory agencies on criteria for determining whether survivorship has stabilized following translocation. Discussion on frequency of sampling is also needed, particularly in the early stages of post-relocation

monitoring and emphasis should be placed on determining a sampling schedule that can help differentiate mortality from translocation vs. unsuitable habitat.

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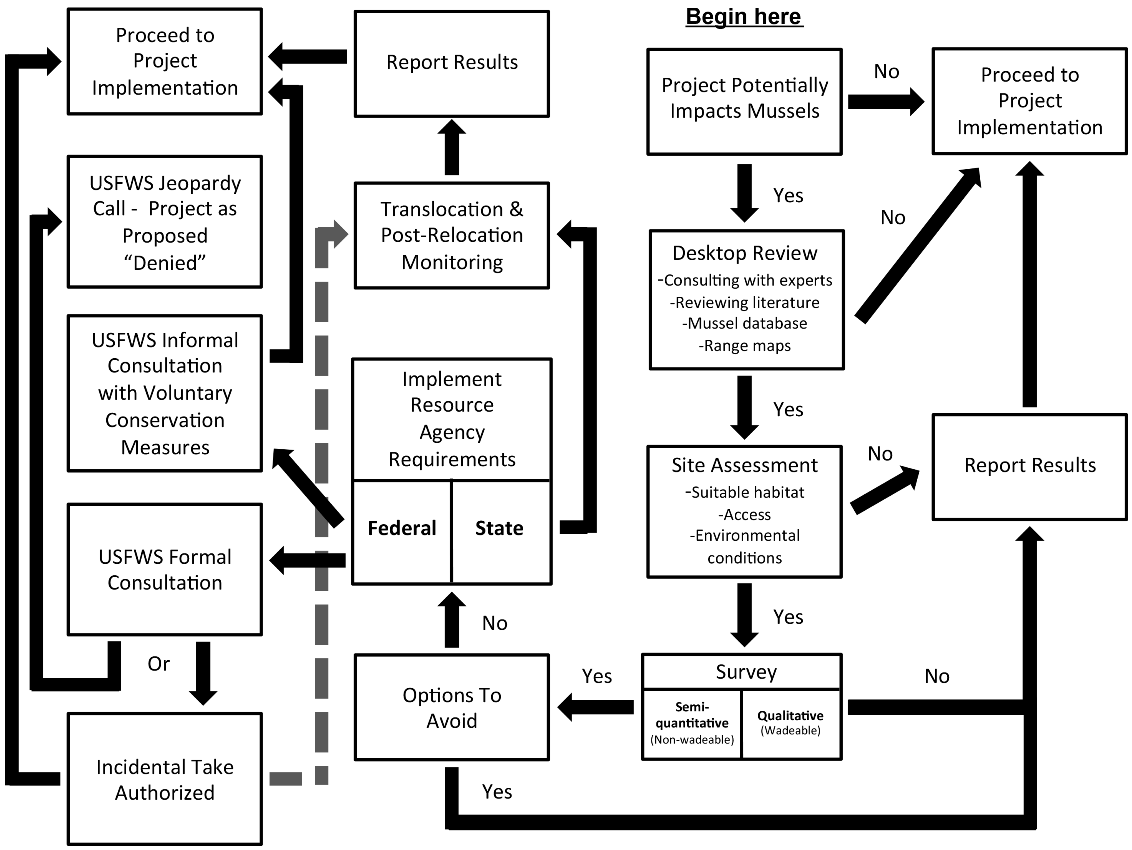
Supplemental 1



Proposed flow chart for mussel surveys, adapted from Piette (2005) and Mackie et al. (2008). The terms "Yes" and "No" refer to whether mussels are determined to be present at a given project site.