VISUAL SURVEY PROTOCOL FRAMEWORK FOR WESTERN NORTH AMERICAN FRESHWATER MUSSELS

Version 1, JUNE 2024

Credit: USFWS/Roger Tabor

Visual Survey Protocol Framework for Western North American Freshwater Mussels

Researched and prepared by

Xerces Society for Invertebrate Conservation (Xerces): Emilie Blevins, Jack Fetters
Confederated Tribes of the Umatilla Indian Reservation (CTUIR): Alexa Maine, Zach Seilo
Bureau of Land Management (BLM): Bryce Frank, Emily Johnson, Scott Miller, Anna Smith
United States Fish and Wildlife Service (USFWS): John Erhardt, Doug Nemeth, Courtney Newlon
United States Forest Service (USFS): Barbara Adams, Jeff Moss

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1 Introduction

1.1 Purpose

Several species of freshwater mussels inhabit rivers and streams in western North America (states west of the Continental Divide), including the western ridged mussel (*Gonidea angulata*), western pearlshell (*Margaritifera falcata*), and several species of floater mussels (*Anodonta nuttalliana, A. californiensis, A. oregonensis,* and *A. kennerlyi; Sinanodonta beringiana*; Williams et al. 2017). There is an increasing need and capacity among state and federal agencies, non-governmental organizations (NGOs), tribes, and others to monitor freshwater mussel populations in western North American waterbodies using standardized methods. Because freshwater mussels may constitute a significant biomass in freshwater habitats (Sansom et al. 2018), play a multi-faceted role (Vaughn 2017), live on the order of decades to a century, are relatively immobile and sensitive to changes in habitat or water quality (Blevins et al. 2017b), better documentation of freshwater mussel populations is important. Additionally, recent analyses of occurrence data at the watershed scale have revealed that multiple species of freshwater mussels that occur in western North America have declined relative to their historic ranges (Blevins et al. 2017a).

Western North America's freshwater mussel fauna is less speciose and occupies a wide range of freshwater habitats that differ from mussel fauna and watersheds in eastern North America, where many freshwater mussel survey protocols have already been developed. As such, there is a need to develop a protocol framework that is effective and adaptive across the range of freshwater ecoregions in western North America in which freshwater mussels occur. Some earlier efforts have documented survey approaches for western North American mollusks (e.g., Duncan 2008), and several existing projects in the Pacific Northwest have conducted similar surveys or are already monitoring population trends in various waterbodies or watersheds for western species of freshwater mussels (e.g., Brim Box et al. 2006; Stagliano 2010; Davis et al. 2013). However, this protocol framework seeks to align and standardize the methods and objectives to enable comparison and analysis of data across species and across their ranges. This document is also intended to provide step-by-step methods usable in a variety of projects by biologists, land managers, and others seeking to conduct freshwater mussel surveys. Therefore, the purpose of this visual survey protocol framework is to establish a standardized visual survey methodology for the collection of distribution and population data for western North American freshwater mussels.

This protocol framework contains survey methods that provide instructions on how to collect visual survey data for freshwater mussels. Throughout the document, **bolded words in the text** direct you to words defined in the glossary in Appendix A. The different sections in this document cover project design, pre-survey implementation steps, detailed instructions for implementing surveys using **core methods**, **contingent methods**, and for the collection of **covariate** data. Supplemental information and a discussion of other sampling methods are also covered, as is data documentation and reporting. Appendices also cover the necessary equipment, adaptations for special situations, paper data forms that supplement the methods, and a discussion of the GRTS sampling design.

This protocol framework takes a **modular, nested** approach to data collection to enable increasingly finer-scaled collection of data. This nestedness also allows surveys to be tailored to account for particular considerations, including those that are specific to the,

- user:
  - interest in target species or sites;
  - limitations such as existing workplans or agency requirements, or availability of permits;
  - goals specific to a project or program;
  - preferred sampling designs;
  - or constraints of time or geography;
- environment: such as the distribution or presence of challenging and highly variable habitat or habitat conditions; and
- freshwater mussels: such as the presence, distribution, or density of species or communities.

1.2 How to Use This Protocol Framework

Following vocabulary adopted by the BLM's Aquatic Inventory Monitoring (AIM) National Monitoring Framework (BLM 2021), **core methods**[1] included here are standardized procedures for collecting data that are applicable across many different ecosystems, management objectives, and agencies and are recommended for use wherever the agencies implement inventory, monitoring, or assessment of freshwater mussels. The protocol framework also includes **contingent methods,** whose usage is *contingent* upon specific needs, such as for management purposes, and do not refer to "contingencies." Contingent methods are not expected to be informative or cost effective for every monitoring application and may impact sensitive resources. The use of contingent methods should be considered during the design phase of a monitoring project and be selected to address specific management or monitoring objectives.

This protocol framework is also focused on largely visual survey methods at wadeable, lotic sites where such surveys are possible. It is not designed for sites where surveys occur in water that is overall too deep, dark, swift, or turbid to visually observe mussels. It is therefore important to note that there are some limitations to the collection of visual observation data for freshwater mussels, given that these methods will not apply at a subset of waterbodies in their native range. Additionally, mussels can form aggregations in which smaller individuals, certain species, or a portion of the overall population will remain burrowed most of the time, out of sight of the visual surveyor. Despite these potential limitations, this protocol framework takes a conservative approach, focusing methods on the visual observation of mussels at the expense of perhaps more accurate population estimates. Where core methods are combined with contingent methods, visual estimates may be augmented by data collected through careful excavation of plots. Additionally, where visual surveys are not possible due to poor visibility, **tactile** searches or **eDNA** sampling may be necessary. Refer to Section 2.10, which covers **other sampling methods**. Figure 1 outlines the suggested workflow to implement this protocol framework.

This protocol framework may not be appropriate for detailed research projects; please refer to Strayer and Smith (2003) for information on conducting such studies. Note that data collected using other

[1] Definitions for **bolded text** can be found in the glossary in Appendix A.

sampling methods provide important information that can be used in combination with data collected through this protocol framework. Please refer to Section 3 for more details about submitting data from projects using this protocol framework or using other methods. Note also that documentation of "zero" data, in which survey methods in this protocol framework are implemented but no mussels are observed, is also of value. Refer to Section 2.3 for more information.

1.3 Protocol Requirements

This protocol framework includes several *requirements* for implementation with which users should be familiar before proceeding further, particularly if this protocol framework is adapted for specific projects. Each requirement is hyperlinked to the section in which it is described in further detail:

- minimum quality standards (Section 1.5.4)
- required project metadata (Section 3.1)
- required covariates (wetted width and channel unit) (Sections 2.8.1 and 2.8.2)

1.4 Project Design

This protocol framework can be used across a range of sampling designs and a variety of **target universes** or **sampling frames**. Some limited information is provided below on objectives, sampling design, target universe, and **sampling unit** selection. See Section 3.1 (**required project metadata**) for important notes regarding documentation during the project design stage.

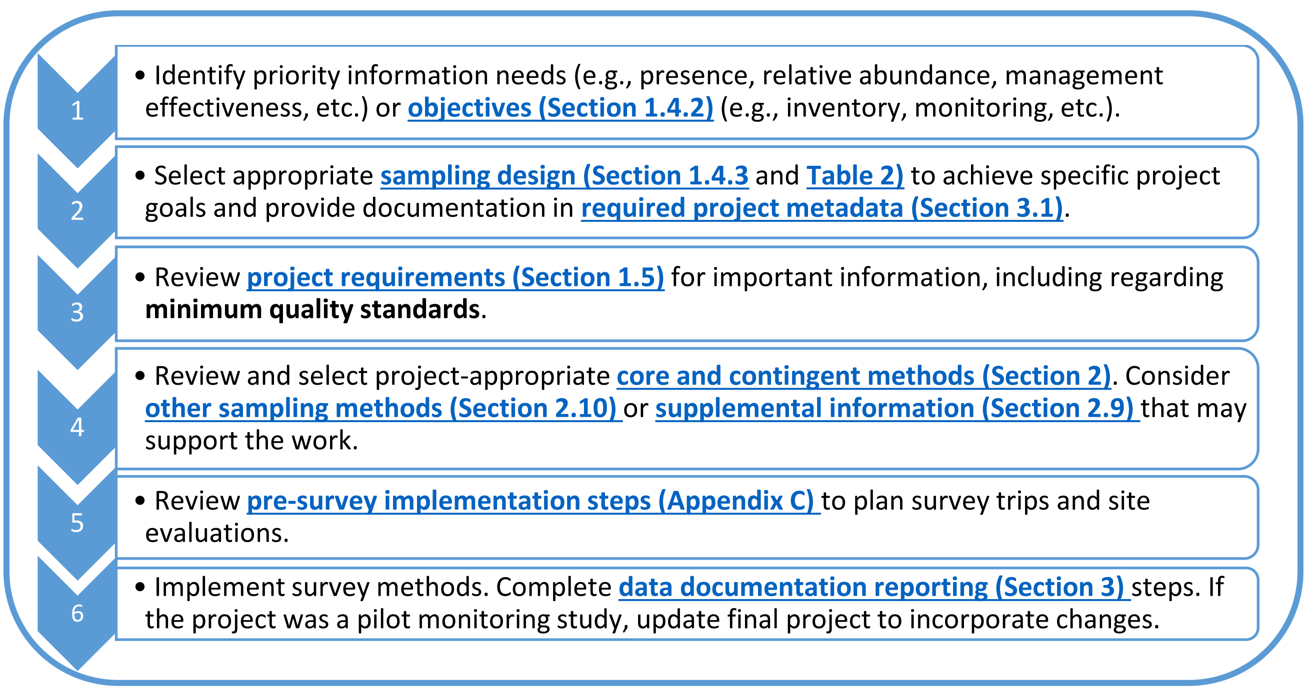

Figure 1 Suggested workflow to implement the protocol framework. Bolded words indicate a topic covered in following sections.

1.4.1 Survey Methods Overview

This protocol framework consists of **core methods**, **contingent methods**, and **covariates**, with additional information provided on **supplemental information** or **other sampling methods** (Table 1). Each of these methods contributes to an increasingly comprehensive understanding of freshwater mussel community characteristics applicable to a range of objectives. Methods can be used independently or in combination to meet specific objectives, such as those related to inventory and monitoring.

The core methods are focused on data associated with **reach** surveys. **The reach is the fundamental unit used to locate and document information, and it is defined in this protocol as a 50 m length of stream.** The sampling design will determine where reaches are established. For example, **exploratory** surveys may serve as an initial inventory for planning a reach survey. By documenting exploratory surveys in a manner that enables future reach surveys, this protocol framework allows the user to later make use of other sampling methods. Methods for selecting reach locations where statistical balance is necessary are also described below. **Incidental** observations or data lacking additional information can be valuable for planning future sites. However, surveys lacking basic **metadata** or not meeting quality standards (Section 1.5.4) precludes use of the data for a wide range of applications.

Methods described in this document are appropriate for a wide range of waterbodies, although special or unusual situations (e.g., **braided channels**, presence of beaver, dry channels, turbid water, or deep pool habitat) may warrant modifications; refer to Appendix D (Special Situations) for more information.

1.4.2 Project Objectives

The specific objectives established during planning will ultimately determine the number of sample sites, methods for site selection, and appropriate survey methods. Visual surveys for freshwater mussels can be implemented under this protocol framework to meet a range of objectives (Table 2). Refer to Section 2.10 for information about other sampling methods that may incorporate additional project objectives relevant to freshwater mussel survey efforts. Also refer to Section 2.8 for information about covariate data collection at sample sites.

1.4.3 Sampling Design Considerations

For statistical purposes, the primary unit of replication (**sampling unit**) for this protocol framework is focused on the **reach** (50 m length of stream) (Frissell et al. 1986; Davis et al. 2013; Stagliano 2010), with other nested methods capable of supplementing the information collected at that level. The exploratory method is most appropriate for inventory purposes, while reach, transect, and plot methods are appropriate for monitoring. In some cases, such as when conducting an impact assessment or determining the proportion of mussels buried or of a certain size class, a combination of methods is preferred.

Randomized **sampling designs** enable rigorous, unbiased estimation of quantities such as population size, presence/absence rates, and densities (e.g., Dorazio 1999) across large geographic regions, along with associated confidence intervals. Collecting reach-level observations using a randomized sampling

Table 1 Core and contingent methods and their characteristics

Method	Sampling Unit	Description	Geospatial Product	Databased Information
Exploratory	Unit 1 km in length, but adaptable to other lengths	A distance-based mussel-focused survey; generally, one or a series of 1 km reaches that are inventoried for mussel species presence and relative abundance	• Series of points denoting upstream/downstream (start/end) of exploratory survey • Point locations at sites where reach surveys could occur	• Broad-scale counts or lineal density estimates by genus/species (or absence data) • Covariates collected at locations within the exploratory sampling unit
Reach	50 m reach	A 50 m reach that is surveyed for mussels in detail; the primary unit for establishing transect and plot locations	• Point documenting the location of the reach	• Counts or lineal density by genus/species (or absence data) within a discrete 50 m reach • Covariates documenting the reach and mussel distribution in detail
Transect	Transect located within a 50 m reach	A transect consisting of at least 10 quadrats measuring 0.25 square meters in size, placed according to a random or non-random method, with a minimum of two transects recommended	• Point documenting the starting point of a transect (if applicable) • Point documenting a **monument** to locate transect (if applicable)	• Attributes documenting the location and orientation of transects, placement approach (random or non-random), number of quadrats, or length of transect • Areal density by genus/species (or absence data) collected from within each quadrat along a transect
Plot[2]	Plot located within 50 m reach	One or more plots where mussels are excavated and enumerated	• Point documenting the location of a plot (if applicable) • Point documenting a monument to locate plot (if applicable)	• Attributes documenting the location of plot in the reach • Areal density by genus/species, as well as information collected on location (surface or subsurface), size, condition, etc.

[2] Contingent method

Table 2 Example project objectives and the recommended methods for use in this protocol framework. Covariates that can be incorporated into each method, dependent on the project objectives, can be reviewed in Section 2.8

If you want to…	consider using…	because…	Line #
document mussel presence (e.g., inventory)	Exploratory	this method is characterized by an adequate survey effort to efficiently cover larger distances or areas lacking any mussel information. It is also easily combined with other species' inventory efforts and can provide preliminary information to support reach-level surveys. This method is not appropriate for monitoring purposes.	1
	Reach	this method provides a measurement of constant length, where the time investment is typically 2-3 hours or less, and is useful when mussels have already been observed but not assessed. This method is not appropriate for covering large areas and is inefficient with respect to inventory unless part of a sampling design.	2
document distribution	Exploratory	Refer to line 1 in this table.	3
document relative abundance	Reach	Refer to line 2 in this table.	4
	Transect	this method is useful when mussels are exceptionally abundant or dense within a reach and improves statistical analyses of count data, including estimates of density. This method must be paired with a reach survey.	5
document changes in populations from baseline conditions resulting from anthropogenic or environmental stressors, or management actions	Reach	this method is implemented at an appropriate scale within which other data can easily be collected by incorporating other core and contingent methods. Where mussels are exceptionally abundant or dense, this method should be supplemented with transects to improve accuracy.	6
	Transect	this method improves statistical analyses of count data, and includes estimates of density. It is also useful when information specific to a mussel bed is of particular interest. This method must be paired with a reach survey.	7
	Plot	this method can be used to track population size distributions, condition, individual fates, species composition, or to improve estimates of relative abundance. Review the checklist in Section 2.7.1 for additional considerations to determine whether this method is advisable or appropriate. This method must be paired with a reach survey.	8
monitor populations	Reach	this method is characterized by an adequate survey effort to detect, identify, and enumerate freshwater mussel species in a repeatable and replicable design. This method is not appropriate for covering large areas unless part of a sampling design.	9
	Transect	Refer to line 7 in this table.	10
	Plot	Refer to line 8 in this table.	11
conduct a survey before and after implementing a restoration or management project	Exploratory	this method covers larger distances or areas lacking any mussel information efficiently. When performed before restoration implementation, care should be taken to document mussel occurrence specifically where actions are proposed.	12
	Reach	Refer to line 9 in this table.	13
	Transect	Refer to line 7 in this table.	14
	Plot	Refer to line 8 in this table.	15
conduct an impact or damage assessment	Exploratory	this method implements an adequate survey effort and covers larger distances efficiently. Refer to line 1 in this table.	16
	Transect	Refer to line 7 in this table.	17
	Plot	this method can be used to estimate the proportion of live/dead individuals. This method should be paired with a transect survey.	18

design is not strictly required by this protocol framework, but organizational objectives may require or strongly suggest the use of a randomized sampling design. This is especially the case when statistically robust trend analysis of populations at large scales (i.e., entire river systems) is a desired outcome of the project. Reach sites selected for re-surveys as part of a monitoring project can also be identified according to a random or stratified random sampling design.

Sparse mussel populations introduce challenges for projects that use random sampling, which may result in a large number of sites that do not contain mussels. These challenges can be mitigated through an effective stratification of the study area, which partitions stream networks such that abundance or density is similar within strata but dissimilar between strata. Information such as gradient, elevation, or stream order may be used to develop a stratification. If a stratification is used, take care to properly weight data (see Appendix E).

1.4.4 Project Duration and Survey Frequency

Projects implemented using the methods provided in this document can be conducted over a single year (within the appropriate sampling index period; see Section 1.5.4), over the course of several years, or long-term. It is recommended that monitoring projects implementing core methods occur on a rotational 3 or 6-year interval (CTUIR 2022; Boon et al. 2019) and that surveys occur at the same time of year or under similar temperature or flow conditions if possible. If ecological events such as drought, wildfire, or flood occur between intervals, or human activities such as in-water construction or habitat restoration occurs, surveys should occur soon after the event to document any effects. For projects involving excavation or handling of mussels, extended periods between sampling can limit impacts to the study population. Note that recruitment of western North American freshwater mussel species may require several years to detect due to the small size and potential slow growth of juvenile mussels.

1.5 Project Requirements and Considerations

1.5.1 Species Identification, Habitats to Survey, and Survey Techniques

Generally, identification of freshwater mussels to species or genus is considered best practice, and surveyors should be trained or familiarize themselves with species identification materials. A Quick Identification (ID) Guide is provided in Appendix F with the data forms. Good photographs (see Quick ID Guide) of mussels or **shell** vouchers can assist with training or confirmation of species identification. **Removing live mussels from the substrate for identification of species is not desirable, and in many cases requires a permit (Section 1.5.2). Surveyors using the methods described in this document are expected to have the expertise to either identify the species or genera on site or take underwater photos of the mussel on site in order to identify to species or genus when shells are not available.**

Freshwater mussel surveys also require surveyors to examine a wide range of microhabitats and positions, as mussels are commonly observed in underbank habitat if burrowing substrate is present; within aquatic rooted plants; under, between, or behind boulders; under or adjacent to large wood; and many other locations instream where stream power, adequate flow, and burrowing material allow. In areas of loose or fine substrate, mussels that are otherwise covered can be revealed by lightly fanning the substrate. Surveyors should also examine bars, banks, and areas of deposition for shells or midden piles for evidence of mussel presence instream.

Surveyors should develop a search image and be trained in proper survey technique by others who have performed surveys. If mussels are handled, training in proper handling technique is also necessary to reduce impacts from sampling. **In general, surveyors should aim to avoid disturbing the site prior to sampling and avoid impacts to freshwater mussels and their habitat, such as walking on mussels, which could result in crushing of fragile shells.**

1.5.2 Permits and Equipment

Permits may be required to touch or otherwise handle live freshwater mussels, even for simple identification purposes. Core methods included in this document do not require any handling of freshwater mussels, but handling is necessary when employing contingent methods. Before implementing the methods in this protocol framework, first determine whether a permit is required, and if the use of contingent methods is necessary for project success.

A detailed gear list is provided in Appendix B, as is a description of equipment disinfection, which is critical when equipment is used across watersheds or from downstream to upstream sites in order to reduce the potential transport of invasive species and pathogens such as viruses or bacteria.

1.5.3 Safety

Because this protocol framework involves activities in flowing water, including wading, swimming, and use of a snorkel and mask, it is highly recommended that surveyors receive safety training to work in water. Before embarking on surveys, supervisors should evaluate swimming proficiency of staff. Topics that should be reviewed include recognizing hazards and evaluating the safety of a site (Appendix C), navigating flowing water under a range of conditions, and avoiding impacts to other aquatic species, such as avoidance of redds (a fish spawning nest) during surveys, among other topics. A job hazard analysis may be useful for review of risk (available upon request).

Credit: USFWS/Roger Tabor

1.5.4 Required Quality Standards for Conducting Surveys

This protocol framework seeks to maximize the precision of field measurements and reduce sampling error by specifying an index period within which data should be collected. With few exceptions, all data collection should occur between **June 1 and September 30**. This time period generally falls during appropriate flow, visibility, and temperature conditions. Note that there may be a narrower optimal site-specific time period for sampling. Surveyors should record the date of the survey and provide justification if the survey occurs beyond the index period in associated notes. A reference questionnaire for these quality standards is also provided in Appendix C.

1.5.4.1 Flow

The index period generally corresponds to **baseflow** water levels (although exceptions exist), when streams can be safely navigated (see also Appendix C). Exceptions can be made where climatic conditions preclude sampling during this time period (e.g., depending on snowmelt influence, regional climate variation, local water management activities, etc.). Factors, such as precipitation, irrigation return flows, or dam releases, can cause discharge to be elevated for short durations during the index period. As such, users should consult local discharge gages (e.g., https://waterdata.usgs.gov/nwis/rt), weather stations, and field offices to determine how recently such an event may have occurred.

Following high flow events, consider whether data should be collected or whether the waterbody should be revisited at a later date. Sampling should be delayed until discharge recedes to baseflow water levels and turbidity levels return to baseline conditions after significant rain events. If sites are particularly difficult to access or require exceptional travel, collect data as long as discharge is below **bankfull** and other minimum quality standards are met. Surveyors will record that the site meets the required hydrologic condition at the time of the survey.

1.5.4.2 Visibility

In order conduct a visual survey that minimizes errors associated with imperfect detection, basic visibility requirements must be met. In accordance with other state and regional freshwater mussel survey protocols, visibility must be at minimum 0.5 meters (approximately 20 inches) or visible to bottom if shallower than 0.5 meters. Although visibility can be obscured by depth or objects providing cover, such as large woody debris or boulders, this quality standard refers specifically to characteristics of water clarity. Surveyors will record that the site meets the required visibility condition at the time of the survey.

1.5.4.3 Water Temperature

Water temperature is an important variable to measure at sites to ensure conditions are not too cold, as freshwater mussels may burrow more deeply and be less visible under (relatively) colder water temperatures that may also coincide with

Does your sampling effort meet the described minimum requirements?

- take place between June 1 and September 30
- occur at baseflow or sufficiently low water for safety and visibility requirements
- occur at sufficiently warm water temperatures

If not, reschedule your sampling effort.

periods of higher flows. To avoid undercounting freshwater mussels, and to ensure the safety of surveyors, combined water and air temperatures should add up to at least 100°F (20°C). Surveyors will record the instantaneous water temperature at the beginning of all surveys. Record time of day and submerge the thermometer at mid-depth (neither the deepest nor shallowest portion of the waterbody) for several minutes while setting up the survey. Avoid stagnant water in favor of flowing (unless representative of the site). Consider deploying a temperature probe to document continuous water temperature at sites where monitoring will occur (Section 2.8.7).

1.5.5 Minimum Search Effort

Survey methods described in this document are based on completing surveys over a set distance (or within a set area) rather than time, and making use of specific survey techniques. These techniques include snorkeling, wading with or without use of an aquascope or view bucket, using a standardized **0.25 m² quadrat** (Figure 2), and a sieve. Considerations for the preferred technique include water depth (deep enough to submerge a mask, for example), glare, type of habitat, and specific core or contingent sampling method employed, and are discussed later in this document. For the reach method, a minimum amount of search time is also recommended. At least 30 minutes per person should be spent surveying at sites where mussels occur, or 15 minutes per person when a careful survey can still determine that mussels are not present at the site (generally only where waterbodies are exceptionally small and/or shallow). Additionally, in situations such as large river sites, where surveying the middle of the channel is not feasible, or where special situations must be considered (Appendix D), only a subset of the reach may be surveyed and preferred habitats can be targeted. Where applicable, details on survey area are provided elsewhere in this document.

1.5.6 Pre-Survey Steps

It is recommended that surveyors review Appendix C before embarking on surveys. This section provides guidance on general pre-survey steps, regardless of the project design or selected methods. It is assumed that project design information has already been reviewed and completed.

Credit: Xerces Society/Emilie Blevins

2 Protocol Survey Methods

2.1 Background

The following sections provide detailed methods for conducting freshwater mussel surveys. Information about the purpose, appropriate use, and method-specific steps are also provided in each of the following sections. **Each methods section is written such that users may print specific methods pages for standalone use in the field.** Core and contingent methods are adaptable for surveys using various techniques to detect and enumerate freshwater mussels (described in Section 1.5.5). When pairing the core methods with contingent or other sampling methods (Section 2.10), such as eDNA sampling, consider the order in which sampling should occur (e.g., collection of eDNA samples prior to a reach survey) or the direction in which sampling will occur (e.g., conducting an exploratory survey from downstream to upstream when also collecting eDNA samples). Be sure to reference the protocol framework requirements (Section 1.5) and the quality standards (Section 1.5.4), as well as relevant appendices before implementing a survey.

2.2 Providing Incidental Observation Data

Incidental observations are generally observations that are incidental to other surveys, are opportunistic, and provide limited information. As such, they are not a key component of this standardized protocol framework. However, reporting incidental observations can, like other supplemental information, support planning and implementation of a survey or facilitate other research (e.g., Scully-Engelmeyer et al. 2023). The level of required expertise to report incidental observations is generally lower, and observations made both in-stream and out-of-stream (e.g., while hiking or as with a **midden** observation) can be valuable. Data points collected for incidental observations can be brief and are easily captured in existing data forms or through an online form available at https://arcg.is/KuS8H or using the QR code below (Figure 2). Additionally, a paper data form is provided in Appendix F.

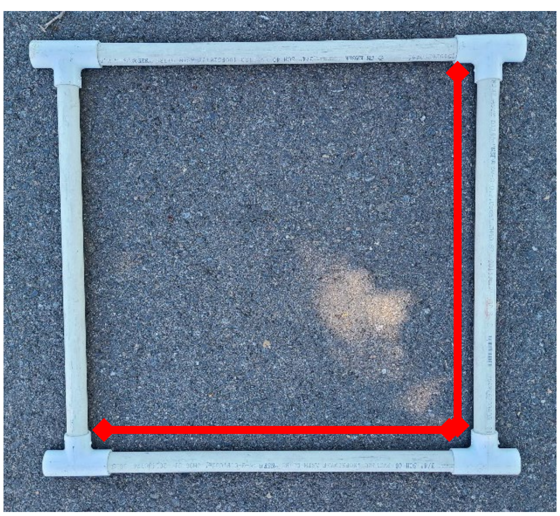

Figure 2 Left: Example of a 0.25 m^2 quadrat with red lines depicting the interior lengths and widths measuring 0.5 meters each; Right: QR code for submitting incidental observation data.

2.3 Reporting Surveys Resulting in No Observations

This protocol framework is designed to set an acceptable standard for conducting visual surveys for freshwater mussels. As such, it includes minimum quality standards (Section 1.5.4). Therefore, when a survey is completed according to the core methods outlined in this document, but no mussels are observed, it is appropriate to report that as "zero" data. Reporting of zero data is valuable particularly for indicating when a required survey, such as a survey that is part of pre-project clearance, is completed, and no freshwater mussels are found to be present. Refer to Section 3 for more information on data documentation and reporting.

Credit: USFWS/Courtney Newlon

2.4 Core Method: Exploratory

2.4.1 Overview

The purpose of an exploratory survey is to gather information on the freshwater mussel assemblage, including the presence, relative abundance, or distribution of freshwater mussel species, within a portion or the entirety of a waterbody. In comparison to an incidental observation, where freshwater mussels are not the explicit survey target, or the observation is not necessarily part of a survey, an exploratory survey has a stated goal of actively surveying for and documenting freshwater mussels; it subsequently captures a greater amount of information than collected from incidental observations. Because exploratory surveys are adaptable to a range of situations, it is easy to begin an exploratory survey at any time. The information collected through an exploratory survey is intended to be gathered in a manner that is efficient for:

- implementing adequate survey effort to detect, identify, and estimate species for inventories,
- covering a larger distance or area,
- incorporating into existing biological surveys, providing greater detail and confidence than incidental observations,
- conducting pre- or post-project surveys, including in-stream construction or restoration work, and/or
- providing preliminary data to support reach-level surveys, including for stratification purposes.

Exploratory surveys are particularly valuable because mussel distribution is often patchy or sparse. When little or no information on mussel presence or species distribution is available for a watershed, the exploratory survey enables larger distances or areas to be covered quickly for inventory purposes, while also capturing information across a range of microhabitats. Additionally, the greater the length of stream surveyed, the greater the opportunity to provide important stream-level context for mussel populations, which may be variable across habitats and stream gradient, even within a single waterbody. However, exploratory surveys are not recommended as the primary method where monitoring is intended. In these cases, the increased effort and specific metrics collected at reaches, or within transects or plots in reaches, is more appropriate.

Exploratory surveys that are not associated with a limited project footprint, such as a small in-stream habitat restoration project, are <u>recommended to cover at least 1 km of contiguous river or stream length</u>. When ownership boundaries or other factors limit what can be surveyed, document the actual length surveyed and reason. In such cases, <u>surveys of shorter lengths still provide valuable information and should be conducted</u> according to the same methods described below. Keep in mind that, because freshwater mussels are long-lived and relatively immobile, a 1 km exploratory survey need not occur over a single day or even consecutive days. Within the index period of a single year, an exploratory survey could be stopped and started over multiple days in order to be completed. Exploratory surveys can also be adapted to skip unsurveyable portions of waterbodies as needed (described further below).

2.4.2 Preparing to Survey

It is strongly recommended that you read through the entirety of the methods, including pre-survey steps, information on conducting the survey, and information on collecting covariate data, before beginning a survey to ensure you have the appropriate equipment and understand how the survey will be conducted. Ensure that you are conducting the survey during the appropriate index period and are meeting minimum quality standards (Section 1.5.4). See comment in Section 2.4.1 about conducting an exploratory survey over multiple visits if needed. Assemble the necessary equipment (Appendix B) and review the *Project Metadata*, *Survey Event Details*, and *Exploratory Survey* data forms (Appendix F).

If possible, plan the survey for at least 1 km in channel length. If a distance longer than 1 km is planned, the survey reach can be split into 1 km portions for reporting during data entry using a geographic information system (GIS). If a distance of 1 km falls within the middle of a habitat unit of interest, such as a pool, the surveyor can continue the survey of the habitat unit and simply report the total distance surveyed. If a distance shorter than 1 km is planned, document the distance surveyed and the reasons for the shorter distance. Where side channels or braided channels occur, make a note of what channels were surveyed and provide an estimate of the length of these surveyed channels on the data form. Surveyors should record information for only one named waterbody per data form. If the waterbody is a tributary to another waterbody also surveyed, each should be reported independently.

2.4.3 Conducting the Survey

1. Determine the appropriate number of surveyors for the chosen location, or determine if a waterbody can be sampled given the number of surveyors available. For waterbodies with a wetted width of 10 m or greater, at least two surveyors should work in tandem.
2. Determine the survey technique. This will be a function of the waterbody, available equipment, and conditions, and as such may need to be modified on the day of the survey. Techniques include snorkeling or wading, with or without use of an aquascope or view bucket. **In general, surveyors should aim to avoid disturbing the site prior to sampling and avoid impacts to freshwater mussels and their habitat.** Under conditions where a waterbody can be snorkeled (i.e., the snorkel mask can be submerged), this is considered the optimal method for detecting freshwater mussels, particularly where overhanging habitat, such as vertical bank or large rocks, could obscure visual observations from above the water, or where abundance is particularly low and freshwater mussels could be easily missed. Where snorkeling is not feasible or preferable, wading with an aquascope or viewing bucket can be implemented, although this method will generally undercount the mussels present. Wading without the use of an aquascope or viewing bucket is not recommended in most cases. However, experienced surveyors can conduct surveys in this manner, generally using polarized sunglasses or a waterproof flashlight as aids, although this technique should be limited in use.
    a. **Special note:** In order to document multiple GPS coordinates while surveying, surveyors can use a variety of methods to transport the GPS unit. Examples can include having one surveyor snorkel while the other wades, placing the GPS unit in a rolltop drybag or waterproof hip pack or flagging locations to return on land to collect coordinates with the GPS. The specific method will depend on survey logistics and available tools or staff.
3. Navigate to the starting location. This can be upstream or downstream of the intended endpoint, keeping in mind that visibility may be impacted if surveyors walk through the waterbody or otherwise disturb bottom sediments during the survey. If surveying in a downstream direction reduces visibility, surveyors should survey working upstream or report observations as **incidental** only (Section 2.2). Additionally, if pairing with eDNA collection, the

survey should occur from downstream to upstream to avoid contamination. See Section 2.10.1 for more information.

4. At the starting location, document the geographic coordinates and start time. There is no need to flag the starting location unless it is useful for other purposes. Record preliminary information on the data form, including the wetted width, reporting to the nearest 0.10 meters (see Section 2.8.1).
5. Surveys can be conducted by surveying the entire wetted width of the stream from bank to bank, either by zigzagging or, if the stream is narrow enough, covering the area completely.
   a. If surveying the entire width is not feasible due to the size of the stream, swift flows, or a deep channel in the **thalweg**, surveyors may opt for using the zigzagging approach from banks, but skipping the middle of the waterbody (see also Appendix D). In this case, surveyors should report the average distance from each bank that was surveyed so that future surveyors can repeat the same level of effort. This method is recommended for pre-project clearance surveys to ensure that the most likely places mussels might occur are searched.
   b. Alternatively, a survey may be conducted by having surveyors each count within a "lane," in which a swath at least 1 m wide or full arm's width (per surveyor) is examined, with the two surveyors working in tandem. Surveyors should not zigzag using this approach but instead follow a consistent upstream (or downstream) path (Figure 3). Surveyors should report the number of surveyed 1 m lanes.
   c. If only one person conducts the survey, they may focus on surveying one or more lanes at least 1 m wide where mussels are most likely to occur, such as within, below, and behind boulders, rooted vegetation, banks, etc. The surveyor should report the number of surveyed 1 m lanes.

Figure 3 In this example, two surveyors are working in tandem to survey two separate 1 m wide lanes (thick black lines). The middle of this river is not surveyable, and rather than zigzag out from the bank, the surveyors chose to survey a consistent line in the river. For this example, mussels occurred in large numbers and nearly continuously along the edges of the river, so surveying a lane ensured mussels were not double counted. Had mussels been more patchily distributed, a zigzag method would have enabled them to survey more potential microhabitats. If the middle of the river had also been surveyable, adding a third surveyor lane would be advisable for a more complete survey.

6. Conduct the survey in this manner until the first live freshwater mussel is observed. Note that if no live mussels are observed, but a **shell** is, the shell should be documented as an **incidental** observation. When live mussels are detected, take a GPS point or record the coordinates and continue to conduct the survey carefully, counting the number of mussels by species or genus up to 100. When more than 100 individuals of a mussel species are present, surveyors may estimate the number observed and select the appropriate estimate "bin" provided on the data form (e.g., 101-500 or >500). Surveyors may also report a larger estimate than >500 individuals per mussel species if preferred. Always report at least the first point where a new species of

mussel is observed to ensure that presence is represented geospatially for the waterbody. A dive slate or clicker counters can be valuable for maintaining a tally during the survey (Figure 4).

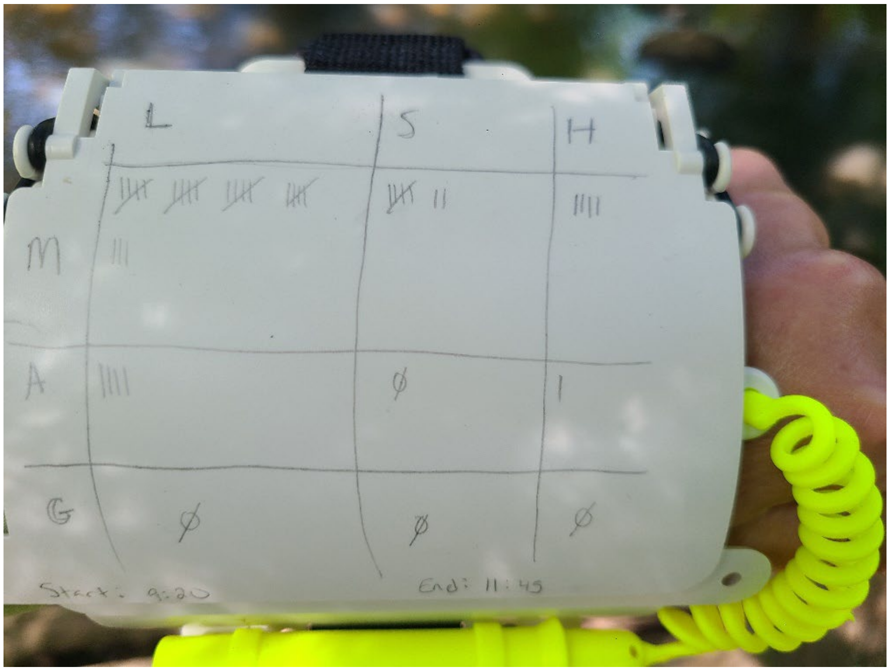

Figure 4 Example of a dive slate used for tracking the number of each species or genus (M, A, G) by live count, shell count, and number handled (L, S, H). Credit: Xerces Society.

7. Because this data is not collected over a specific distance, as in the reach survey, surveyors can determine where to start or stop counts/estimates based on their observations at the site. For example, at a site where habitat restoration is planned, specific counts/estimates could be limited to within the footprints of project elements or activities. Where discrete **mussel beds** occur, these may naturally serve to delineate points and their associated counts/estimates. Mussels may also occur continuously or may be patchily distributed. If channel units (Section 2.8.2) naturally transition during the survey and appear correlated with a change in mussel presence, this can also serve as a natural break for counts/estimates. Surveyors can also document their counts/estimates by reporting the upstream and downstream coordinates of the observation rather than a single point. The goal should be to collect general location and count or estimate by genus, with a greater emphasis on covering a longer distance, and with less emphasis on accurately mapping the extent of any mussel beds. Instead, be as descriptive as possible in the notes section of data forms where applicable. Data collected through the exploratory survey can be targeted for future reach surveys, which are better equipped to characterize the population or community.
8. Report the general location of live mussels in-stream at this location, whether **left bank, right bank, or in the middle** (when oriented facing downstream). Also estimate the number of shells present (if applicable). Take voucher photos of freshwater mussels for species documentation (see the Quick ID Guide for examples of good photographs).

   a. OPTIONAL: Complete the Human Influence checklist for the entire survey or for specific aggregations of mussels if of interest (Section 2.8.5) on the *Human Influence Covariate* data form (Appendix F).
   b. OPTIONAL: Record the presence of mussel host fish within the survey area or near specific aggregations of mussels if of interest (Section 2.8.6) on the *Host Fish Presence Covariate* data form (Appendix F).
   c. OPTIONAL: Collect water quality data or samples or near specific aggregations of mussels if of interest (Section 2.8.7) and record on the *Water Quality Covariate* data form (Appendix F).
9. When mussels are not encountered, surveyors should report their absence on the *Survey Event Details* data form (Appendix F). If mussels are observed during the survey but point data or GPS tracks are not recorded (e.g., due to insufficient time or other reasons), surveyors may still document the survey using the *Incidental Observation* data form (Appendix F) and report general information.
10. If surveyors observe any obvious differences in habitat or conditions that could contribute to a change in mussel occurrence between habitat units, record in the notes section and reference the relevant location where mussels were last observed before the change.
11. If the exploratory survey cannot be thoroughly conducted through any portion of the waterbody longer than 50 m, whether due to unsurveyable areas (e.g., especially deep or dangerous conditions) or other constraints (e.g., change in ownership boundary, change in conditions, etc.), mark an endpoint to the exploratory survey. If conditions improve upstream or downstream, the surveyor should resume surveying but record it as a new exploratory survey with new starting coordinates on the data form in order to capture what portion was surveyed (as opposed to including the unsurveyable portion in the total distance).
12. Upon completion of site data collection, refer to Data Documentation and Reporting (Section 3) for next steps.

2.5 Core Method: Reach

2.5.1 Overview

The purpose of a reach survey is to gather information on the freshwater mussel assemblage, including species composition and relative abundance, and other characteristics at and within a discrete location (50 m reach). In comparison to an exploratory survey, the reach survey is designed to accurately enumerate mussels at a site and provide a survey effort of constant length for comparison with other reaches across waterbodies and watersheds in an efficient manner. Features of this method include:

- adequate survey effort to detect, identify, and enumerate freshwater mussel species,
- a survey effort of constant length, where the time investment is typically 2-3 hours or less,
- a repeatable and replicable design for use in most streams and for monitoring purposes, and
- an appropriate scale within which other data can easily be collected by incorporating other core and contingent methods.

In particular, this method supports opportunities for both real-time inventory and long-term monitoring, while also sampling at a scale at which additional research efforts can be simultaneously implemented. For example, reach surveys can provide the basis for long-term monitoring at sites to detect changes in presence, relative abundance, or species composition over time or to monitor the status of rare species at a specific location. It is also valuable for evaluating localized threats or responses to change, or for developing population estimates (using an appropriate sampling design and target universe).

Due to the oftentimes patchy distribution or low density of freshwater mussels, reach surveys are not recommended where little or no information is available regarding freshwater mussel presence within a waterbody or watershed, where search effort is constrained by time or resources, or where the goal is to establish presence or absence of mussel species within a larger context (inventory). In those situations, an exploratory survey provides a more efficient means to document areas where mussels do or do not occur, providing preliminary data for use in follow-up investigations. As an exception to this, projects that are designed for large-scale monitoring, where large numbers of reach surveys can be conducted and where the statistical design, such as a GRTS design (see Appendix E), supports population-level assessments, should employ the reach method.

Data collected through incidental observations or exploratory surveys can be used to select locations where reach surveys occur. Additional core (transect) and contingent (plot) methods, though not required, are designed to nest within the reach survey location, and can be added to a project at the time of the initial reach survey or at a later date. These methods, which can provide detailed **mussel bed** metrics, can supplement information gathered at a 50 m reach, particularly for projects involving mussel translocation or restoration. If additional methods are conducted at a reach site, those methods should be implemented after the reach survey. Additionally, adaptive sampling methods used to increase the total distance surveyed have been successfully implemented elsewhere (e.g., Stagliano 2010) and should be considered during project planning and sampling design development stages.

2.5.2 Preparing to Survey

It is strongly recommended that you read through the entirety of the methods, including pre-survey steps, information on conducting the survey, and information on collecting covariate data, before beginning a survey to ensure you have the appropriate equipment and understand how the survey will be conducted. Ensure that you are conducting the survey during the appropriate index period and meeting minimum quality standards (Section 1.5.4). Before implementing, consider which other nested core (transects) or contingent (plot) methods might also be implemented. Assemble the necessary equipment (Appendix B) and review the *Project Metadata*, *Survey Event Details*, and *Reach Survey* data forms (Appendix F).

2.5.3 Conducting the Survey

1. Determine the appropriate number of surveyors for the chosen location, or determine if a waterbody can be sampled given the number of surveyors available. For waterbodies with a wetted width of 10 m or greater, at least two surveyors should work in tandem. As with exploratory surveys, reach surveys can be conducted by surveying the entire wetted width of the stream from bank to bank, either by zigzagging or if the stream is narrow enough, covering the area completely.
    a. If surveying the entire width is not feasible due to the size of the stream, swift flows, or a deep channel in the **thalweg**, surveyors may opt for using the zigzagging approach from banks, but skipping the middle of the waterbody (see also Appendix D). For the purposes of analysis and interpretation, the reach consists only of the surveyable portion of the river. In this case, surveyors should report the average distance from each bank that was surveyed throughout the 50 m reach so future surveys can repeat the same level of effort.
    b. Alternatively, a survey may be conducted by having surveyors each count within a "lane," in which a swath at least 1 m wide or a full arm's width (per surveyor) is examined, with the two surveyors working in tandem. Surveyors should not zigzag using this approach but instead follow a consistent upstream (or downstream) path (Figure 3). Surveyors should report the number of surveyed 1 m lanes.
    c. If only one person conducts the survey, they may focus on surveying multiple lanes at least 1 m wide where mussels are most likely to occur, such as within, below, and behind boulders, rooted vegetation, banks, etc. The surveyor should report the number of surveyed 1 m lanes.
2. Determine the survey technique. This will be a function of the waterbody, available equipment, and conditions, and as such may need to be modified on the day of the survey. Techniques include snorkeling or wading, with or without use of an aquascope or view bucket. **In general, surveyors should aim to avoid disturbing the site prior to sampling and avoid impacts to freshwater mussels and their habitat.** Under conditions where a waterbody can be snorkeled (i.e., the snorkel mask can be submerged), this is considered the optimal method for detecting freshwater mussels, particularly where overhanging habitat, such as vertical bank or large rocks, could obscure visual observations from above the water, or where abundance is particularly low and freshwater mussels could be easily missed. Where snorkeling is not feasible or preferable, wading with an aquascope or viewing bucket can be implemented, although this method will generally undercount the mussels present. Wading without the use of an aquascope or viewing bucket is not recommended in most cases. However, experienced surveyors can conduct

surveys in this manner, generally using polarized sunglasses or a waterproof flashlight as aids, although this technique should be limited in use.

3. Navigate to the reach. Using a GPS, record the geographic coordinates for the starting location and whether they represent the upstream end of the reach, the downstream end of the reach, or the midpoint. To visually assist in determining the survey length, surveyors may want to place flagging along the bank at both endpoints (Figure 5).
4. Confirm that the survey can be conducted along the full 50 m (refer to Section 5.3.2). Note that the 50 m distance should attempt to follow the **thalweg**, not the bank. If more than 5 m in waterbody length is totally unsurveyable, shift the reach upstream or downstream so that less than 5 m is unsurveyable. Reference the information on minimum search effort (Section 1.5.5).
    a. **Special note:** For waterbodies where flow is frequently only intermittent or isolated pools are common, or where vegetation or debris prevents a continuous 50 m survey, reach surveys can still be conducted. Simply follow the same methods but report the number of isolated wetted habitats surveyed, their approximate length and width, and sum up the total surveyable length. Provide some explanation of the situation in the notes section of the data form. The start and end points for the survey should still cover 50 m in length, even if the actual surveyed area is less than 50 m.

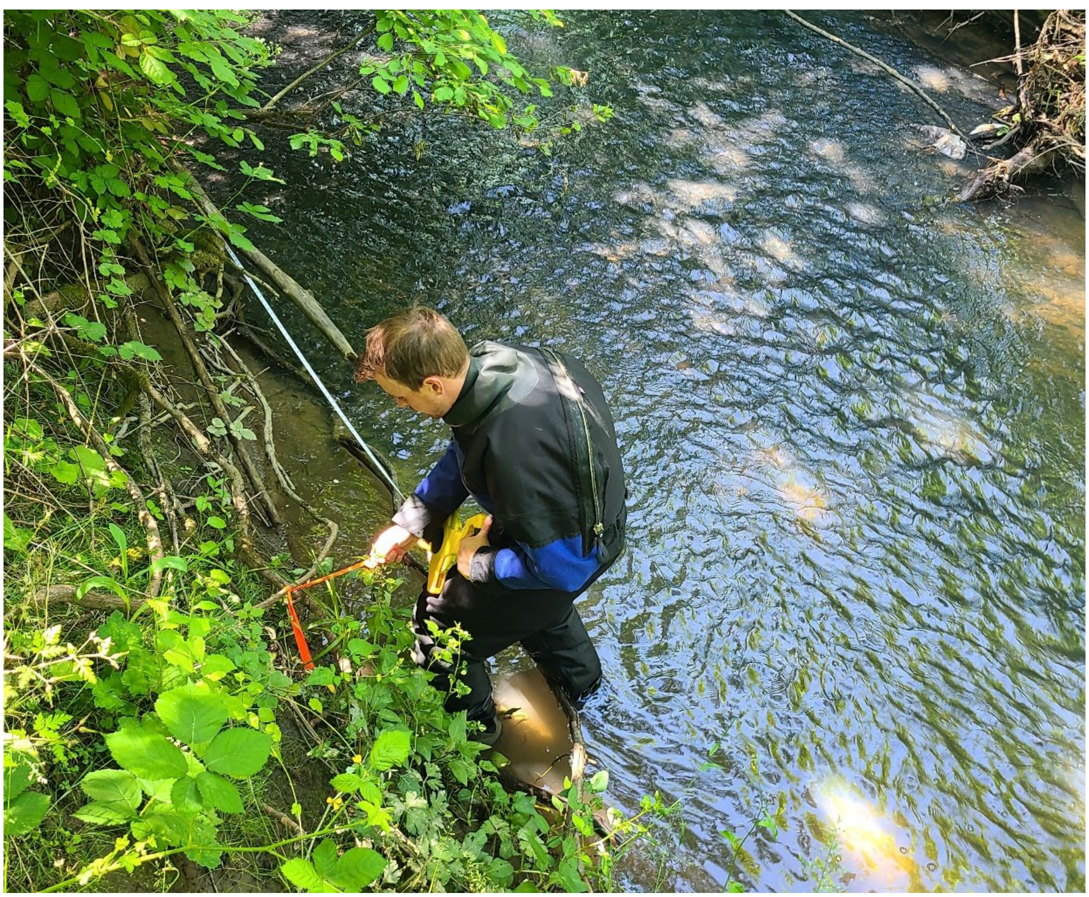

Figure 5 Surveyor flagging the bank at both ends of the reach to delineate the survey area. Credit: Xerces Society.

5. If sites are selected for monitoring, it may be helpful to take two **monument** photos, with a white board or paper with the site name clearly written for documentation and for scale (Figure 6). One photo should be at the coordinates recorded on the data sheet oriented upstream, and one should be oriented downstream. Photos should be oriented so that obvious features can be identified in the event of a revisit. Orient photos to show prominent features of the area (e.g., large tree, boulder, or human structure). Do not zoom in for the photo and make sure both banks are visible in the photo. Try to include **scour line**, **bankfull**, and **floodplain** surfaces in at least one photo. During or after the survey, additional photos should be taken of any key features of the reach.
6. Observers can survey in an upstream or downstream direction (making note of which on the data form), keeping in mind that visibility may be impacted if surveyors walk through the waterbody or otherwise disturb bottom sediments during the survey, such as by dislodging silt accumulating on aquatic plants. If surveying in a downstream direction reduces visibility, surveyors should only perform the survey working upstream.
7. Record the required information on the *Survey Event Details* and *Reach Survey* data forms, including the wetted width at the upstream, downstream, and midpoint of the 50 m reach, reporting to the nearest 0.10 meters (see Section 2.8.1) and the channel unit, as described and classified in Section 2.8.2.
    a. OPTIONAL: Measure lengths and widths of wet wood pieces (wet, large wood) in the reach (Section 2.8.4) and record on the *Wet Wood Covariate* data form (Appendix F).
    b. OPTIONAL: Complete the Human Influence checklist in the reach (Section 2.8.5) on the *Human Influence Covariate* data form (Appendix F).
    c. OPTIONAL: Record the presence of mussel host fish in the reach (Section 2.8.6) on the *Host Fish Presence Covariate* data form (Appendix F).
    d. OPTIONAL: Collect water quality data or samples at or near a specific aggregation of mussels in the reach (Section 2.8.7) and record on the *Water Quality Covariate* data form (Appendix F).
8. If surveyors have a permit to do so (where required), visual searches may be supplemented with minimal **tactile** searches, making note of this under "Survey Methods, Other:_____" on the *Survey Event Details* data form. If tactile searching reveals a large number of mussels not otherwise detected, take detailed notes to ensure future estimates do not result in a substantial undercount at the site. Take voucher photos of freshwater mussels for species documentation (see the Quick ID Guide for examples of good photographs).
9. During the survey, conduct a complete count (not an estimate) of the number of live freshwater mussels per species or genus present. Also, provide a count/estimate of the number of **shells** present. A dive slate or clicker counters can be valuable for maintaining a tally during the survey (Figure 4). If mussels are handled for any reason (following permitting guidelines), keep track of the number of live mussels of each species or genus handled and report on the *Reach Survey* data form.
10. Upon completing the survey, make note of the end time. Document the general location of mussels in-stream, whether **left bank, right bank, or in the middle** (when oriented facing downstream). Use the notes section to record any other relevant information about the mussels (e.g., if mussels occur mostly along the stream margin, in the bottom of deep pools, associated

with large structures that modify flow like large wood or large boulders, etc.). If surveyors observe any obvious changes in habitat or conditions within the 50 m reach that could contribute to variation in mussel distribution within the site, record in the notes section. If live mussels appear to continue beyond the reach, but no additional reaches are added, indicate that on the *Reach Survey* data form. Record any general observations about the survey or site in the notes section.

11. When live mussels are not encountered, the reach survey shall conclude upon completion of the maximum search effort of distance (50 m), and surveyors should report their absence on the *Survey Event Details* data form. Surveys lacking adequate documentation will be treated as incidental. If surveyors observe potential habitat or are interested in examining additional areas outside of the reach upon completion of the reach survey, any incidental observations can be reported using the *Incidental Observation* data form.
12. Prior to departing a site, remove all flagging. Upon completion of site data collection, refer to Data Documentation and Reporting ([Section 3]) for next steps.

Figure 6 Example monument photo ensuring the depiction of features that could be used to find the site in the future, such as the location of trees and cliffs in the background. The whiteboard depicts the reach's unique ID. In this case, a flag was placed and two photos were taken at the midpoint in addition to the upstream and downstream boundaries. Credit: Xerces Society.

2.6 Core Method: Transect

2.6.1 Overview

The purpose of a transect survey is to gather information on the freshwater mussel assemblage, including species composition and density, and other characteristics at and within a discrete location (two or more transects consisting of a continuous series of at least 10 adjacent quadrats measuring 0.25 square meters in size). Transects should not be established independent of reaches. When monitoring sites using the transect method, repeat both the reach survey and transect counts to ensure consistency in methods across monitoring periods. Additionally, the plot contingent method (Section 2.7) can be coordinated with transect installations. The transect method is most valuable and appropriate:

- when mussels are exceptionally abundant or dense within a reach, where accurate enumeration is not feasible during a reach survey alone,
- when there is interest in information specific to a **mussel bed**, in addition to mussel distribution within a 50 m reach, and/or
- when subsampling within a reach is valuable for monitoring (such as at mussel restoration sites or other long-term monitoring locations) or for improving statistical analysis of count data.

The use of the transect method is not necessarily appropriate for some projects. For example, if mussels occur in deep or swift water portions of waterbodies or where transect installation is not feasible, this method should not be employed (but see **Special note** in Section 2.6.3). Additionally, when mussel abundance or density is low, reach surveys may be adequate for monitoring purposes without the increased effort required by the transect method. In streams too narrow for transects, consider just establishing plots (Section 2.7).

This method includes descriptions for both non-randomly and randomly installed transects. Non-random transects focus on counts within a mussel bed at a reach site and can be used to establish a long-term monitoring location to track a mussel bed's characteristics through time. Because it is non-randomly located, results may not be comparable across sites, but when collected across monitoring events, the data can be used to assess site-specific trends. This approach is advantageous when density is low and/or aggregations are sparse.

In comparison, the random allocation of transects enables the aggregation of transect-level observations into unbiased large-scale estimates of relative abundance, especially when large errors in mussel count are expected during the reach surveys. It may be particularly applicable at sites where mussel density is relatively higher or more continuously distributed, or if observers are unsure where to place a non-random transect (e.g., unable to determine optimal placement for monitoring within a bed). This approach also allows for error estimation of mussel abundance at a reach site and is comparable across reach sites. Determining whether to use non-random, random, or a mix of transect installations should be decided during the project development and sampling design phases of a project. However, as mentioned previously, transects can be added to an established reach site at any point.

Of special note is monitoring of an acute mass mortality event (MME), a situation in which a large number of dead or dying mussels is encountered (DuBose et al. 2019). Collection of mortality data is especially important for such events (e.g., USDOI 2023). When encountered, it is recommended that one or more transects be established within the zone of impact and that plots be excavated to enumerate the number of live versus dead mussels (**fresh dead** or **shell**) in situ in each quadrat within a transect.

2.6.2 Preparing to Survey

It is strongly recommended that you read through the entirety of the methods, including pre-survey steps, information on conducting the survey, and information on collecting covariate data, before beginning a survey to ensure you have the appropriate equipment and understand how the survey will be conducted. Ensure that you are conducting the survey during the appropriate index period and meeting minimum quality standards (Section 1.5.4). Assemble the necessary equipment (Appendix B) and review the *Project Metadata*, *Survey Event Details*, and *Transect Survey* data forms (Appendix F).

For establishment of new sites, determine whether non-random placement of transects, random placement, or a combination of the two approaches will be used at a site and plan accordingly. See *Overview* for discussion of each approach. For random transects, have a list of random numbers pre-generated or be prepared to access a random number generator.

Transects are monitored through use of a temporarily-placed 0.25 m^2 quadrat, often comprised of PVC piping, which may be weighed down with the use of dive weights, rocks, or other equipment (Figure 2). Use of a surveyor's tape or other tool may help to keep the transect oriented perpendicular to the **thalweg** while spanning the wetted width, bank to bank.

2.6.3 Conducting the Survey

1. After completing a reach survey at the site, locate the start points for the transects and record the information on either the *Non-random Transect Survey* or *Random Transect Survey* data form. When first establishing a transect, the start point will be established for either a random or non-random transect as described below. Regardless of method, ensure that during transect installation all transects remain within the 50 m reach and do not extend beyond. If plots will be sampled in association with the transect (see plot contingent method, Section 2.7, for details), determine the way in which plots will be established before beginning the transect survey.
    a. OPTIONAL: Collect water quality data or samples at or near a specific aggregation of mussels (Section 2.8.7) and record on the *Water Quality Covariate* data form (Appendix F).
2. **<u>When establishing transects that will be non-randomly placed</u>**, determine the number of transects and the number of quadrats per transect that will be installed according to the following guidelines:
    a. A transect is established at one or more mussel beds of interest to the surveyor within a reach (Figure 7). All non-random transects consist of at least 10 quadrats (but see **Special note** below). A transect is centered on the densest portion of the bed and is oriented any direction from that center point along the longest axis of the bed. Note that the "starting point" of the transect will be located five flips of the quadrat from this location and that the location on which the transect is centered should not be considered the starting point. Record the transect start coordinates or the distance of the transect start from the reach upstream boundary, midpoint, or downstream boundary, and record the transect direction and angle (i.e., "downstream and parallel to

the bank" or "at a 45^{o} angle to the right bank, oriented facing downstream"), providing sufficient details for future surveyors to install and orient the transect in the same manner. Take at least two **monument photos** at the transect start and indicate the orientation the photographer is facing. Include a white board or paper with the site and transect IDs clearly written for documentation and for scale.

i. **Special note:** If the longest axis of the mussel bed is less than 10 quadrats in length (e.g., 10 quadrats would extend beyond the bank in a small stream), sample only as many quadrats as fall within the waterbody and make a note of the reason it is less than 10.

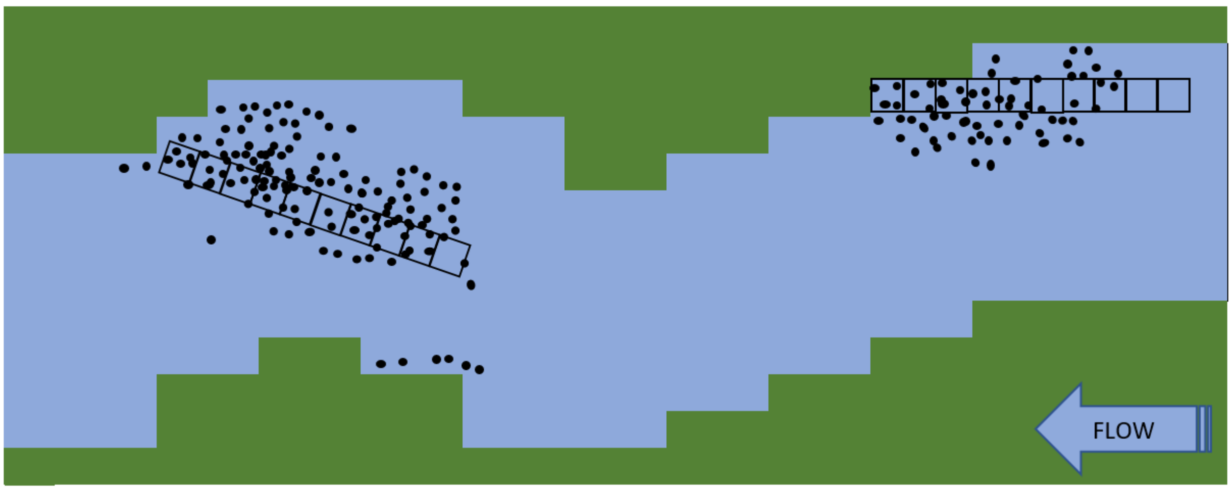

Figure 7 Example of a 50 m reach with two non-random transects placed along what the surveyors identify as the densest portions of the mussel beds (mussels depicted as black dots). Black squares depict the quadrats placed within each bed, oriented along the longest axis of the bed.

b. Surveyors can aim to place transects on discrete mussel beds, such as aggregations separated by uninhabited areas like the **thalweg** (e.g., along opposite banks). If mussels are continuously distributed and there are no discrete aggregations, indicate on the data form that transects are replicates for the same aggregation. Transects can also be placed side by side if it is possible to ensure plots are not sampled twice, for example using a double quadrat.
c. A transect can also be extended another 10 quadrats in the same orientation to increase the amount of data collected from a single bed, such as capturing the "edge" of the bed, where density begins to decrease or mussels cease to occur. This is particularly recommended for longer beds. For the transect extension, indicate on the data form that the two (or more) transects are replicates for the same aggregation. In this case, quadrats 10 and 11 should be centered on the densest portion of the bed. Consider flagging a rock or otherwise marking the anticipated locations of quadrats 10 and 11 to ensure the transect lands where intended.
d. Conduct sampling steps beginning at #4 below.

3. **<u>When establishing transects that will be randomly placed</u>**, plan to install at least 2 but not more than 12 transects (note: *it is recommended that at least 6 transects be established*) perpendicular to the **thalweg**. Surveyors may determine the number of transects they will place based on the percentage of the reach they wish to cover or the amount of time they can allocate to transect surveys.
    a. To establish the transect locations, first select the number of transects that will be installed, n. Then, select a random number between 0 and $\frac{50}{n} - 0.25$, rounding to the nearest tenth. This random number is referred to as the random starting distance and determines the distance from the upstream or downstream edge of the reach to the closest edge of the first transect. Record the random starting distance on the data form.
    b. Next, calculate $\frac{50}{n} - 0.5$, also rounding to the nearest tenth. This number represents the distance between subsequent transects. Refer to the example (Figure 8 below and text box on the next page) using n = 6. Record this number under "Distance Between Transect Flags" on the *Random Transect* data form.
    c. The transects will be oriented perpendicularly to the flow, from one bank to the opposite, placing the quadrat edge starting at the selected random start number in meters from either the upstream or downstream end (record direction on data form) of the reach at the wetted edge of the waterbody. After the first transect is installed, transects will be installed until the opposite boundary of the reach is encountered. In rare cases, due to measurement error, the final transect might fall outside the 50 m reach. In these cases, do not install the last transect. For each transect, record the starting bank from which sampling begins.
    d. Conduct sampling steps beginning at #4 below.

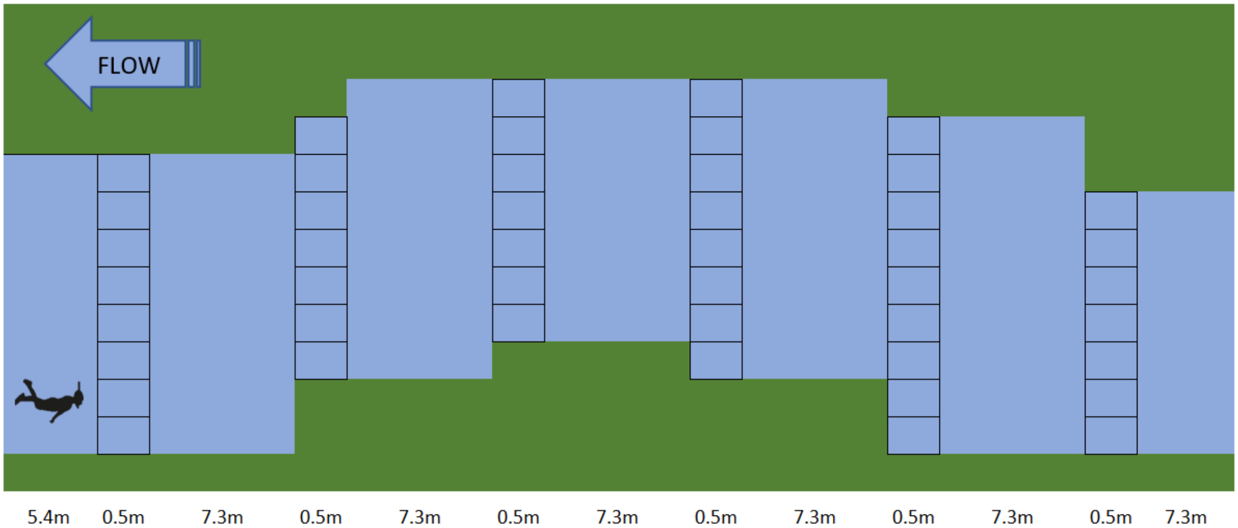

Figure 8 Example of a 50 m reach with six random transects placed perpendicular to the thalweg (not to scale). Black-outlined squares depict the quadrats placed within each transect from bank to bank, starting at the wetted edge of the waterbody. The edges of the diagram represent the upstream and downstream edges of the reach, where flags would be placed prior to sampling. See box below depicting the math used to calculate spacing.

Random Transect Example

In this example, the surveyor has elected to establish six random transects in the 50 m reach (n = 6). To minimize turbidity, the reach and transect surveys will be conducted from downstream to upstream. Therefore, in this example, the random starting distance will be measured and transect number 1 will be installed starting from the downstream boundary of the reach, beginning at the left bank each time. All transects will be flagged at the downstream end of the transect. Therefore, each quadrat will be placed so that the downstream edge of the quadrat aligns with the flag.

1. To calculate the random starting distance, first calculate the maximum possible random number, rounding to the nearest tenth: $\frac{50}{6} - 0.25 = 8.1$. Then, use a method for generating random numbers between 0 and the number calculated (8.1). In this example, the random number selected using a random number generator was 5.4. The surveyor should then measure 5.4 meters from the downstream boundary flag to the edge of the first transect.
2. To calculate the distance between transects: $\frac{50}{6} - 0.5 = 7.8$. This number will remain constant between each transect. The surveyor should measure 7.8 meters from the first transect flag to the next. To reduce field measurement error, measure out each 7.8 m distance between each flag. Figure 8 depicts the distance between each flag as $0.5 + 7.3 = 7.8$. This is just to indicate that the placement of the 0.5 m quadrat falls within the 7.8 m distance between flags.

4. **For either transect method,** to begin the survey, place the first quadrat in the starting position for transect 1. **In general, surveyors should aim to avoid disturbing transects prior to sampling, and avoid impacts to freshwater mussels and their habitat. Avoid walking within or around the transect or moving materials (such as rocks to weigh down the quadrat) as much as possible when implementing this method**. Record data (count of visible mussels per species/genus) within each quadrat, flipping the quadrat end over end until data for all quadrats in the transect have been recorded. Count all mussels touching the inner edge of the quadrat. It may help to fan the substrate lightly to improve visibility during sampling. If a mussel spans two adjacent quadrats, record that mussel only for the first quadrat in which it is counted (do not double count). Repeat for each transect.
    a. **Special note:** If portions of a transect, or an entire transect cannot be accessed due to extreme turbidity or depth, skip those quadrat flips and indicate the number of inaccessible quadrats or transects on the data form. This number can be approximated if necessary.
    b. **Special note:** Mussels may be especially dense in underbank habitat, occurring far back in areas difficult to place a quadrat. In these instances, quadrats may be placed at an angle that allows for counting the number of mussels within the quadrat. These are important microhabitats that should not be skipped during transect surveys. If quadrats land on partially dry substrate, such as a boulder or at the transition from wet to dry, such as at a gravel bar, sample but make note that the quadrat was partially dry.
    c. OPTIONAL: Estimate the substrate composition (percent) in a subset of quadrats in the transect (Section 2.8.3) and record on the *Substrate Covariate* data form (Appendix F).

   d. OPTIONAL: Record the number of quadrats per transect covered by wet wood (wet, large wood) (Section 2.8.4) on the *Wet Wood Covariate* data form (Appendix F).
5. Provide a sketch on the *Survey Event Details* data form depicting the number and location of all transects relative to the reach boundaries, each other, banks, and other relevant permanent stationary features (such as boulders or trees). Also, generally depict and describe the mussel beds in which transects are placed.
6. Upon completion of site data collection, refer to Data Documentation and Reporting (Section 3) for next steps.

Credit: Xerces Society/Emilie Blevins

2.7 Contingent Method: Plot

2.7.1 Overview

The purpose of the plot method is to collect data on the composition of the freshwater mussel assemblage at a very fine scale within a reach and/or a transect, including:

- the sizes of individuals (a proxy for age, and in some cases, evidence of recent recruitment that may not be observable without excavation),
- more accurate estimates of relative abundance (incorporating both visual and burrowed mussels),
- condition (e.g., Crosby and Gale 1990),
- the proportion of live versus dead individuals at a site where an acute mass mortality event (MME) has occurred,
- species composition (in cases where some species may only be detected by excavation), and/or
- individual fates, when paired with a unique tag (e.g., shellfish or PIT tag).

A plot survey, like a transect survey, makes use of a temporarily placed 0.25 m^2 quadrat. However, this contingent method differs from the transect method in that it is both a **visual and a tactile survey**, which requires excavation within the quadrat. It is important to note that excavation of sensitive mussel species in areas where populations are already in decline is a conservation and management concern, and the negative impacts of disturbing mussels should be thoroughly considered before conducting plot surveys. As such, before plot surveys are undertaken, review the following questions and directives. Additionally, because this method departs from other methods in this protocol framework, requiring handling of mussels for purposes beyond identification, ensure that surveyors are covered under any necessary permits before utilizing this method.

1. Do regulatory requirements prohibit excavation?
    a. YES: Do not excavate.
    b. NO: Proceed to 2.
2. Has a disturbance already occurred, such as an acute mass mortality event (MME), or is expected to occur, such as a planned habitat restoration project?
    a. YES: Proceed to 4.
    b. NO: Proceed 3.
3. Do the objectives of the study require: accurate estimates of relative abundance, demographic metrics, evidence of recent recruitment, or assessment of condition?
    a. YES: Proceed to plot methods.
    b. NO: Do not proceed.
4. Will there be direct impact of the disturbance to the mussels (e.g., mortality, crushing by large equipment, burial from sedimentation, dewatering, etc.) or irreparable damage to their habitat (e.g., loss of fine burrowing sediment, removal of substrates from the river channel, fill of existing habitat, etc.)?
    a. YES: Proceed to plot methods.
    b. NO: Do not proceed.

As with transects, this method should not be employed without first conducting a reach survey. Further, plots are best paired with a transect survey, although this is not strictly necessary. Details are provided in the next section for either scenario.

2.7.2 Preparing to Survey

It is strongly recommended that you read through the entirety of the methods, including pre-survey steps, information on conducting the survey, and information on collecting covariate data, before beginning a survey to ensure you have the appropriate equipment and understand how the survey will be conducted. Ensure that you are conducting the survey during the appropriate index period and meeting minimum quality standards (Section 1.5.4). Assemble the necessary equipment (Appendix B) and review the *Project Metadata*, *Survey Event Details*, and *Plot Survey* data forms (Appendix F).

Plot surveys are conducted through use of a temporarily placed 0.25 m^2 quadrat, often comprised of PVC piping, which may be weighed down with the use of dive weights, rocks, or other equipment. Excavated mussels from the sediment within the bounds of the quadrat are often sieved by hand or paired with a net or mesh bag to capture lightweight juveniles as they are dislodged into flowing water. Collected mussels are generally placed within a mesh bag and held in water until excavation is complete and data recording can begin. **In general, surveyors should aim to avoid disturbing the plot prior to sampling.**

Before beginning the survey, any metrics other than counts by species/genus at the surface and subsurface (see *Plot Survey* data form) should be selected (refer to discussion in *Overview*). Space is provided on the data form to report pre-selected metrics, including any unique species tag number, length, condition, etc., under "Other Mussel Data."

The number of plots to be excavated should be determined during development of the project and sampling design. Project goals should aim to excavate the minimum number of plots, handling the minimum number of mussels to achieve necessary analytical requirements. A power analysis or other statistical methods can be used to determine these requirements (see Smith et al. 2000). However, for sites where an acute mass mortality event (MME) has occurred, in which a proportion of the freshwater mussels present appear to be suddenly dead or dying (e.g., DuBose et al. 2019), it is recommended that an entire transect's worth of plots be excavated to accurately determine the number of live versus dead mussels (**fresh dead** or **shell**) in situ.

2.7.3 Conducting the Survey

1. After completing the reach survey method during a site visit, but before starting transect surveys, determine if the plot method will be paired with transects or not. Pairing with a transect can assist with establishing appropriate plot placement. However, if the transect method is not employed at the time of the plot method, plots can be placed within random locations at the site or non-random locations within **mussel beds** selected by the surveyor.
    a. **If not paired with transects,** determine where plots will be located. The quadrat used to sample a plot is best placed in areas with lower flow and shallower depth, where surveyors can easily position themselves with little damage to the mussels present, both prior to quadrat placement and during the survey. Document the location (distance)

relative to the reach midpoint, downstream, or upstream boundary, or to a monument. Surveyors can also report the geographic coordinates of the plot. Record the position instream (left bank, right bank, or middle).

   b. **If paired with transects,** determine where plots will be placed within transects according to the study design. Document the ID of the associated transect and the quadrat flip number.
      i. **Special note:** In the case of an acute mass mortality event, transects should be established and every quadrat should be excavated, with surveyors recording the number of live versus dead (**fresh dead** and shells) in situ.

2. Once the quadrat is placed, use dive weights, rocks, or hands to hold it in place. Surveyors should first record the number of live mussels and shells of each species/genus visually observed within the plot, including all mussels touching the inner edge of the PVC pipe. If adjacent plots are sampled and a mussel spans both plots, include the mussel in only the first plot's count (do not double count). It may help to fan the substrate lightly to improve visibility during plot sampling. Remove all "surface" mussels from within the plot and place into a mesh bag that will remain underwater during sampling.
   a. OPTIONAL: Estimate the substrate composition (percent) in the plot (Section 2.8.3) and record on the *Substrate Covariate* data form (Appendix F).
3. Next, carefully excavate live mussels and shells from the substrate within the plot to collect buried animals, digging until hardpan is reached or no additional mussels are retrieved. Place these "subsurface" mussels into a mesh bag that will remain underwater during sampling. Excavated mussels and sediment are often sieved by hand or paired with a dipnet to capture lightweight juveniles as they are dislodged into flowing water. After the sediment clears, check that no mussels remain on the surface after digging around in the substrate. All substrate should also be returned to the location of the quadrat prior to replacing the mussels.
4. After counting mussels, take voucher photos of mussels (see the Quick ID Guide for examples of good photographs). Record "surface" and "subsurface" mussels separately, counting and identifying to species or genus. At this point, additional metrics can be recorded on the data form. If of interest, each mussel can be measured for length along the longest axis of the shell, from posterior (aperture) to anterior (foot) (Figure 9), or be tagged for research or monitoring purposes. After all data is recorded, mussels should be placed carefully back on the substrate within the quadrat, with a valve flat against the bottom, which will enable the mussel to burrow back into the substrate.
5. Any shells collected during plot surveys could be retained for vouchers or for use in future studies (e.g., ageing studies; Haag and Commons-Carson 2008).
6. Indicate the locations of plots on the *Survey Event Details* data form in the "Site/Survey Sketch" box, depicting plot placement relative to any transects, or to the reach upstream boundary, downstream boundary, or midpoint, banks, or other relevant permanent stationary features (such as boulders or trees). Also, generally depict and describe the mussel beds in which plots are located. Take monument photos of the plot placement instream if not associated with a transect and record on the *Survey Event Details* data form.
7. Upon completion of site data collection, refer to Data Documentation and Reporting (Section 3) for next steps.

Figure 9 Freshwater mussels should be measured along the long axis using a tool like calipers, rounding down to the nearest tick mark. Credit: USFWS/Courtney Newlon.

2.8 Covariates

This document provides information on required and recommended covariates to include in data collection at the time of surveys. Covariate information collected at surveyed sites can prove valuable for understanding freshwater mussel habitat associations or documenting issues of concern. The intent is to include the fewest number of relevant or potentially relevant covariates that can be measured accurately, will not be especially burdensome to collect, and do not require special expertise or tools on the part of the surveyors. Covariates that could be measured via remote sensing or using other GIS applications have been excluded. For information on Supplemental Information or Other Sampling Methods that may be of interest, refer to Section 2.9 and Section 2.10. The following covariates are identified as either REQUIRED for a particular survey method or OPTIONAL. For OPTIONAL covariates, data forms are provided in Appendix F.

2.8.1 Wetted Width

(REQUIRED for **EXPLORATORY** AND **REACH**)

Wetted width is an important covariate for interpretation of mussel count data in reaches. It is influenced by streamflow, which varies throughout the year and interannually. Because freshwater mussels do not persist in areas that dry, measurements of wetted width at time periods when flows are above baseflow will overestimate potential mussel habitat. However, wetted width provides an easily measured metric for use in interpreting general stream size, and this metric could be combined with stream order in a GIS for further interpretation. The purpose for collecting this information is to allow for normalization of mussel density estimates.

When collecting this data, it should be reported for both an **exploratory** survey and a **reach** survey. To measure and report for the exploratory survey, document at the start and anywhere considered relevant (i.e., where any major change is observed). If these locations are not representative of the overall 1 km portion (or actual distance, if less) of the waterbody that is surveyed, document that in the notes. To measure and report for the reach survey, document at the upstream, downstream, and midpoint of the 50 m reach. Where side channels or braided channels occur, document in the notes the wetted width of each channel encountered at the location of measurement. This covariate should be reported directly on the *Exploratory Survey* or *Reach Survey* data forms.

2.8.2 Channel Unit

(REQUIRED for **REACH**)

The concept of a channel unit incorporates complex site variables and interactions among flow and substrate, with resulting differences in characteristics such as gradient, bed roughness, and turbulence (CHaMP 2016). As a result, it is a valuable descriptor for reaches and may provide insight into mussel habitat preferences across geographic scales (e.g., Hegemen et al. 2014). Therefore, this information must be reported for a **reach** survey. Report the channel units present in the **reach overall and make note of the specific channel units in which mussels occur**. The following descriptions can be used to determine the channel unit classification (repeated or adapted from CHaMP 2016; Section 7 therein):

- **Fast Water Turbulent** channel units are topographical high points in the bed profile that feature gradients >1%, broken water surface, coarse substrate, and tend to have *consistently* turbulent flow. The bedform of these units generally lacks longitudinal and/or lateral concavity.
- **Fast Water Non-Turbulent** channel units feature low gradients, dominantly sand to cobble substrate, and a *consistently* smooth unbroken water surface. Often, fast water non-turbulent units have a gentle slope, similar to pools, but are distinguished from pools by their general lack of lateral and longitudinal concavity. These channel units are generally deeper than riffles. [Note that exceptionally low water may result in a ripply water surface within this channel unit.]
- **Slow Water** channel units are topographical low points in the bed profile that feature gradients <1%, a *consistently* smooth unbroken water surface flow, and possess lateral and longitudinal concavity. [Note that exceptionally low water may result in a ripply water surface within this channel unit.]
- **Off Channel** units include backwaters and alcove type units that are connected to the main channel or large side channel but have little (<1%) to no flow through them. The **thalweg** never passes through Off Channel units.
- **Pool** channel units include the following: Scour Pool, Plunge Pool, Dam Pool, Beaver Pool.
- **Small Side Channel** units are small side channels that contain <16% of the total stream flow.

Identifying and Reporting Channel Units

1. If you have clearly identified any channel units in the reach based on the above definitions, indicate these on the data form. Select as many units as were identified.
2. For channel units observed in the reach that you struggle to identify based on definition alone, review Figure 10, which is reproduced and adapted from CHaMP (2016; Section 7 therein) to assist in classifying channel units for freshwater mussel surveys. Upon identifying the channel unit in question, indicate this on the data form. If surveyors do not agree or are unsure of whether a specific channel unit is present after reviewing the figure, make a note and describe the observation.
3. Next, report which channel units (from those selected as present in the reach) contained live mussels. Again, select as many units as apply.
4. This covariate should be reported directly on the *Reach Survey* data form.

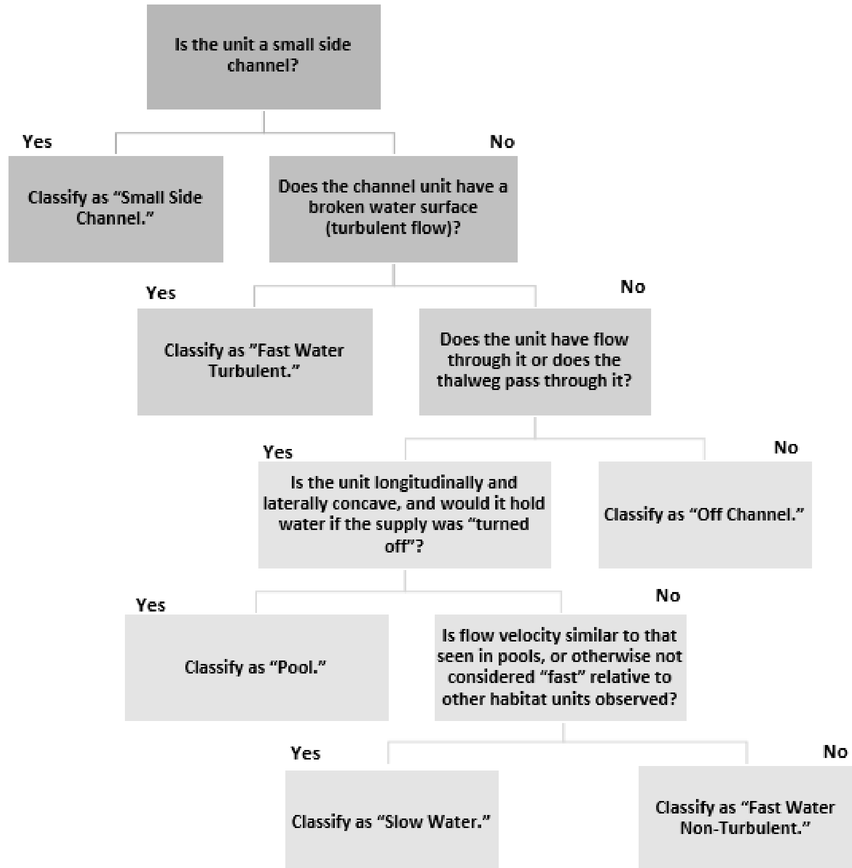

Figure 10 Classification Tree adapted from CHaMP (2016; Section 7 therein).

2.8.3 Substrate

(OPTIONAL for **TRANSECT** or **PLOT**)

Substrate is an important component of freshwater mussel habitat and may be worth documenting within transects or plots to better understand microhabitat use by mussels or be used to inform habitat restoration to support mussels at restoration sites. However, because it may take too much time to collect at sites, it is considered optional in this protocol framework. If this information is collected, it is recommended that it be reported at the **transect** level, such as reporting the percent composition in every third quadrat of a transect as one example. Be sure to document the chosen sampling approach. It can also be reported at the **plot** level, reporting the percent composition in each quadrat excavated. Refer to Figure 11, which is reproduced and adapted from CHaMP (2016; Section 8.2 therein) to assist in measuring and reporting substrate for freshwater mussel surveys. To estimate the percent composition within a quadrat/plot:

- Visually observe the substrate composition and record the percent of each type in Figure 11 below on the *Substrate Covariate* data form provided in Appendix F. Indicate if it is measured as part of a survey and list associated survey IDs. Record coordinates for the location sampled or the transect and quadrat number, as applicable.

- Round each estimate to the nearest 5%. The total for all types should equal 100% within the quadrat.
- If a thin layer of fine sediment overlays larger substrates, measure the fine sediment. However, if only small particles rest atop larger substrates, measure the larger substrate.
- Clay hardpan can be classified as bedrock.

Substrate Type	Size class (mm)	Description
Bedrock	N/A	Exposed bedrock surface
Boulders	> 256	Basketball size and greater
Cobbles	64 to 256	Tennis ball to basketball size
Coarse gravel	16 to 64	Marble to tennis ball size
Fine gravel	2 to 16	Small pebble to marble size
Sand	0.06 to 2	Smaller than ladybug size, but visible as particles and gritty between fingers
Fines	< 0.06	Silt and clay that is not gritty between fingers

Figure 11 Ocular substrate size classes for a variety of substrate types, including a description of each.

2.8.4 Wet Wood

(OPTIONAL for **REACH** or **TRANSECT**)

Where large wood commonly occurs, influences fish habitat use, covers a significant portion of the streambed, or plays a visible role in localized hydraulics, it may be valuable to document wet wood presence and size (length and width). Additionally, the presence of a significant volume of large wood can obscure visual surveys, which may result in an undercount of the mussels within a reach. Therefore, it is optional to document wet wood during surveys.

When collecting this data, it can be reported for **reach** and **transect** surveys. Large wood (wood volume) is classified as having a midpoint diameter ≥10 cm and is ≥1 m in length. It is considered wet if a portion of the main stem or root (diameter ≥10 cm) touches the water (CHaMP 2016; Section 8.4).

To record wet wood within a **reach**, take a measurement of the length and width in meters of each piece of wet, large wood. To record wet wood within a **transect**, document the quadrats covered by wet, large wood. Record data on the *Wet Wood Covariate* data form provided in Appendix F.

2.8.5 Human Influence

(OPTIONAL for **EXPLORATORY** or **REACH**)

Human influence in freshwater habitats can have a wide range of effects on freshwater mussels and may be useful for monitoring or management purposes. When collecting this data, it can be reported for an **exploratory** survey or a **reach**. Human influences are considered present if they can be seen while standing instream during an exploratory survey or within a reach. A checklist (adapted from BLM 2021) is provided on the *Human Influence Covariate* data form in Appendix F. For each type of influence that is detected at a marked exploratory survey point or within a reach, the surveyor should determine its proximity to the stream:

- **Streambed:** Present within the defined **reach** streambed (i.e., within **scour line** on left bank to scour line on right bank) or at sites within **exploratory** surveys where mussels are georeferenced. For the "streambed" class, all land and water from the centerline of the stream channel to the edge of the scour line is considered.
- **Contained:** Within sight of the stream but not located in the streambed (i.e., not within scour line on left bank to scour line on right bank).
- **Absent/Unknown:** Not present within or adjacent to the stream (or unknown).

If present in the streambed or contained, include specific notes and take photos.

2.8.6 Host Fish Presence

(OPTIONAL for **EXPLORATORY** or **REACH**)

The presence of particular species of fish has important implications for the presence and viability of freshwater mussel populations. This metric should only be measured when conducting surveys via snorkeling. It is recommended that surveyors spend five minutes snorkeling to observe and record the fish community before a **reach**-level mussel survey is complete. Observations made during other surveys covered in this document are also valuable to record.

During the survey, record observations of documented host fish on the *Host Fish Presence Covariate* data form provided in Appendix F. If these or other fish species are present, both native and nonnative, and are able to be identified by the surveyor, include those species identifications in the comments.

2.8.7 Water Quality

(OPTIONAL for **EXPLORATORY** or **REACH** or **TRANSECT**)

Freshwater mussels can be sensitive or respond to a range of chemical or physical water conditions. Collecting water quality measurements or samples at survey sites can provide insights into mussel population health and stream conditions. This protocol is divided into **intensive** and **non-intensive** methods for collecting water quality parameters, which were selected based on a literature review, an assessment of their feasibility to collect in the field, and their relatively low cost to collect (Table 3).

This protocol is to be used on the assumption that that the requisite skills for protocol implementation will be obtained through additional training. In order to maintain sample quality and accuracy, use of a chain of custody form is recommended when sending samples to a lab. The following water quality protocol should always be implemented prior to the survey, and all single sample events should only be collected at base flow conditions. When collecting grab samples (such as Chlorophyll a, 2-4D, total organic carbon, and phosphorus), it is sufficient to collect them annually, but if possible, collecting at least once per month during the growing season/protocol index period is best (June 1 – September 30). Record where sampled by providing sample location coordinates or listing any "Associated Survey IDs" on the *Water Quality Covariate* data form provided in Appendix F.

Non-intensive methods/parameters include the following: temperature, specific conductance, pH, dissolved oxygen, and ammonia. Collection of non-intensive parameters would be appropriate for reach surveys visited once or only infrequently. For example, when implementing the **reach** and **exploratory** protocols, collect only the non-intensive parameters, but be sure to follow guidelines regarding when and where these parameters should be measured. For the **reach** protocol, collect water quality parameters at the midpoint, prior to sampling of the survey. For the **exploratory** protocol, collect all parameters prior to sampling at the starting point, where wetted width will be taken, or at specific survey locations.

Intensive methods/parameters include the following: continuous temperature, total organic carbon, alkalinity, phosphorus, 2-4D, and chlorophyll a. These parameters would be appropriate for more frequently visited sites, such as at annually monitored **reach** sites or those visited in 3-6 year intervals. Additionally, when implementing the protocol for **transect** surveys and/or regularly visited reach sites, collect the intensive parameters along with the non-intensive parameters. Similar to the non-intensive parameters, these parameters should be sampled prior to mussel surveys, but if possible, nearest to any known mussel locations. If intensive water quality parameters are collected at monitored sites, consider recording depth at the sample collection location during each visit or with a continuous monitoring instrument. This could enable calculation of flow for cross referencing to temperature and dissolved oxygen levels.

Table 3 Parameters to be collected based on non-intensive and intensive methods

Non-intensive Parameters (Exploratory, Reach, or Transect)	Intensive Parameters (Transect)
Temperature	Continuous Temperature (ex. Hobo logger)†
Specific Conductance	Total Organic Carbon (TOC) *
pH†	Alkalinity
Dissolved Oxygen†	Phosphorus *
Ammonia NH_3	Pesticides/Herbicides - 2-4D *
	Chlorophyll a *

* Indicates grab sample; † indicates a sample that could be collected continuously

Selection of Parameters

The following section ranks the parameters in order of importance. Review Table 2, which outlines different project types and their respective goals, as well as the descriptions above to ensure that appropriate methods and parameters are implemented at a particular site. The definitions and level of importance for each parameter provided below are based on published literature related to water quality and freshwater mussels (e.g., Haag et al. 2019; Boon et al. 2019; Bakshi et al. 2023).

1. **Continuous Temperature (°C)** - This parameter is related to seasonal or annual mean temperature using passive equipment that collects instream water temperature in regular intervals. Temperature is directly related to mussel growth and time of reproduction.
2. **pH** - This parameter is a measure of how acidic/basic the water based on hydrogen ion concentration. pH is directly related to mussel shell health.

3. **Dissolved Oxygen or DO (mg/L**) – This parameter is a measure of how much oxygen is dissolved in the water, and how much is available to aquatic organisms. DO is directly related to a mussel's ability to absorb oxygen through the gills. Low DO can be lethal.
4. **Specific Conductance (µS/cm)** – This parameter is an indirect measure of the collective concentration of dissolved ions and heavy metals in the water. It can affect mussel shell health and energy storage.
5. **Ammonia NH_3 (mg/L)** – This parameter is a measure of an un-ionized form of nitrogen that is toxic to freshwater mussels. Juvenile mussels and glochidia are more sensitive to this form of nitrogen than adults; therefore, the effects of ammonia on mussel populations can be misleading if effects on only adult mussels are considered.
6. **Phosphorus (mg/L)** – This parameter is a measure of a limiting nutrient in aquatic ecosystems and controls the production of primary producers/food for freshwater mussels.
7. **Total Organic Carbon (mg/L)** - This parameter is a measure of the amount of total carbon present as organic molecules within the water. It can control the number of primary producers and, in high levels, decrease DO levels due to a large increase in consumption of oxygen by primary producers.
8. **Chlorophyll a (mg/L)** - This parameter is a measure of a specific form of chlorophyll used in oxygenic photosynthesis and can be used to measure phytoplankton in the water column. Phytoplankton play a large role in freshwater mussel diets. This measurement can help determine potential adequate levels of food availability.
9. **Dichlorophenoxyacetic acid 2-4D (mg/L)** – This parameter measures a broadly used herbicide that can control broadleaf plant growth. The ester forms of this compound can be toxic to mussels and other aquatic animals.
10. **Alkalinity ($CaCO_3$ mg/L)** – This parameter is used to measure the buffering capacity of a waterbody and its ability to neutralize acids and bases and thus maintain a fairly stable pH level. It is also related to the underlying geology in a riverine system. For example, if the landscape is in an area containing rocks such as limestone, runoff picks up chemicals such as calcium carbonate ($CaCO_3$).[3] This molecule can affect shell health and a river's ability to maintain balanced pH levels during high or low flow events.

Suggested Supplies

- Coolers and ice
- Sterilized bottles 125 ml-500 ml
- Permanent markers and pencils
- Rite in the Rain paper
- Electrical Tape
- Nitrile Gloves

[3] https://www.usgs.gov/special-topic/water-science-school/science/hardness-water

Collection Protocol for Parameters

Non-intensive: Temperature, pH, Specific Conductance, Dissolved Oxygen, and Ammonia

1. Standing on shore and near the flowing water, lower the logger's probe to a depth of 0.5 m below the water surface, taking care to avoid contacting the stream bottom. If water depth is <1 m, take measurements at mid-depth.
2. Wait for the readings on the screen to stabilize (this could take up to a few minutes).
3. Record the pH, temperature (°C), specific conductance (µS, not mS), dissolved oxygen (mg/L), and ammonia (mg/L). Ensure the handheld logger is set to take measurements in the appropriate units and that temperature-corrected conductivity (i.e., specific conductance) is being measured. Measurements of pH, temperature, specific conductance, and dissolved oxygen must be taken at the site.
4. Record the model number and serial number of the instrument used so that any issues with the device can be tracked.
5. Note any odd water smells, surface films, or water discoloration observed during sampling and photograph for documentation if possible.

Intensive: Continuous Temperature, Total Organic Carbon (TOC), Alkalinity, Phosphorus, 2-4D, and Chlorophyll a

Continuous Temperature

1. Temperature loggers should be launched during low flow conditions so that the logger will always be submerged and in a place of representative stream flow.
2. Calibrate loggers prior to launch, and make sure there enough battery life for the desired launch period.
3. It is preferred to attach the logger to rebar that is hammered to the river's substrate with a coated wire cable. If the substrate depth is low and bedrock is present, attach logger to a cement block with a coated wire cable.
4. If time and funds allow, place two loggers at each site for back-up, in case of loss, damage, or malfunction.
5. Check and download temperature data on a monthly basis to avoid data loss due to possible malfunction or loss of equipment.

Phosphorus and TOC

1. Take a single "grab sample" that will be analyzed for total organic carbon and phosphorus, and stabilize the sample with concentrated sulfuric acid. Sample kits may differ between labs. Be sure to take sample based on your laboratory's preferred procedure.
2. Take the sample from an area of flowing water. Use new nitrile gloves, and be careful not to contaminate the outside of the gloves with substances such as sunscreen. Dispose of gloves after use.
3. Fill out a bottle label based receiving lab procedure. For internal purposes it is recommended to use the following label techniques:
    - On waterproof paper, record Site ID, waterbody name, date, and your initials.

- Record the day, month, and year, making sure to use letters rather than numerals for the month and to use four digits for the year (e.g., "27Aug2015").
- Water quality labels must be on Rite in the Rain paper and filled out with a pencil. Labels that are not on Rite in the Rain paper will not withstand wet and frozen environments, while ink from pens will leach and become illegible.

4. Tape the label on the outside of the bottle with clear packing tape, making sure the tape is wrapped completely around the bottle.
5. Stabilize all samples appropriately. This will be dependent on the receiving lab's methods.

2-4D

1. Samples should be collected in appropriately sized and labeled glass VOC vials with minimal headspace.
2. After collection, samples should be tested as soon as possible, within 30 days maximum. If this is not possible, storage at 4°C (39°F) is recommended to minimize evaporative losses.
3. If the means are available for lab analysis within your own organization, consider using this protocol: https://www.modernwater.com/assets/Technical%20Support/Environmental/User%20Guides/Z00094.2%20User's%20Guide%20RA%202,4-D.pdf.

Chlorophyll a

1. Samples are prepared by filtering a known volume of water through microfiber filters with a vacuum filtration device, then wrapping the filters in foil and storing in a freezer for future chlorophyll a lab analysis using a fluorometer or spectrometer.
2. Frozen samples must be analyzed in a lab within 30 days. If the means are available for lab analysis with a fluorometer within your own organization, follow the EPA protocol: https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=309417.

Alkalinity

1. This parameter can vary depending field kit purchased.
2. Samples should be taken from a flowing area that is representative of the site.
3. Most field-testing kits require alkalinity to be measured immediately on site.

2.9 Supplemental Information

The following supplemental information may be used to assist in application of the methods described in this document.

2.9.1 Existing Distribution Data

2.9.1.1 Western Freshwater Mussel Database

The Xerces Society and the Confederated Tribes of the Umatilla Indian Reservation have compiled a database of western North American freshwater mussel occurrence records to gather information on species' distribution and support research and conservation efforts. More information on the database can be found at https://www.xerces.org/endangered-species/freshwater-mussels/database.[4] This database is a valuable tool for the development of visual survey projects, and data can be accessed by contacting mussels@xerces.org.

2.9.1.2 Western North American Freshwater Mussel Data Portal

As part of protocol methods development and in line with future planned projects to implement this document, a one-stop data portal and database will be available in the future to accept data collected through these methods. When available, details will be provided in Section 3.2 of this document.

2.10 Other Sampling Methods

2.10.1 eDNA Samples

This tool is valuable for identifying potential sampling locations for projects, similar to supplemental information provided by incidental observations or other occurrence data. It can also be utilized in areas where visual surveys are not feasible, such as waterbodies or subsets of a waterbody that are too deep, dark, swift, or turbid to visually observe mussels. It is also a cost-effective means for collecting occurrence data equivalent to incidental data described in this document. More information on eDNA data collection can be found through a literature search or through resources available online at the Aquatic eDNAtlas.[5]

When pairing eDNA sample collection with core and contingent methods in this document, consider the order in which sampling should occur (e.g., collection of eDNA samples prior to a reach survey) or the direction in which sampling will occur (e.g., conducting an exploratory survey from downstream to upstream when also collecting eDNA samples).

If eDNA samples have been collected but the locations of live mussels are not yet known, exploratory surveys are recommended to maximize the area covered before more intensively sampling for mussels.

2.10.2 Tactile Searches

As with eDNA sampling, tactile surveys can supplement investigations both at sites where visual surveys occur and where visual surveys are not feasible, filling an important gap not addressed by visual

[4] A list of the many contributors, including individuals, institutional collections, organizations, and literature is also available at https://www.xerces.org/endangered-species/freshwater-mussels/database/contributors.
[5] https://www.fs.fed.us/rm/boise/AWAE/projects/the-aquatic-eDNAtlas-project.html

methods alone. Methods for implementing tactile freshwater mussel surveys, like the plot method described in this document, should be used with care due to the potential impacts to mussels from handling. Tactile sampling has been described by the Texas Parks and Wildlife Department (TPWD) as

> "hand-grubbing into the top 1-4 inches of substrate to increase detection of more-deeply buried mussels… [especially in] areas of loose or fine substrate…All live mussels collected during the…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)."

Additionally, information gathered through tactile searches will need to be evaluated for its comparative utility under this protocol framework, since tactile searches are generally far more limited in how much area can be reliably surveyed compared to visual survey methods, or may result in differences in survey effort between surveys (if methods are not appropriately documented). Before planning to collect tactile data, reach out to the visual protocol framework development team to discuss what methods you intend to use, including the area over which you will use the method.

2.10.3 Lentic or Deep Water Habitat Surveys

This protocol has been developed specifically for linear, wadeable, lotic habitat. For a review of methods specific to surveys planned in lentic, deep water, or turbid habitats, see Dolson et al. (2023).

Credit: USFWS/Roger Tabor

3 Data Documentation and Reporting

3.1 Required Project Metadata

All projects using this protocol framework should provide the information in Table 4. Project metadata should be submitted with other information collected at each site or each survey, including information documenting the final status of sites pre-selected for sampling (Section 5.3.3), if applicable. This information can be documented by filling out the *Project Metadata* data form provided in Appendix F.

Table 4 Required project metadata

Attribute	Expected Information
Project Name	Name of the project
Project Affiliation	Agency or organization leading project
Project Contact	Name of the project contact
Project Timeline	Report the anticipated years over which the project will occur. Indicate if sites will be visited once or repeatedly. If repeatedly, report the survey intervals.
Project Purpose	Report the purpose of the survey (inventory, monitoring, project clearance) and the target species (if applicable).
Project Locality	Report any information specific to the location of a project, including descriptive information, geographic coordinates, or other details.
Sampling Design	Select the methods used to develop the project sampling design, particularly the selection of survey locations. • Random[1] [describe] • Opportunistic [describe] • Systematic [describe] • Purposive[2] [describe] • Other [describe]
Target Universe	Select the geographic area in which survey locations occur. • Ecoregion [names] • Watershed [names and codes] • Political Boundary [names] • Waterbody [names] • Other [describe]
Supplemental Information or Other Sampling Methods	Report any supplemental information or other sampling methods used in combination with the project.
Notes	Describe other information relevant to the project metadata documentation.

[1]For GRTS designs, select "Other" and report as GRTS.
[2]Survey locations are purposefully selected at sites where the target is known to or believed to occur.

3.2 Data Forms and Reporting

Data forms associated with each protocol method are provided in Appendix F. Currently, incidental data can be directly entered online at https://arcg.is/KuS8H. In the future, data entry will make use of a data portal and database (currently in development) to enable data submission, storage, access, visualization, and querying of information gathered. The database will be designed to accommodate visual surveys conducted according to the methods outlined in this document and will serve as a resource for users to contribute freshwater mussel data. Data can be exported from the database and cross-walked for use in internal agency databases (e.g., NRM, GeoBOB, others). **Until this database is available, please submit data to one of the project members listed on the title page of this document.**

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5 Appendices

5.1 Appendix A. Glossary

- **acute mass mortality event:** an observed situation in which a large proportion of mussels appear to be recently dead or dying suddenly, as a result of an environmental change, pathogen, or other circumstance. Enumeration of the proportion of live versus dead (fresh dead or shell) in situ is described in both the transect and plot methods.
- **aquascope**: a tool, like a viewing bucket or glass-bottomed bucket that can be used to break up water surface tension or reduce glare to enable viewing underwater, much like a snorkel mask.
- **bankfull**: the height on the streambanks where water fills the channel and begins to overflow onto the active bench (i.e., floodplain).
- **baseflow**: flow that is maintained in a stream or river channel from groundwater delivery to the channel. In many western rivers, baseflow is all water that is present during summer low flow conditions.
- **braided channel**: river or stream that has multiple mid-channel bars below bankfull that form short and small subchannels, often with no obvious dominant channel.
- **contingent method**: standardized procedure for collecting data, similar to core methods, but is measured only where applicable. Contingent methods are not expected to be informative or cost effective for every monitoring application and may impact sensitive resources. Thus, they are only measured when there is reason to believe the resulting data is important for management purposes.
- **core method**: standardized procedure for collecting data that are applicable across many different ecosystems, management objectives, and agencies. Core methods are recommended for application wherever inventory, monitoring, or other research is planned.
- **covariate**: measured or derived parameter used to account for natural spatial or temporal variation in a core or contingent method or indicator (e.g., gradient); covariates may help determine the potential of a given stream or river reach to support freshwater mussels or to assist in interpreting monitoring data.
- **cut bank**: outside portion of a river bend or meander that is actively eroding and often near vertical in slope and/or has erosional features present (e.g., fracture, slump, eroding).
- **dive slate**: a tool useful for recording and tracking observations in water. Dive slates can be affixed to surveyors and can be cleaned for repeat use underwater.
- **eDNA**: a tool for assessing the presence of a species. eDNA samples can be used as supplemental information or provide another sampling method to support this protocol framework. Primers necessary for use of this tool have been developed for all species of western North American freshwater mussel.
- **exploratory method**: a core method of this protocol framework whose purpose is to gather information on the freshwater mussel assemblage, including the distribution of freshwater mussel species within a surveyed portion of a waterbody.
- **floodplain**: see bankfull.
- **fresh dead:** freshwater mussels appearing to have been recently predated or dead, often with flesh still attached to the shell interior.

- **glochidia**: larval freshwater mussels, which must attach to host fish to complete metamorphosis. Different species of fish may serve as hosts for western North American species of freshwater mussels.
- **incidental**: an observation that is in addition (incidental) to other surveys or is opportunistic in nature and may not be associated with a particular survey project.
- **inventory**: a survey documenting presence, relative abundance, or distribution of freshwater mussel species at a particular time.
- **intensive water quality parameter**: parameter collected to provide more seasonal and/or long-term data that is typically collected via grab sample or continuous sampling device.
- **left and right bank**: left and right bank are determined when the observer is oriented (facing) downstream.
- **metadata**: data intended to provide background information, useful for interpreting and tracking details related to this protocol framework.
- **midden**: one or more mussel valves/shells, generally observed without tissue and found piled with other shells and often broken or covered with scratches or bite marks, indicating they are the result of predation.
- **modular**: a feature of this protocol framework, based on the nestedness of each method. This feature enables individualized selection of methods to achieve specific project goals, while collecting data in a standardized fashion.
- **monitoring**: a survey repeated through time to document changes in select attributes of freshwater mussel species, whether for baseline or management purposes.
- **monument:** for the purposes of this protocol framework, a digital (e.g., photograph or gps coordinates) device used to record an accurate location for monitoring purposes
- **mussel bed (also mussel aggregation)**: an imprecise term used to indicate an aggregation or cluster of freshwater mussels. There is no agreed upon, standard number of animals for a cluster of mussels to be considered a bed.
- **nestedness:** a feature of this protocol framework, building from the modular nature of each method. This feature enables pairing of methods within an area of interest to achieve specific project goals, while collecting data in a standardized fashion.
- **non-intensive water quality parameter:** parameter collected to provide baseline water quality data with a single sample event that is typically collected via a thermometer or probe.
- **in situ:** meaning “in position,” this term is intended to indicate that the shells of freshwater mussels, to the best of the surveyor’s ability, are deemed to be from mussels that lived on site, rather than referring to those that appear to have been washed in. Shells that remain lodged in the sediment as though burrowed in life would be indicative of in situ shells.
- **plot method:** a contingent method of this protocol framework whose purpose is to gather information on both the surface and subsurface composition of a freshwater mussel bed. This method makes use of a quadrat. Information gathered may include one or more of the following: the sizes of individuals, more accurate estimates of relative abundance, condition, species composition, or individual species fates, when paired with a unique tag.
- **quadrat**: a tool used to delineate a discrete area for collecting data for this protocol framework. A quadrat is used to complete either the transect method (multiple quadrats placed for visual

sampling) or plot method (a quadrat that is excavated). A quadrat's purpose is to gather information on the freshwater mussel assemblage within a defined area (rather than a linear distance).

- **reach method**: a core method of this protocol framework whose purpose is to gather information on the freshwater mussel assemblage, including species composition and relative abundance, and other characteristics of the mussel assemblage at and within a discrete location (50 m length). The focal unit of replication in this protocol framework.
- **sampling method**: standardized procedure for collecting data that are specific to a given objective or support the collection or interpretation of core methods.
- **sampling unit**: a unit in which samples are collected. In this protocol framework it is considered the reach level, the unit of replication.
- **shell:** the hard outer covering common to many mollusks, including freshwater mussels. When counting and reporting shells in this protocol framework, a shell is defined as one or both of the two valves (a mussel is a "bivalve"), as well as weathered fragments. Estimates of the number of shells should be conservative when not observed as attached valves. For example two individual, unattached valves or an intact valve and a fragment should in both cases be estimated as one shell.
- **supplemental information**: other information used to support the collection or interpretation of core or contingent methods.
- **sampling frame:** the set of elements from which a sample is collected. In this protocol framework, reaches are collected from the set of points composing the stream network.
- **scour line:** the lowest consistent limit of perennial or sod-forming vegetation, or the ceiling of undercut banks.
- **target universe**: in statistical surveys, the target universe refers to the group of things that one seeks to make inference to (e.g., wadeable streams in Oregon).
- **thalweg**: the line along a stream channel (up and downstream) connecting the lowest elevations or deepest water depths. The thalweg would be the last portion of the stream or river to contain water if it were to dry up, and it tends to move back and forth across the stream channel.
- **tactile search**: another sampling method for conducting surveys where conditions are not conducive to visual surveys. Described as "hand-grubbing" into the upper layers of the substrate to encounter freshwater mussels. Often requires a permit to cover "take."
- **transect**: a core method of this protocol framework whose purpose is to gather information on the freshwater mussel assemblage, including species composition and relative abundance, and other characteristics of the mussel assemblage at and within a discrete location (a transect consisting of a continuous series of at least 10 quadrats measuring 0.25 square meters in size) within a reach.
- **wetted width**: a measure of waterbody width from the far right wetted edge to the far left wetted edge including all bars.

5.2 Appendix B. Equipment and Disinfection Methods

5.2.1 Equipment List

- Printed copies of the approved state fish and wildlife scientific take permit (as needed)
- Mussel shells and guide to review ID
- Printed copy of this document, including data forms, clipboard, and pencils
- Dry erase board and marker for monument photos
- Dive slate (to record data underwater) or clicker counters (for use above water)
- Alcohol hand sanitizer for cleaning the dive slate
- Disinfectant (see next page) and plastic tub for submerging equipment
- Drysuit or wetsuit, boots and neoprene socks, as well as a neoprene hood and gloves (as needed)
- Snorkel and mask with defogging liquid or polarized sunglasses and aquascope (as needed)
- Personal flotation device (as needed)
- Thermometer
- Waterproof flashlight, batteries, and charging cord
- Waterproof camera and charging cord
- GPS and spare batteries
- Surveyor’s tape (meters) or laser range finder capable of measuring to a tenth of a meter
- Metal cable or rope for delineating surveyor lanes (as needed)
- Watch
- Dry bag/rolltop backpack and/or snorkeler fanny pack (comes in waterproof and non-waterproof options)
- Dive weights (bean bags) for securing quadrat in place (as needed)
- Flagging tape (two colors)
- Whistle
- Knife
- 0.25 m^2 quadrat
- Mesh bags (at least two)
- Calipers

5.2.2 Equipment Disinfection

Before entering any waterbodies, it is best practice to review which, if any, invasive aquatic species are likely present in the watersheds that will be surveyed. If specific invasive aquatic species are present for which there is a specific decontamination protocol, follow those steps. Very few decontamination protocols are effective against all organisms, so it is important to evaluate the effectiveness of selected methods for each situation. Refer to Section 5.3.1 for more information on including decontamination considerations in planning. The following general methods can be used to decontaminate equipment where risk is lower.

- Cleaning:
    - Remove any debris such as plant materials or mud from equipment before leaving a site. Use a high-pressure spray or scrubbing brush to remove materials that are stuck on or in cracks or seams.
    - Disinfect equipment using the following:
        - Materials that would be damaged by a bleach solution: Use a mild disinfectant like Virkon® Aquatic or quaternary ammonia compounds (quat) such as Bardac®, following label guidelines for treatment. This is the preferred method for Washington Department of Fish and Wildlife (WDFW 2022), which recommends that gear is soaked in a 1% solution for at least 10 minutes, making sure it is totally saturated.
        - WDFW also recommends that "decontamination for larger aquatic organisms such as New Zealand mudsnails and zebra/quagga mussels requires soaking gear thoroughly with 2% solution so that it is completely saturated for a minimum of 20 minutes. Rinse thoroughly in a contained area and dispose of rinse water down a sewage drain, not a storm drain" (WDFW 2022).
        - Materials that can be exposed to a bleach solution: Soak equipment in a 10% bleach solution for 10 minutes. Bleach solutions are recommended where exposure to whirling disease is a higher risk.
    - Draining: Drain water from containers before leaving a site. If relocating mussels to a new waterbody, limit additional transfer of water during relocations. For example, do not pour water from coolers into new waterbodies. Instead, return water to the original site or dispose of water upland and far away from waterbodies.
- Drying: Allow equipment to fully dry (24 hours is best practice) before use in new waterbodies.

5.3 Appendix C. Survey Planning and Site Evaluation

Surveyor success in accessing survey locations and sampling the waterbody will rely heavily upon pre-departure investigation or "office evaluation." The evaluation process is useful for determining which locations will be sampled; how a crew will access a given waterbody or reach; whether field crews ultimately successfully sample an area; and documentation of the reasons why particular sampling locations (particularly reach sites) are ultimately rejected. Proper project design management, the process of documenting office evaluation and field-based outcomes, is necessary to maintain the statistical validity of a sample design (if required) and the subsequent inferences drawn from collected data. It relies on documentation of Required Project Metadata (Section 3.1). Table 5 should also be used to document the status of pre-selected sites as part of standard project documentation.

5.3.1 Office Evaluation and Pre-Survey Planning

The purpose of office evaluation is: 1) to determine whether a pre-selected location (if applicable) should be sampled; 2) to assess the accessibility and safety of a location; 3) to plan travel routes to the location; and 4) assess the need for specific decontamination steps. Field crew efficiency and productivity is often directly related to how well locations are evaluated, and thus the value of this process cannot be underestimated. Office evaluation involves using available geospatial information and local knowledge to determine if a location is surveyable or if it should be excluded because it is inaccessible or does not meet other criteria. If the site is selected for sampling, directions, landowner contacts, and other access information should be provided.

Consider the following questions when evaluating possible survey locations and the order in which they will be surveyed:

- Stream Flow Considerations:
  - Is the system likely to be perennial?
  - Is there a specific time of year that this location should be sampled based on when the stream might be influenced by runoff, weather, dam operations or irrigation withdrawals and returns?
  - Is the location likely to be safe to wade or snorkel?
- Access Considerations:
  - Is the location safe to access?
  - What are the driving directions to the access point and what will road conditions be like?
  - How long (in miles) is the hike from the access point to the sample point?
  - How difficult is the hike (easy/moderate/hard/very difficult)?
  - Are there unique challenges that might affect access (e.g., cliffs or private land)?
- Private and Public Land Considerations:
  - Where is any private land in relation to the location (e.g., upstream, east, etc.)?
  - Who is the point of contact to gain access and how should the person be contacted?
  - What are the access stipulations/directions from the landowner?
  - Is at least 50 m of stream on land of the target ownership (if applicable)?
- Decontamination Considerations:
  - Are there any invasive aquatic species documented or likely to be present?
  - Will surveyors be working at multiple sites within a watershed (and if so, will they work from

upstream to downstream, which reduces the need for equipment decontamination)?
- Will surveyors visit sites in different watersheds during the same trip (and if so, have they accounted for the time necessary for equipment decontamination or use of separate equipment for each watershed)?

Once potential survey sites are office evaluated, trip planning begins. Trips should be planned in advance to allow adequate time to contact private landowners, field office personnel, or other staff regarding access or for follow up questions. During trip planning, evaluate conditions to ensure that quality standards will be met (Section 1.5.4).

5.3.2 Field Condition and Pre-Survey Safety Evaluation

Upon arriving at a site, determine if it can be visually sampled. During the evaluation, consider whether shifting the point could enable sampling, avoiding the following issues:

1. Flow:
   - Is the site dry throughout, or is >5 m of the 50 m reach dry?
     - ☐ No. Evaluate next condition.
     - ☐ Yes, shift site to encompass 50 m of wet channel. If isolated pools or areas of wetted habitat are typical habitat for the region, conduct survey and report length and width of surveyed pools or wetted habitats. If site is dry throughout, drop site permanently. Completely dry sites should be dropped from mussel surveys, as temporary or seasonal drying of substrate is likely to exclude mussels.
   - Is flow elevated above bankfull and/or safe levels? High flows can be recognized by whitewater, the presence of substrate that is the size of boulders, and/or high slope or drop-offs. Water should not exceed flows at which a surveyor can comfortably cross, or exceed speeds at which a surveyor can comfortably choose a route, stop or slow progress if needed, or pick a safe point of exit and reach it. Surveyors are expected to be conservative in this evaluation.
     - ☐ No. Evaluate next condition.
     - ☐ Yes, the site exhibits high flows. Condition is thought to be a permanent limiting factor to the survey. Drop site permanently.
     - ☐ Yes, the site exhibits high flows, but the condition is not thought to be a permanent limiting factor to the survey. Revisit site at another time and reevaluate.
2. Visibility: Is visibility generally less than 0.5 meters (approximately 20 inches), or in shallower streams is the streambed obscured, even with the use of a dive flashlight? Factors affecting visibility temporarily may include low light conditions, suspended particles, depth as a result of higher than normal water levels, water discoloration, or other factors.
     - ☐ No. Evaluate next condition.
     - ☐ Yes, and the condition is thought to be a permanent limiting factor to the survey. Drop site permanently.
     - ☐ Yes, but the condition is not thought to be a permanent limiting factor to the survey. Revisit site at another time and reevaluate.

- ☐ Yes, but the condition is limited to the thalweg or middle portion of the waterbody, while the edge is surveyable (a common condition in large rivers). Refer to Appendix D a discussion regarding dealing with sites meeting this criterion.

3. Temperature: Do the combined air and water temperatures add up to at least 100°F (or 20°C)? Consider whether water temperatures may still be too cold or air temperatures may be too hot to conduct work safely.
   - ☐ No. Evaluate next condition.
   - ☐ Yes. Revisit site at another time and reevaluate.

Next, conduct a site evaluation for additional safety considerations. Be sure to continually re-evaluate the site for safety during the survey in case conditions change or new hazards are identified.

4. Potential contamination: Are there any obvious potential sources of contamination such as unidentified drums, containers appearing to contain toxic substances, pipes or hoses with unknown waste, toxic algae, areas of concentrated human use (i.e. feces present), or other similar issues?
   - ☐ No. Evaluate next condition.
   - ☐ Yes. Drop site permanently.

5. Warning signage: Are there any signs or markers present indicating a warning that potential hazards may be present onsite?
   - ☐ No. Evaluate next condition.
   - ☐ Yes. Determine the kind of hazard and evaluate according to the next condition.

6. Hazards: Is a hazard such as strong current, rapid, strainer, deep pool, lowhead dam, or a waterfall present within the site? If hazards are present upstream or downstream of a site but potentially still dangerous to the surveyor, surveyors should assess the situation to determine if the site should be dropped. Surveyors are expected to be conservative in this evaluation.
   - ☐ No. Evaluate next condition.
   - ☐ Yes. Drop site permanently.

Upon completion of this review, surveyors are ready to implement the survey.

5.3.3 Post-Evaluation Documentation

It can be helpful to track the status of potential sample points, and it is particularly important to do so for GRTS designs. To assign a final status for each possible survey location, determine which of the following apply to pre-selected locations. Table 5 provides more information about each situation.

- **Sampled**: When a point is sampled, it is considered a member of the target population and the data is used for analysis and reporting.
- **Non-Target**: The point was not sampled because the selected reach location was not considered a part of the target population. Non-target points are distinguished from points that are simply "not sampled" as a result of error or bias, which represent "holes" in the dataset. Instead, non-target points will not be used for estimating mussel community composition or density, but they can be used to estimate errors in the base data set and the amount of surveyed system. Therefore, it is very important to accurately record why a point was classified as non-target.

- **Permanently Inaccessible**: The point was not sampled for one of several possible reasons. The decision to classify a point as permanently inaccessible should not be taken lightly, as omitting points can create a "hole" or a gap in the design thus reducing the design's statistical rigor.
- **Too Close:** Points that were skipped, categorized as "too close" to another sample point (i.e., would overlap in survey area).
- **Unknown ("Unk")**: Points that were skipped, despite occurring earlier in the order of sampling (i.e. "holes"). Unknown points can have a negative effect on the statistical rigor and inference of the sample design and efforts should be taken to avoid having points with such a final status.
- **Not Needed (NN)**: Points not needed to meet desired sample sizes. These points will typically be oversample points that fall after the last evaluated point and are not considered in analyses.

Table 5 Reasons for which sample points in a study design are rejected or not sampled

Status	Reason not sampled	Description
Revisit	Different route or permission needed	The crew was unable to access the point but could gain access later with landowner permission or by taking a different route.
	Does not meet minimum quality standards	The site does not meet minimum survey quality standards, but could be sampled at a future date.
	Other	The crew started to access or sample but ran out of time, the crew was turned back by inclement weather, the point will require a more capable truck or ATV because it is remotely located or access road is too rugged, or various reasons not listed above.
Permanently Inaccessible	Access denied private	This point can only be accessed by crossing private land, and landowner permission was explicitly denied.
	Access no response private	This point can only be accessed by crossing private land, and landowner permission could not be verified.
	Inaccessible terrain	All routes were attempted, but natural barriers such as cliffs, steep slopes, or extremely dense vegetation prevented access.
	Not surveyable	This point will *always* be unsafe to wade or snorkel per Appendix C or will never meet minimum quality standards.
Non-Target	Dry	The stream was determined to be dry, either by field visit, or by two lines of evidence reviewed during office evaluation. Provide detailed notes if dry due to irrigation withdrawal.
	Lentic	The stream is a wetland, pond, or is otherwise impounded and no defined channel is present. Do not use this classification for lotic points inaccessible due beaver ponds.
	Frame error	There is no evidence that a waterbody or stream channel exists, the stream is actually an artificial channel such as a diversion ditch, or the reach is not on land of targeted ownership.
	Reach too short	The sample point falls on a stream reach containing less than 50 m of contiguously accessible or target waterbody.
Too Close	Too close	Too close to other points (e.g., overlapping the survey area of another point within the same design).
Unk	Unknown	The reason a sample point was skipped is unknown. This situation is best avoided for statistical purposes.
NN	Oversample	The sample point is part of a GRTS design and served as an oversample to enable the project to meet the desired sample size.

5.4 Appendix D. Special Situations

Some special situations may be encountered in the field when implementing the methods described in this document. For instance, a reach may fall within a portion of a river comprised of beaver ponds or **braided channels**, or a stream channel may naturally or regularly consist of a series of isolated pools separated by dry habitat. Surveyors should attempt to survey all wetted portions of the waterbody at a site, and it is important to capture the nature of the special situation, recording via photographs or site sketches. This information should also be described in the notes section and entered into the database so future surveyors can interpret observed differences at sites from one survey period to the next.

In some waterbodies, such as large rivers, *a portion* of the waterbody (e.g., the thalweg or middle of the channel) is unable to be surveyed due to

- depth (and subsequent lack of visibility), or
- swift flow that is either unsafe or does not allow for surveyors to move at an appropriate pace to observe and count mussels

In these cases, the survey can proceed instream along the banks, where mussels may still occur and be observable and able to be counted (see discussion of surveyor “lanes” and other survey modifications) in the exploratory and reach methods. Reporting on the area surveyed, combined with reporting of the wetted width, can provide an estimate of the proportion of the reach actually surveyed.

For sites consisting of naturally or regularly isolated wetted habitats, where flow is frequently only intermittent or isolated pools are common, follow the same methods described for other surveys, but report the number of isolated pools or wetted habitats surveyed and their approximate lengths and widths. Where vegetation or debris limits a continuous survey, similarly, report the number of isolated sections surveyed and their approximate lengths and widths.

In some waterbodies, sites may also be actively undergoing change (e.g., aggrading, experiencing sedimentation following fire or dam removal, or drying). This information is important for conservation and management purposes, and should be documented in the notes section of the data form.

5.5 Appendix E. GRTS Sampling Design

One goal of large-scale environmental monitoring efforts is to minimize error in estimates of quantities of interest. For the case of monitoring freshwater mussels in the western United States, metrics such as mussel densities and presence/absence rates are some examples of desired quantities for large geographic regions. Error is introduced when these quantities are estimated from sample data. Some sources of error may incur bias into the final estimate, such as measurement error, including imperfect detection, error due to unobservable site locations (e.g., due to access issues, turbidity, etc.) or other factors. These sources of error are typically minimized using state-of-the-art measurement techniques and meeting minimum quality standards during data collection. Other sources of error do not incur bias into the final estimate, such as the proper use of a randomized sampling design to observe a subset of the sampling frame. Error of this kind is minimized by selecting a sufficient sample size and using appropriate sampling designs that select sample sites in a statistically efficient manner.

GRTS is a widely accepted sampling design used broadly in the western United States for environmental studies (Stevens Jr 1997; Stevens Jr and Olsen 2003), and has an out-of-the-box software implementation in the R programming language via the *spsurvey* package, which is maintained and published by the Environmental Protection Agency (Dumelle et al. 2023). GRTS renders a set of sample locations along the stream network that are spatially balanced, and can consider varying sampling intensities via stratification, which are useful features for large-scale mussels sampling. This section provides high-level guidance for the GRTS sampling design including software and data requirements.

5.5.1 Definition of the Sampling Frame

The sampling frame defines the locations from which a sample of reaches is selected. In contrast to the target universe, which specifies the population of interest, the sampling frame is the practical tool by which samples are collected from this population. In the case of stream networks, a shapefile representing the stream network as polylines is a sufficient starting point for constructing the sampling frame. We recommend the use of the National Hydrography Dataset (NHD), which is publicly available using the National Map Downloader[6] to obtain such a dataset of the project area.

As a first step, areas of the sampling frame can be eliminated from consideration, such as areas outside of the organization’s ownership, areas that are known to be devoid of mussels or streams that are not covered by this protocol framework due to extreme turbidity or depth. Estimates made from the sample data will be contingent on the sampling frame produced after this step. For example, if only BLM land is considered inside the stream network, then the sampling frame is composed entirely of BLM land, and estimates made from the data collected from the sampling frame will only apply to this land.

Once a sampling frame has been established, the study region can be optionally stratified into regions of homogenous mussel density or presence/absence. For example, if mussel density is known to be high in a subregion of the sampling frame, it can be made into a separate stratum and sampled with a higher intensity. While prior information about mussel density is rare, ongoing mussel sampling efforts and other data sources such as eDNA can be used to develop prior information about mussel density, which can inform the development of an effective stratification. Other proxy variables such as stream order,

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[6] https://apps.nationalmap.gov/downloader/

hydrologic unit and presence or absence of certain types of substrate may all be used to develop strata. Strata need not be contiguous. For example, the *STREAMORDER* feature in NHD might be useful as a stratum, but not all stream orders of the same value are contiguous.

5.5.2 Determination of Sample Size

Sample size determination can take on a variety of forms. A final sample size is often selected after considering multiple factors, such as the desired statistical precision for an estimate of interest, operational feasibility, and budgetary constraints. Below we document how to determine the optimal sample size for the simple scenario of optimizing the standard error of a presence/absence rate *without stratification*. This is one of a multitude of possible scenarios analysts may face, and interested readers may refer to Strayer and Smith (2003) for more complex sample size determination methods.

Under this scenario a sample size that will achieve the desired level of precision is

$$n = \left(\frac{Z \cdot p}{MOE}\right)^2$$

where Z is the z-score corresponding to the desired level of confidence, p is a prior estimate of the presence rate and MOE is the desired margin of error. For example, if an MOE of 0.1 is desired for the presence/absence rate estimate at a 95% confidence level, the sample size becomes

$$n = \left(\frac{1.96 \cdot p}{0.1}\right)^2$$

For presence/absence estimation, the most conservative sample size using the above expression is obtained by setting $p = 0.5$, which may be prudent when no information about is available.

$$n_{Base} = \left(\frac{1.96 \cdot 0.5}{0.1}\right)^2 \approx 96$$

This indicates that approximately 96 sample reaches are needed to reach an MOE of 0.1 at a 95% confidence interval, assuming the presence / absence rate is 0.5. Analysts are free to adjust these values based on the desired uncertainty and available prior information on p.

Analysts must also define the number of oversample points to accommodate for nonresponse, i.e., when a planned sample site cannot be visited due to access, safety or other limitations. In these cases, oversample points should be sampled in order to maintain the spatial balance of the GRTS design. The *spsurvey* feature handles oversample points out-of-the-box, and we suggest adding 10 to 20% of the original sample size as oversample points, i.e.,

$$n_{Plan} = n_{Base} + n_{Over}$$

where $n_{Over} = n_{Base} \cdot 0.1$. If large nonresponse rates are expected, analysts should consider increasing n_{Over} to accommodate for more potentially missed sites.

5.5.3 Allocation of Sample Points to the Sampling Frame

Once the sampling frame, sample size and stratification have been made, points can be randomly allocated using the GRTS software. We refer the reader to the *spsurvey* documentation for further information on how to run the GRTS sample allocation (Dumelle et al. 2023). Analysts should take care to inspect several trial sample allocations to ensure that samples are allocated to the region of interest. These trial allocations are used to observe the nature of the sampling design, i.e., to inspect any errors or flawed assumptions in the GIS processing. Once the sampling design is deemed adequate, it should be re-run a final time to ensure proper randomization.

5.5.4 Necessary Metadata

Sampling designs of all forms need documentation to be useful. Sampling design documentation enables end users to compile estimates from the sample, such as presence/absence rates and mussel density estimates for the study region. For each stream reach, the *survey weight* is the primary piece of information needed for this process. The survey weight is responsible for "expanding" the reach-level observation to larger scales. Under GRTS using stratification the survey weight takes the form

$$w_i = \frac{n_i}{L_i}$$

where L_i is the stream length of stratum i and n_i is the number of stream lengths sampled in the stratum. The survey weight, w_i, represents the length of the stream network that the reach "represents" and are in units of length, e.g., kilometers. The *spsurvey* package produces weights directly, and are given as the *wgt* feature of the output point feature class. If a different sampling design is used, then the analyst is responsible for producing *wgt*. We recommend that all studies that implement a sampling design describe the sampling frame, design, stratification, and other necessary details in a short narrative document in addition to tracking the weights.

All sample points will also need a final status at the end of the study (Table 5). See also Section 3.1, which discusses required project metadata, which is applicable to all projects, including those employing a GRTS design.

5.6 Appendix F. Data Forms

Data forms and the freshwater mussel Quick ID Guide are provided in the following pages. **Be sure to complete the required *Project Metadata* data form for all projects and the Survey Event Details data form for every survey/site.** Complete other forms for each survey method conducted at the site. **Note that some survey methods, such as Exploratory, Transect, and Plot, may require pre-printing multiple copies in order to complete the survey method.**

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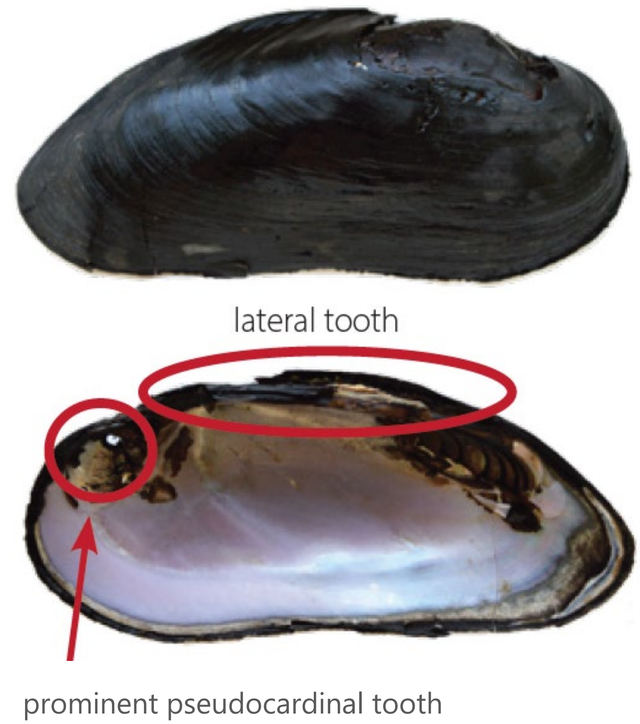
Western Pearlshell (*Margaritifera falcata*)



Photos: Left: views of western pearlshell papillae (top row), appearance in silty habitat when open and closed (middle row, left), young mussels (middle row, right), and as seen in an aggregation (bottom). Right: notable shell characteristics.

- Incurrent aperture: papillae fleshy and “tree-like," ~2–5 cm long
- Excurrent aperture: no papillae, ~2–4 cm long, *note similar (long) length of two apertures
- Shell size: up to 185 mm
- Shell interior: prominent pseudocardinal tooth
- Papillae color: variable, ranging from translucent to purple, orange, or striped purple and orange
- Shell color: variable, ranging from dark brown or black to lighter brown; older mussels may have significant shell erosion to the periostracum (outer layer), with calcium underlayer more visible

Credits: Shells: Xerces Society/Michele Blackburn; Live mussels: USFWS/Roger Tabor (top 2, bottom), Xerces Society (middle left), Nez Perce Tribe Watershed Division (middle right)

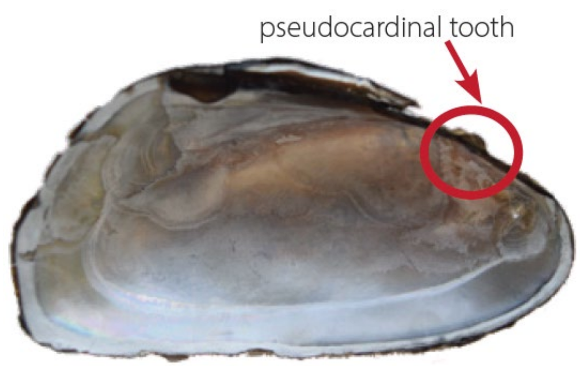
Floater Mussels (*Anodonta* sp.)

Photos: views of floater papillae (top row, left and right), appearance in silty habitat when open and closed (middle row, left), size ranges from a creek (middle row, right), and view from the side (bottom). Top right: notable shell characteristics.

- Incurrent aperture: singular and "finger-like," ~2–4 cm long
- Excurrent aperture: no papillae, ~1 cm long, *note shorter size of excurrent aperture
- Shell size: up to 180 mm but highly variable depending on species, location, and age. Adults of some species may be half the maximum length in certain habitats.
- Shell interior: lacking teeth
- Papillae color: variable, ranging from translucent to honey-colored or accented with purple
- Shell color: variable, ranging from yellow with green rays to dark or light brown

Credits: Shells: Xerces Society/Emilie Blevins; Live Mussels: Xerces Society/Emilie Blevins (top left and middle), USFWS/Roger Tabor (top right and bottom), Xerces Society/Candace Fallon (right)

Western Ridged Mussel (*Gonidea angulata*)



Photos: views of western ridged mussel papillae in sandy and silty habitats (top left and middle), size ranges (right, top and bottom), and interior shell view (bottom middle).

- Incurrent aperture: papillae bifid, branched and non-uniform along "trunk," ~2–4 cm long
- Excurrent aperture: no papillae, ~1-2 cm long, *note shorter size of excurrent aperture
- Shell size: up to 180 mm
- Shell interior: has minimal or absent pseudocardinal tooth
- Papillae color: variable, ranging from translucent to light or dark purple, occasionally pink
- Shell color: variable, ranging from yellow with green rays to dark or light brown; older mussels may have significant shell erosion to the periostracum (outer layer), with calcium underlayer more visible
- Shell shape: angular in appearance with often prominent ridge

Credits: Shell: Xerces Society/Michele Blackburn; Live Mussels: Xerces Society/Emilie Blevins

QC Date:__________ Initials:_______
Date Photos Entered (https://arcg.is/KuS8H):_______

Western Freshwater Mussel Visual Survey Protocol:
Incidental Observation Data Form (V. 1 June 2024)

This form is appropriate for incidental observations of freshwater mussels in the following US states and Canadian provinces: Alaska, Washington, Oregon, California, Idaho, Utah, Nevada, Arizona, Wyoming, Montana, and British Columbia. Observations collected through this form should be entered online at https://arcg.is/KuS8H or using the QR code below. For questions related to this form, or to share a large number of observations, please email: mussels@xerces.org.

Please note that states, provinces, tribes, or agencies may have specific limitations to handling live freshwater mussels, even for identification purposes. Please consult these regulations before handling live freshwater mussels.

Project Name: **Project Affiliation:** **Observation Date (MMDDYYYY):**
Lead Observer: **Other Observers:**
Contact Phone #: **Contact Email:** **Contact Organization:**

Waterbody Name			**Locality Description**			
Observation Coordinates	X:	Y:	**Datum or UTM Zone**		**Coordinate Source**	

Species Observed: Unknown, *Anodonta* sp. (floater), *Gonidea angulata* (western ridged mussel), or *Margaritifera falcata* (western pearlshell).

Photo required for incidental observations for online entry.

Species 1: ______________________________ **Voucher Photo #:**___________
☐ **Live Mussel** **Count:**________ **OR SELECT:** ☐ **101-500** ☐ **>500**
☐ **Shell** **Count:**________ **OR SELECT:** ☐ **101-500** ☐ **>500**

Species 2: ______________________________ **Voucher Photo #:**___________
☐ **Live Mussel** **Count:**________ **OR SELECT:** ☐ **101-500** ☐ **>500**
☐ **Shell** **Count:**________ **OR SELECT:** ☐ **101-500** ☐ **>500**

Species 3: ______________________________ **Voucher Photo #:**___________
☐ **Live Mussel** **Count:**________ **OR SELECT:** ☐ **101-500** ☐ **>500**
☐ **Shell** **Count:**________ **OR SELECT:** ☐ **101-500** ☐ **>500**

Observation Techniques	☐ Wading Visual ☐ Snorkel	☐ Wading + Aquascope ☐ Other:__________
Location Shells Observed	☐ In water	☐ On bank

Notes:

QC Date:__________ Initials:_______
Date Entered Online: _______

Section 3.1, Table 4

Project Name: **Project Affiliation:** **Project Contact:**

Contact Phone #: **Contact Email:** **Contact Organization:**

Project Timeline	
Project Purpose	
Project Locality	
Sampling Design (check box and describe)	☐ Random*: ____ ☐ Opportunistic: ____ ☐ Systematic: ____ ☐ Purposive**: ____ ☐ Other: ____
Target Universe (check box and describe)	☐ Ecoregions: ____ ☐ Watersheds: ____ ☐ Political Boundaries: ____ ☐ Waterbodies: ____ ☐ Other: ____
Supplemental Information or Other Sampling Methods	
Notes	

*For GRTS designs, select "Other" and report as GRTS.
**Purposive indicates that survey locations are purposefully selected at sites where the target is known to or believed to occur.

QC Date:__________ Initials:_______
Date Entered Online: _______

Western Freshwater Mussel Visual Survey Protocol: Event Data Form (V. 1 June 2024)
REQUIRED FOR ALL SURVEYS

Project Name:	**Project Affiliation:**	**Date (MMDDYYYY):**
Lead Observer:	**Other Observers:**	
Contact Phone #:	**Contact Email:**	**Contact Organization:**

Survey Types		**Waterbody Name**		**Survey Location**	**X:**	**Y:**
☐ **Exploratory**	☐ **Plot**	**Locality Description**		**Datum or UTM Zone**		**Coordinate Source**
☐ **Reach**	☐ **Transect**			**Coordinates for**	☐ Exploratory ☐ Reach	☐ Other:___________

Survey IDs:

Exploratory:________________________ **Plots:**____________________________

Reach:___________________________ **Transects:**_________________________

Covariates Documented

☐ **Wetted Width** ☐ **Substrate** ☐ **Human Influence** ☐ **Host Fish Presence**
☐ **Channel Unit** ☐ **Wet Wood** ☐ **Water Quality**

Quality Standards Met (check boxes)			
☐ **Flow below bankfull**		**Water Temp (°C)**	
☐ **Visibility >0.5 m or to bottom**		**Time Recorded**	

Monument Photos	☐ Yes	☐ No	Photo #s:
Species Voucher Photos	☐ Yes	☐ No	Photo #s:

Species Observed:		
	☐ ***Anodonta sp.*** **(floater)**	☐ ***Margaritifera falcata*** **(western pearlshell)**
	☐ **None**	☐ ***Gonidea angulata*** **(western ridged mussel)**

Site/Survey Sketch:

Survey Methods	☐ Wading Visual	☐ Wading + Aquascope
☐ Snorkel	☐ Other (e.g., tactile):_____________________	
Survey Directions	☐ Upstream	☐ Downstream
Survey Locations*	☐ Left bank	☐ Right bank

*Survey location is oriented looking downstream

Survey Coverage (Select one)

☐ **Entire Width (bank to bank)**

☐ **Bank Edges Only:** Average Distance Surveyed (Bank to Middle)
Left Bank ______m Right Bank ______m

☐ **Surveyor Lanes**: Total # of Surveyed 1m Lanes ______

Survey Notes (including special situations, Appendix D)**:**

QC Date:__________ Initials:_______
Date Entered Online: _______

Western Freshwater Mussel Visual Survey Protocol:
Exploratory Survey Data Form (V. 1 June 2024)

Project Name: **Date (MMDDYYYY):**

Lead Observer: **Exploratory Survey ID:**

Other Observers:

Event Details Form Completed ☐	**Survey Start**	X:	Y:	**Datum or UTM Zone**	
	Survey End	X:	Y:	**Coordinate Source**	
Wetted Width @ Start	m	**Exploratory Survey Distance**		m	
Survey Start Time		**Survey End Time**		**Survey Time** (minutes x # observers)	

Point #		Mussel Obs. Start	X:	Y:
General location of mussels, oriented facing	☐ Left bank	☐ Right bank	☐ Middle
Large wood present and wet within segment? Optional: Wood Volume (Section 2.8.4)	☐ Yes	☐ No	

Species 1:______________________________		Voucher: ☐ Photo ☐ Shell		Notes:
☐ Live Mussel	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
☐ Shell	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
Species 2:______________________________		Voucher: ☐ Photo ☐ Shell		
☐ Live Mussel	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
☐ Shell	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
Species 3:______________________________		Voucher: ☐ Photo ☐ Shell		
☐ Live Mussel	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
☐ Shell	Count:_____	OR SELECT: ☐ 101-500	☐ >500	

Point #		Mussel Obs. Start	X:	Y:
General location of mussels, oriented facing	☐ Left bank	☐ Right bank	☐ Middle
Large wood present and wet within segment? Optional: Wood Volume (Section 2.8.4)	☐ Yes	☐ No	

Species 1:______________________________		Voucher: ☐ Photo ☐ Shell		Notes:
☐ Live Mussel	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
☐ Shell	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
Species 2:______________________________		Voucher: ☐ Photo ☐ Shell		
☐ Live Mussel	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
☐ Shell	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
Species 3:______________________________		Voucher: ☐ Photo ☐ Shell		
☐ Live Mussel	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
☐ Shell	Count:_____	OR SELECT: ☐ 101-500	☐ >500	

Point #		Mussel Obs. Start	X:	Y:
General location of mussels, oriented facing	☐ Left bank	☐ Right bank	☐ Middle
Large wood present and wet within segment? Optional: Wood Volume (Section 2.8.4)	☐ Yes	☐ No	

Species 1:______________________________		Voucher: ☐ Photo ☐ Shell		Notes:
☐ Live Mussel	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
☐ Shell	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
Species 2:______________________________		Voucher: ☐ Photo ☐ Shell		
☐ Live Mussel	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
☐ Shell	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
Species 3:______________________________		Voucher: ☐ Photo ☐ Shell		
☐ Live Mussel	Count:_____	OR SELECT: ☐ 101-500	☐ >500	
☐ Shell	Count:_____	OR SELECT: ☐ 101-500	☐ >500	

*Species Observed: *Anodonta* sp. (floater), *Gonidea angulata* (western ridged mussel), or *Margaritifera falcata* (western pearlshell).

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Western Freshwater Mussel Visual Survey Protocol: Reach Survey Data Form (V. 1 June 2024)

QC Date:__________ Initials:_______
Date Entered Online: _________

Project Name: **Date (MMDDYYYY):**

Lead Observer: **Reach Survey ID:**

Other Observers:

Event Details Form Completed ☐	**Reach Coordinates**	X:	Y:	**Survey Start Time**	
	Datum or UTM Zone		**Coordinate Source**	**Survey End Time**	
Coordinate Location	☐ Upstream Reach Boundary	☐ Midpoint	☐ Downstream Reach Boundary	**Survey Time (mins x # obs):**	

Monument Photos are required, taken from the reach coordinates: Mark Upstream/Downstream when completed	☐ Upstream	☐ Downstream	
General Location of mussels within survey area, oriented facing	☐ Left bank	☐ Right bank	☐ Middle
Wetted Width	Upstream Reach Boundary __________m	Midpoint __________m	Downstream Reach Boundary __________m
Channel Units (Section 2.8.2) Present in Reach	☐ Fast Water Turbulent ☐ Off Channel	☐ Fast Water Non-Turbulent ☐ Pool	☐ Slow Water ☐ Small Side Channel
Channel Units Where Mussels Occur	☐ Fast Water Turbulent ☐ Off Channel	☐ Fast Water Non-Turbulent ☐ Pool	☐ Slow Water ☐ Small Side Channel
Evidence that mussels occur adjacent to the reach, either upstream or downstream?	☐ Upstream ☐ Did not check upstream	☐ Downstream ☐ Did not check downstream	☐ Neither

Species Observations: Count total number of live mussels (do not estimate). Count or estimate number of shells. Estimates of the number of shells should be conservative when not attached valves. For example two individual, unattached valves or an intact valve and a fragment should in both cases be estimated as one shell.

Species 1:______________________________ Voucher ☐ Photo ☐ Shell ☐ Live Mussel Count:__________ # Handled:_____ ☐ Shell Count:__________ OR SELECT: ☐ 1-100 ☐ 101-500 ☐ >500	Notes:
Species 2:______________________________ Voucher ☐ Photo ☐ Shell ☐ Live Mussel Count:__________ # Handled:_____ ☐ Shell Count:__________ OR SELECT: ☐ 1-100 ☐ 101-500 ☐ >500	
Species 3:______________________________ Voucher ☐ Photo ☐ Shell ☐ Live Mussel Count:__________ # Handled:_____ ☐ Shell Count:__________ OR SELECT: ☐ 1-100 ☐ 101-500 ☐ >500	

*Species Observed: *Anodonta* sp. (floater), *Gonidea angulata* (western ridged mussel), or *Margaritifera falcata* (western pearlshell).

Reach Channel Unit Definitions

• Fast Water Turbulent channel units are topographical high points in the bed profile that feature gradients >1%, broken water surface, coarse substrate, and tend to have consistently turbulent flow. The bedform of these units generally lacks longitudinal and/or lateral concavity.

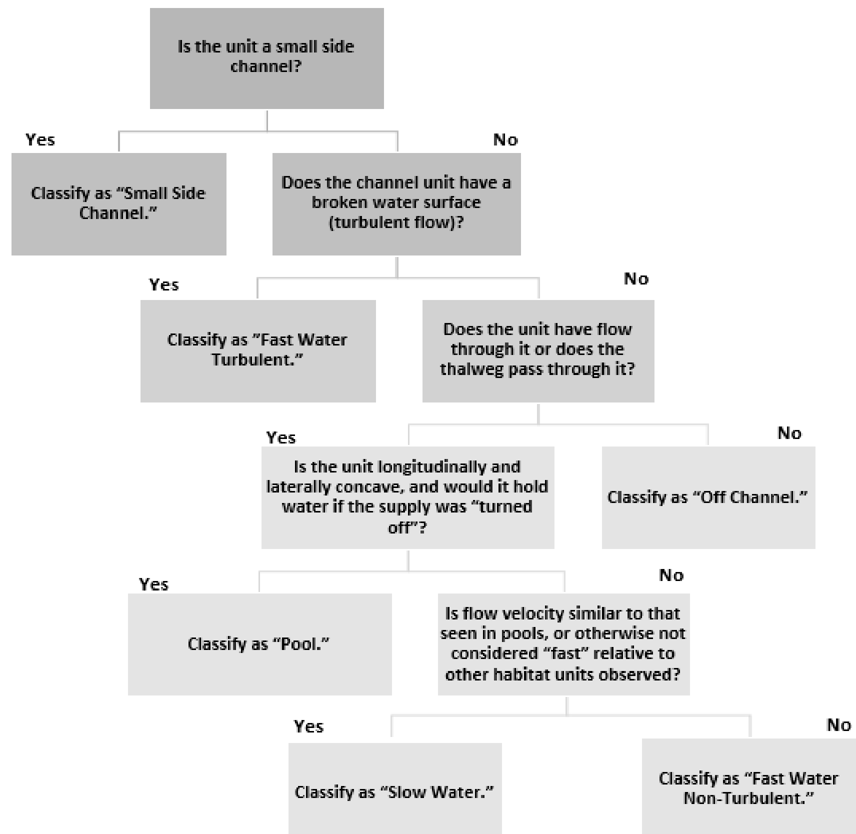
• Fast Water Non-Turbulent channel units feature low gradients, dominantly sand to cobble substrate, and a consistently smooth unbroken water surface. Often, fast water non-turbulent units have a gentle slope, similar to pools, but are distinguished from pools by their general lack of lateral and longitudinal concavity. These channel units are generally deeper than riffles. [Note that exceptionally low water may result in a ripply

• Slow Water channel units are topographical low points in the bed profile that feature gradients <1%, a consistently smooth unbroken water surface flow, and possess lateral and longitudinal concavity. [Note that exceptionally low water may result in a ripply water surface within this channel unit.]

• Off Channel units include backwaters and alcove type units that are connected to the main channel or large side channel but have little (< 1%) to no flow through them. The thalweg never passes through Off Channel units.

• Pool channel units include the following: Scour Pool, Plunge Pool, Dam Pool, Beaver Pool.

• Small Side Channel units are small side channels that contain <16% of the total stream flow.



Western Freshwater Mussel Visual Survey Protocol:
Non-random Transect Survey Data Form (V. 1 June 2024)

QC Date:__________ Initials:_______
Date Entered Online: _________

Project Name: **Date (MMDDYYYY):**

Lead Observer: **Reach Survey ID:**

Other Observers:

Event Details Form Completed ☐

Transect ID		**Transect Start** ____ (m) from Reach Boundary ☐ Upstream ☐ Midpoint ☐ Downstream				
Transect Start Coords.	X:	Y:	**Datum or UTM Zone**		**Source**	
Monument Photos for Transect Start (Take at least two photos and indicate the orientation the photographer is facing. Orientation relative to looking downstream.)			☐ Left bank ☐ Right bank ☐ Upstream ☐ Downstream			
Transect Direction (Provide sufficient details for future surveyors to orient transect in same manner.)			☐ Upstream ☐ Downstream ☐ Bank to Bank ☐ Other:__________________________ Angle:_________			

Quad #	*Anodonta* sp.		*Gonidea angulata*		*Margaritifera falcata*		**Comments** (report dropped quads if applicable)
	# Live	# Shell	# Live	# Shell	# Live	# Shell	
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Notes:

Transect ID		**Transect Start** ____ (m) from Reach Boundary ☐ Upstream ☐ Midpoint ☐ Downstream				
Transect Start Coords.	X:	Y:	**Datum or UTM Zone**		**Source**	
Monument Photos for Transect Start (Take at least two photos and indicate the orientation the photographer is facing. Orientation relative to looking downstream.)			☐ Left bank ☐ Right bank ☐ Upstream ☐ Downstream			
Transect Direction (Provide sufficient details for future surveyors to orient transect in same manner.)			☐ Upstream ☐ Downstream ☐ Bank to Bank ☐ Other:__________________________ Angle:_________			

Quad #	*Anodonta* sp.		*Gonidea angulata*		*Margaritifera falcata*		**Comments** (report dropped quads if applicable)
	# Live	# Shell	# Live	# Shell	# Live	# Shell	
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Notes:

*Species Observed: *Anodonta* sp. (floater), *Gonidea angulata* (western ridged mussel), or *Margaritifera falcata* (western pearlshell).

Western Freshwater Mussel Visual Survey Protocol:
Random Transect Survey Data Form (V. 1 June 2024)

QC Date:__________ Initials:_______
Date Entered Online: _________

Project Name: **Date (MMDDYYYY):**

Lead Observer: **Reach Survey ID:**

Other Observers:

Event Details Form Completed ☐	Distance Between Transect Flags	__________m

Transect Random Start	____ (m) from Reach:	☐ Upstream Boundary	☐ Downstream Boundary

Transect ID		**Transect Starting Bank** (Surveyor is oriented looking downstream.)				☐ Left ☐ Right	
Quad #	***Anodonta* sp.**		***Gonidea angulata***		***Margaritifera falcata***		**Comments** (report dropped quads if applicable)
	# Live	**# Shell**	**# Live**	**# Shell**	**# Live**	**# Shell**	

*Species Observed: *Anodonta* sp. (floater), *Gonidea angulata* (western ridged mussel), or *Margaritifera falcata* (western pearlshell).

Western Freshwater Mussel Visual Survey Protocol:
Plot Survey Data Form (V. 1 June 2024)

QC Date:__________ Initials:_______
Date Entered Online: _______

Project Name: **Date (MMDDYYYY):**

Lead Observer: **Reach Survey ID:**

Other Observers:

Event Details Form Completed ☐

Plot ID		**If associated with a Transect Survey**	**Transect ID**		**Quadrat Flip #**	

If not associated with a Transect Survey, indicate location according to any of the following	**Reach Midpoint**	Distance ______m Relative to midpoint: ☐ Upstream ☐ Downstream
	Monument	Distance ______m Orientation/Angle:________________________________ Coordinates: X: Y:
	Location (Orient downstream)	☐ Left bank ☐ Right bank ☐ Middle
	Plot Coordinates	X: Y: **Datum or UTM Zone**

If recording mussel lengths: keep surface and subsurface collections in separate bags and report separately. **Source**

	Anodonta sp.		*Gonidea angulata*		*Margaritifera falcata*		Comments
	# Live	# Shell	# Live	# Shell	# Live	# Shell	
Surface							

	Anodonta sp.		*Gonidea angulata*		*Margaritifera falcata*		Comments
	# Live	# Shell	# Live	# Shell	# Live	# Shell	
Subsurface							

Notes:

Other Mussel Data:

*Species Observed: *Anodonta* sp. (floater), *Gonidea angulata* (western ridged mussel), or *Margaritifera falcata* (western pearlshell).

Western Freshwater Mussel Visual Survey Protocol:
Substrate Covariate Data Form (V. 1 June 2024)

QC Date:__________ Initials:_______
Date Entered Online: _________

Project Name:	Project Affiliation:	Date (MMDDYYYY):
Lead Observer:	Contact Phone #:	Contact Email:
Other Observers:		Contact Organization:

Event Details Form Completed ☐		Measured as part of survey	☐ Transect	☐ Plot
Associated Survey IDs				
Coordinates	X:	Y:	Datum or UTM Zone	
Location Notes			Coordinate Source	

Section 2.8.3

Transect #	Quad/Plot #	% Bedrock	% Boulders	% Cobbles	% Coarse gravel	% Fine gravel	% Sand	% Fines

Substrate Type (mm)	Description
Bedrock (NA)	Exposed bedrock to surface
Boulder (>256)	Basketball or larger
Cobble (64-256)	Tennis ball - basket ball
Coarse Gravel (16-64)	Marble - tennis ball
Fine Gravel (2-16)	Small pebble - marble
Sand (0.06-2)	<lady bug, visible/gritty feeling
Fines (<0.06)	Silt/clay - smooth on fingers

Notes:

Western Freshwater Mussel Visual Survey Protocol: Wet Wood Covariate Data Form (V. 1 June 2024)

QC Date:__________ Initials:_______
Date Entered Online: _________

Project Name:	**Project Affiliation:**	**Date (MMDDYYYY):**
Lead Observer:	**Other Observers:**	
Contact Phone #:	**Contact Email:**	**Contact Organization:**

Event Details Form Completed ☐		**Measured as part of survey**	☐ Reach ☐ Transect		
Associated Survey IDs		**Coordinates**	**X:**	**Y:**	
Location Notes		**Datum or UTM Zone**		**Coordinate Source**	

Section 2.8.4

Reach				**Transect**		
Wet, Large Wood Piece #	**Length (m)**	**Width (m)**	**Comments**	**Transect #**	**# Quads Covered by Wet, Large Wood**	**Comments**

Notes:

Western Freshwater Mussel Visual Survey Protocol: Human Influence Covariate Data Form (V. 1 June 2024)

QC Date:__________ Initials:_______
Date Entered Online: _________

Project Name: **Project Affiliation:** **Date (MMDDYYYY):**

Lead Observer: **Other Observers:**

Contact Phone #: **Contact Email:** **Contact Organization:**

Event Details Form Completed ☐	**Measured as part of survey**	☐ Exploratory	☐ Reach		
Associated Survey IDs		**Coordinates**	**X:**	**Y:**	
Location Notes		**Datum or UTM Zone**		**Coordinate Source**	

Human Influence (Section 2.8.5)*	**Streambed**	**Contained**	**Absent/Unk**	**Photo Taken**	**Notes**
1. Wildfire (natural or human caused)					
2. Row crops					
3. Pastures, hay fields, or fences					
4. Presence of livestock or wild horses and burros, including feces, cropped vegetation, or trails from grazing					
5. Logging operations					
6. Mining (e.g., gravel, open pit, placer mining)					
7. Oil and gas wells and associated well pads					
8. Walls, dikes, or bank stabilization structures (e.g., riprap)					
9. Inlet or outlet pipes					
10. Instream habitat restoration (e.g., gabion rock baskets, cabled large wood, beaver dam analog structures)					
11. Hydrologic alterations (e.g., irrigation diversions, impoundments, dams)					
12. Utility/powerline/pipeline corridor					
13. Buildings					
14. Pavement/cleared lots (e.g., paved, graveled, dirt parking lot, foundation)					
15. Roads or railroads, including culverts					
16. Landfills or trash (e.g., cans, bottles, trash heaps)					
17. Parks or maintained lawns					
18. Recreation (e.g., off-highway vehicle use, camping, trails, boating)					

*Please use categories (e.g., streambed, contained, absent/unknown) as checkboxes. Indicate if photos taken. Please include additional explanatory notes if columns are checked.

Additional Notes:

Western Freshwater Mussel Visual Survey Protocol:
Host Fish Presence and Water Quality Covariate Data Form (V. 1 June 2024)

QC Date:__________ Initials:_______
Date Entered Online: _________

Project Name: **Project Affiliation:** **Date (MMDDYYYY):**

Lead Observer: **Other Observers:**

Contact Phone #: **Contact Email:** **Contact Organization:**

Event Details Form Completed	☐	**Measured as part of survey**	☐ Exploratory	☐ Reach	☐ Transect
Associated Survey IDs		**Coordinates**	**X:**	**Y:**	
Location Notes		**Datum or UTM Zone**		**Coordinate Source**	

HOST FISH PRESENCE (Section 2.8.6, five minute snorkel survey)		**WATER QUALITY (Section 2.8.7)**	**Time of Sample Collection:**		
Fish Species*	**Comments**	**Parameter**	**Collected**	**Value**	**Comments**
		Temperature (°C)			
		Specific Conductance			
		pH			
		Dissolved Oxygen (mg/L)			
		Ammonia NH3 (mg/L)			
		Continuous Temperature			
		Total Organic Carbon (mg/L)			
		Alkalinity (mg/L)			
		Phosphorus (mg/L)			
		Herbicide 2-4D (mg/L)			
		Chlorophyll a (mg/L)			
		Other Parameters:			

Notes:

*Fish examples (report actual species if known): salmonids, sculpins, dace, shiners, nonnative species