



Freshwater Mussel Rapid Assessment Sampling Protocol for Wadeable Streams

Alexa M. Hershberger¹, Allison H. Roy², Peter D. Hazelton³, Ayla J. Skorupa⁴, Jason Carmignani⁵, Michael A. Baker³, Stefanie J. Farrington^{1,6}, and Sean Sterrett⁷

- ¹ Organismic and Evolutionary Biology Program, Massachusetts Cooperative Fish and Wildlife Research Unit, University of Massachusetts - Amherst
- ² U.S. Geological Survey, Massachusetts Cooperative Fish and Wildlife Research Unit, Department of Environmental Conservation, University of Massachusetts - Amherst
- ³ Warnell School of Forestry and Natural Resources, University of Georgia
- ⁴ Illinois Natural History Survey, University of Illinois at Urbana-Champaign
- ⁵ Natural Heritage and Endangered Species Program, Massachusetts Division of Fisheries and Wildlife
- ⁶ U.S. Fish and Wildlife Service Richard Cronin Aquatic Resource Center
- ⁷ Biology Department, Monmouth University

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For additional copies or information, contact:

Dr. Allison Roy
U.S. Geological Survey
Massachusetts Cooperative Fish and Wildlife Research Unit
Email: aroy@usgs.gov

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Left photos captured by Dr. James Garner. Right photo captured by Mickala Stratton.

Prepared by: Alexa M. Hershberger¹, Allison H. Roy², Peter D. Hazelton³, Ayla J. Skorupa⁴, Jason Carmignani⁵, Michael A. Baker³, Stefanie J. Farrington^{1,6}, and Sean Sterrett⁷

Affiliations:

¹ Organismic and Evolutionary Biology Program, Massachusetts Cooperative Fish and Wildlife Research Unit, University of Massachusetts - Amherst

² U.S. Geological Survey, Massachusetts Cooperative Fish and Wildlife Research Unit, Department of Environmental Conservation, University of Massachusetts - Amherst

³ Warnell School of Forestry and Natural Resources, University of Georgia

⁴ Illinois Natural History Survey, University of Illinois at Urbana-Champaign

⁵ Natural Heritage and Endangered Species Program, Massachusetts Division of Fisheries and Wildlife

⁶ U.S. Fish and Wildlife Service Richard Cronin Aquatic Resource Center

⁷ Biology Department, Monmouth University

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Preface

In 2018, a standardized protocol for sampling Brook Floater (*Alasmidonta varicosa*) was developed with the goal of understanding occupancy of the At-Risk species across its range (Sterrett et al. 2018). The protocol was developed as one of the first tasks of the Brook Floater Working Group (BFWG), a group of scientists and managers throughout the species' range along the Atlantic coast from Georgia (United States) to Nova Scotia (Canada). The Brook Floater Rapid Assessment Monitoring Protocol has been widely used by state agencies and non-profit organizations over the past decade and has been increasingly used for collecting data on other freshwater mussel species and for purposes other than understanding species occupancy. As such, the new protocol described herein was developed to expand the scope of the Brook Floater protocol for broader applications, while retaining most of the basic survey methods from Sterrett et al. (2018). Importantly, this new protocol offers more flexibility than the Brook Floater protocol in that it does not require random site selection, has no minimum channel width, and can be completed by a single person. This protocol includes additional details on mussel handling and safety, new guidance on site selection, and a new section on data management. We intend that this protocol is used for sampling freshwater mussels in wadeable streams when the goal is not an estimate of species' occupancy; Sterrett et al. (2018) remains a valuable protocol for occupancy assessment.

Author Contributions

AMH: conceptualization, writing-original draft, writing-editing, writing-review, project supervision; AHR: conceptualization, writing-editing, writing-review, funding acquisition; PDH: conceptualization, writing-editing, writing-review, funding acquisition; AJS: conceptualization, writing-editing, writing-review; JC: conceptualization, writing-editing, writing-review, funding acquisition; MAB: writing-editing, writing-review; SF: writing-editing, writing-review; SS: conceptualization, writing-review.

1. Introduction

1.1 Background

Freshwater mussels (Families: Unionidae and Margaritiferidae) are an important but under-assessed group of aquatic biota. Freshwater mussels provide critical ecosystem services, such as biofiltration, nutrient cycling, and habitat modification (Vaughn 2018), but they are less commonly targeted in assessments of ecosystem health compared to aquatic insects (commonly used for water quality assessments; Sumudumali and Jayawardana 2021) and fishes (often used to assess biodiversity and habitat connectivity; Karr 1991). While global declines of population size and distribution of freshwater mussels are well accepted (Böhm et al. 2021), the lack of consistent sampling through time has largely limited managers' understanding of causes of declines and focal areas for conservation (Lindenmayer and Likens 2010; Haag and Williams 2014).

Standardized sampling provides opportunities to compare assessments across surveys to develop more comprehensive descriptions of species distribution, understand drivers of species presence or abundance (e.g., O'Brien et al. 2025), and develop predictive models forecasting species' presence into the future (e.g., Rogers et al. 2025). To date, the most comprehensive review of sampling designs and methods for sampling freshwater mussels is by Strayer and Smith (2003); this book serves as a valuable resource and overview of methods to meet different objectives. However, for managers and biologists implementing and contracting surveys, specific sampling protocols are needed. A protocol that can be implemented with minimal time and effort (i.e., facilitating multiple site visits in a day) has great potential to increase knowledge about mussel distribution and community structure, particularly if the same protocol is implemented widely throughout a species' range.

1.2 Protocol Scope and Applications

This protocol is designed to quickly assess streams for what mussel species are present (i.e., provide confirmed presence) and in what numbers (i.e., provide a minimum number at a stream). This information can be used to calculate species richness and catch per unit effort (CPUE in person hours), understanding the protocol cannot confirm species absence and CPUE is often a poor proxy for population abundance or density (Strayer and Smith 2003). The protocol also includes a rapid method for collecting basic habitat information that can help identify habitat correlates of mussel presence and inform hypotheses about potential drivers. We designed the protocol to be useful for a variety of purposes (see Appendix A), but it is likely particularly valuable when a site has never been surveyed or has not been surveyed for decades. As such, the protocol may be useful in conjunction with environmental DNA (eDNA; Coghlan et al. 2021; Appendix B) collection to collectively sample many locations; if eDNA analysis reveals mussel presence, follow-up sampling with this protocol will provide more information about mussel counts, biological information (mussel size, condition, etc.), and precise locations of mussels to understand habitat associations. This protocol may also be useful to capture baseline information

(e.g., species presence, habitat data) where there are planned disturbances or restoration (including stocking), or to inform site selection for long term population monitoring; such surveys may be followed up with more intensive surveys if species of interest are found.

While this protocol can be used for a wide variety of purposes, it is not intended to serve as a “one stop shop” for all freshwater mussel sampling, and we point readers to other protocols and resources for various purposes. Most notably, the work by Strayer and Smith (2003) provides a helpful framework for manager considerations to be made prior to designing a mussel survey (pp. 1-5, Strayer and Smith 2003). They further categorize sampling designs by relative cost, and goals of the researcher (e.g., species presence, abundance, and monitoring). The protocol described herein would be considered by Strayer and Smith as *Informal Sampling*, useful primarily (with some reservation) for evaluating and monitoring species presence (Table 1, p.7, Strayer and Smith 2003). Selected purposes and study approaches that this protocol does not cover, along with supplementary resources are provided in Table 1; many other studies are available by searching for primary literature and agency reports.

Randomized site selection is not a component of this protocol as we recognize this protocol may be used by researchers and managers for a variety of purposes. However, we acknowledge randomized selection of sites across a species range or study area is critical for statistical inference of habitat use or species occurrence rates. Further discussion of randomized site selection can be found in Appendix B, and readers can find an approach with random site selection in Sterrett et al. (2018) and other primary literature.

Table 1. Example freshwater mussel sampling purposes where the freshwater mussel sampling protocol for wadeable streams would *not* be appropriate.

Purpose	Description	Resources
Studies with a regulatory nexus by any local, state, or federal jurisdiction	Where states or other jurisdictions have specific requirements for survey criteria that are unique to those jurisdictions.	Surveyors can inquire about these directly with each leading agency. Other resources: Freshwater Mollusk Conservation Society n.d.
Occupancy Estimation	When trying to make inference about a population or species distribution, which requires estimates of detection using temporal (preferred) or spatial replicates	Mackenzie et al. 2002, Wisniewski et al. 2013a, Wisniewski et al. 2014, Sterrett et al. 2018
Population Estimation	When a precise estimate of population abundance (or demographic parameters such as recruitment, immigration/emigration rate, population growth rate, or survival) is needed for long term monitoring or understanding effects of restoration. Methods include 1) capture-mark-recapture (CMR) and 2) quantitative density estimates	<i>CMR</i> - Villella et al. 2004, Meador et al. 2011, Wisniewski et al. 2013b, Inoue et al. 2014, Carey et al. 2019, Sterrett et al. 2022 (though see updates by Baker et al. <i>in press</i>). <i>Density</i> - Dorazio 1999, Smith et al. 2001, Smith et al. 2003, Strayer and Smith 2003, Sanchez and Schwalb 2021.

1.3 Protocol Goals and Organization

This protocol aims to provide a standardized method for sampling freshwater mussels in wadeable streams along with basic physical habitat information relevant to interpret mussel data. The protocol can be widely used for a variety of purposes and to target all freshwater mussel species. Specifically, the goals of this protocol are to:

- 1) Provide a standardized sampling protocol for rapid assessment of freshwater mussels in wadeable streams;
- 2) Describe selected habitat variables to supplement freshwater mussel surveys;
- 3) Present a framework for data management after data collection.

The protocol provides information about personnel qualifications and guidance for walking in streams and handling mussels to minimize disturbance. The mussel and habitat survey methods include equipment lists and detailed descriptions of how to sample and what data to collect. We end with a section on best practices for data management and reporting. The protocol is intentionally simple to allow it to be implemented quickly, but we offer information in appendices and citations for more in-depth data collection and analysis. Ultimately, we hope this protocol offers the tools to expand standardized sampling for freshwater mussels to provide valuable data to aid in their conservation and management.

2. Minimizing Impacts on Freshwater Mussels

2.1 Overview

Mussels are sensitive to handling and disturbance, so implementing methods to minimize impacts are important for freshwater mussel conservation. Having an experienced malacologist on site and training other observers can help to ensure proper procedures are being followed. Timing of sampling can be adjusted to minimize disturbance. Care in mussel handling and minimizing stream disturbance are also described below.

2.2. Personnel Training

This protocol can be completed with personnel (also referred to as observers) with a range of levels of expertise; however, there is evidence that detection increases with years of observer experience (Wisniewski et al. 2013a). At minimum, it is critical one person is able to perform species-level identification, which can be challenging (Shea et al., 2011). If people are unfamiliar with mussel sampling, an on-site training on equipment use, survey methods, minimizing disturbance, and mussel handling is necessary prior to sampling. All observers should be familiar with permitting guidelines and safety procedures for safe care and handling of all mussel species. While this protocol can be completed with a single person, at least one additional person is helpful for safety purposes and ensuring that sampling protocols are being followed.

2.3 Survey Timing

The timing of surveys may influence mussel detectability, reproduction, and survival. Some state agencies require surveys to occur within specific seasonal windows, primarily to minimize temperature-related handling stress and mortality. Conducting surveys too late in the year, when water temperatures drop, can reduce mussel mobility and reduce their ability to rebury after being placed in the substrate (Block et al. 2013; Lurman et al. 2014), potentially compromising overwinter survival. Conversely, surveying during abnormally warm periods can add physiological stress to individuals already near their thermal limits (Ganser et al. 2015; Curley et al. 2021).

Handling sensitivity also varies with time of year, depending on the species and reproductive status. Some mussels may expel glochidia when disturbed, so it is important to avoid sampling during peak brooding periods. Detectability can also change seasonally: during spawning, certain species move into shallower habitats or extend further above the sediment surface, increasing the likelihood of detection (Watters et al. 2001; Vilella et al. 2004). Because spawning and glochidia release are temperature-dependent, both the onset and duration of reproductive activity can vary across species and across a species' geographic range (Hastie and Young 2003; Barnhart et al. 2008). Planning surveys to avoid reproductive activity for freshwater mussel species and other sensitive taxa minimize disturbance to a site. If unsure about sampling

windows that account for important reproductive timelines, consult with state and federal biologists.

2.4 Permits

Prior to any mussel handling, be aware of all applicable state and federal permitting requirements. State regulations can vary widely: some states prohibit handling any freshwater mussels without a permit, whereas others only restrict the collection or removal of state-listed threatened or endangered species. Working with federally-listed species adds complexity and requires obtaining a U.S. Fish and Wildlife Service scientific research permit. Federal, and some state, regulations also require observers to have completed a specified number of supervised surveys before independently handling listed species; however, an inexperienced observer may work under the direct supervision of a qualified, permitted individual to gain the experience necessary for full certification.

2.5 Mussel Handling

To ensure mussel survival, special precautions can be placed to ensure mussel safety during identification and measurement. While processing individuals, minimize the time out of water and avoid exposure to direct sunlight. Microhabitats may play a critical role in mussel survival (e.g., mediate burrowing ability, influence fine scale hydraulics, and shape sediment chemistry and associated bacterial processes; Pandolfo et al. 2016; Kubala et al. 2024). Thus, to reduce survey impacts and ensure mussels are returned to appropriate microhabitats, surveyors can temporarily mark each collection location with a bright colored flag (Table 2) placed in the sediment where the individual was removed. This practice is especially important for rare or sensitive species that depend on specific microhabitats or experience heightened handling stress, as returning them to suitable conditions may help offset energetic costs. Flagging also helps prevent foot traffic in areas with sensitive mussels and preserves critical microhabitat structure (see Sterrett et al. 2022). For more common species, returning individuals to the same mesohabitat may be sufficient. In these cases, mussels can be held in mesh bags submerged within the mesohabitat, and the location marked with a bright flag. Avoid placing mussels in channel-edge areas where mud or detritus are easily suspended and may create unsuitable conditions. When completed, check all mesh bags, including turning them inside out, to ensure no animals are inadvertently removed from the site. Removing all flagging at the conclusion of the survey will follow site integrity practices.

After mussel processing, return each individual to the substrate in its natural orientation (Figure 1). Mussels are to be “seeded” into the substrate in a natural, siphoning position with the posterior end of the shell pointing up. Ensure mussel valves are closed prior to seeding to avoid lodging substrate matter inside the valves. Avoid pushing mussels into the substrate, particularly when substrate sizes are relatively coarse and shells are thin. Observers can use two fingers to dig a small hole and then hold the mussel near the substrate in the correct position for a few

seconds to allow the foot to secure the animal. Ensure the mussel stays lodged as some species may trap air between their valves resulting in floating up out of the substrate.

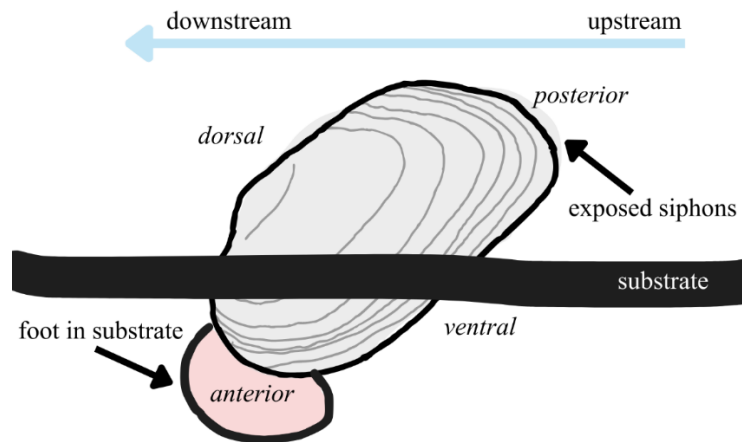


Figure 1. Proper placement of an individual mussel following handling. The mussel is positioned upright and partially inserted into the sediment to facilitate reburial and reduce handling stress.

2.6 Stream Disturbance

Minimizing disturbance to potential freshwater mussel habitat and to other sensitive or imperiled stream species is critical before and during survey work. Snorkel surveys typically reduce substrate contact compared to walking surveys using view buckets and can improve detectability (Nuri et al. 2020). However, view bucket surveys may be the most effective strategy depending on site-specific conditions (e.g., water depth). Regardless of the method used, surveyors should take deliberate steps to limit disturbance when moving through the stream. For example, walk in a single file along the bank to reach survey start and end points, avoid unnecessary substrate contact, and communicate disturbance-minimizing procedures to all personnel prior to beginning surveys.

3. Mussel Survey

3.1 Overview

Mussel surveys consist of a timed search effort using visual (snorkeling and/or view buckets) and tactile methods. If there is more than one observer, then observers follow longitudinal (i.e., parallel with flow) lanes (Figure 2). Study objectives will inform the search rate and mussel data collected. For example, the search rate could be faster if interested in species richness (list of species encountered) compared to counts for each. Surveys may also want to target one or more species of interest, which could modify survey methods and increase search rate when encountering large numbers of non-target mussel species. Information on the collection of relic shells for species of interest is below.

3.2 Site Reconnaissance and Starting Location

Depending on your goals, it might be useful to visit the stream prior to bringing a team to conduct basic site reconnaissance to determine 1) if the stream is appropriate for sampling, and 2) the specific starting location. In some cases, there may be disturbances close to the stream access point that make the location challenging to sample (e.g., beaver dams creating wetland habitat or road-stream crossings creating scouring and introducing runoff) or problematic due to bank or riparian disturbances (e.g., rip-rap on banks, buildings in the riparian area); moving the site upstream or downstream by 100 or more may address those concerns. Even if there are no visible problems or disturbances, if seeking a site representative of the stream, consider using a random distance (e.g., 1–100 m) away from stream access locations as a starting point.

3.3 Equipment

When conducting a mussel survey, supplies are needed for swimming/wading in the stream, collecting mussels, and recording the data (Table 2).

Table 2. Supplies needed for sampling and measuring freshwater mussels.

Item	Quantity	Purpose/Notes
Clipboard	1–2	Data recording
Pencils or waterproof pen	2	Data recording
Waterproof data sheets	1 per site (plus backups)	Data recording; see Appendix C
Masks and snorkels	1 per site (plus backups)	
Wetsuits	Per observer	For warmth, protection, and buoyancy while snorkeling
Weight belts	Per observer	To achieve neutral buoyancy in deeper water
View buckets	Per observer	To supplement when conditions are unsuitable for snorkeling
Mesh bags	1 per site (plus backups)	To hold mussels while sampling and processing. Attach flagging tape to dark-colored bags. Bring backups.
Pin flags	~100	To mark mussel locations. Recommend bright colors. Flagging tape tied to a heavy object (nuts, washers) can be used as a substitute. Quantity is dependent on expected density per site.
Stopwatch or timing device	1	To record survey duration
Tape measures or range finder	2	Measure survey length and stream width. 50 m and 100 m tape recommended
Small calipers or ruler	2	To measure mussels
Waterproof camera or phone	1	To document general conditions
GPS or phone	1	To obtain site coordinates
Ziploc bags	Sites per day	To collect relic shells (if found)
Waterproof labels	Sites per day	To label relic shells

3.4 Snorkel Survey

All survey approaches described here target wadable stream conditions suitable for visual and tactile survey methods, including depths $\leq \sim 1$ m and low streamflow. Ideally, surveys will consist of visual and tactile surveys using mask and snorkel. If the habitat is too shallow to effectively snorkel ($< \sim 25$ cm) and the streambed is visible, then view-buckets (if shallow for the entire site) or visual scanning at the surface (for infrequent shallow areas) are acceptable. Snorkeling allows surveyors to float above habitat with only minor disturbance to the stream bottom (i.e., gentle digging). Tactile methods (such as slightly digging fingers into soft substrate or grazing fingers along the substrate) may be needed in systems with high turbidity or low water

flow. Areas too deep for snorkeling should be avoided in this protocol as deep water could affect detection and safety.

There are three survey approaches covered in this protocol (i.e., Survey A, Survey B or Survey C), determined by the width of the stream or sampleable habitat, and the number of observers available (Figure 2).

Survey A – Full Stream Width Survey: The stream site is divided into lanes of equal size proportional to the number of observers performing the survey (Figure 2). The width of observer lanes is recommended at no more than 3 m (Figure 2). During each survey, try to keep the search lanes used by the observers independent although the lanes may overlap slightly (Figure 2). Keep in mind lane width may vary throughout the site and large wood such as a downed tree may impact the ability to survey a lane. Observers are randomly assigned to lanes to avoid potential observer bias if planning to use lanes to estimate detection.

Survey B – Bank Survey: This approach is identical to Survey A but does not include the full stream width because the sampleable habitat is too large to efficiently sample relative to the number of observers (Figure 2). The selected bank for surveying can be randomly selected or intentionally selected depending on survey objectives. If a survey is targeting a species typically found on the banks of the river, the observers may want to split the lanes to cover both banks and note the middle of the river channel.

Survey C - Single Observer Survey: Single observer surveys might be advantageous in perennial streams < 4 m in stream width, when habitat conditions limit sampling (see Survey B), or to perform site reconnaissance. However, single observer surveys are generally inefficient in streams > 4 m in width (Figure 2). Although one observer may be sufficient for a survey, an additional person is for safety reasons.

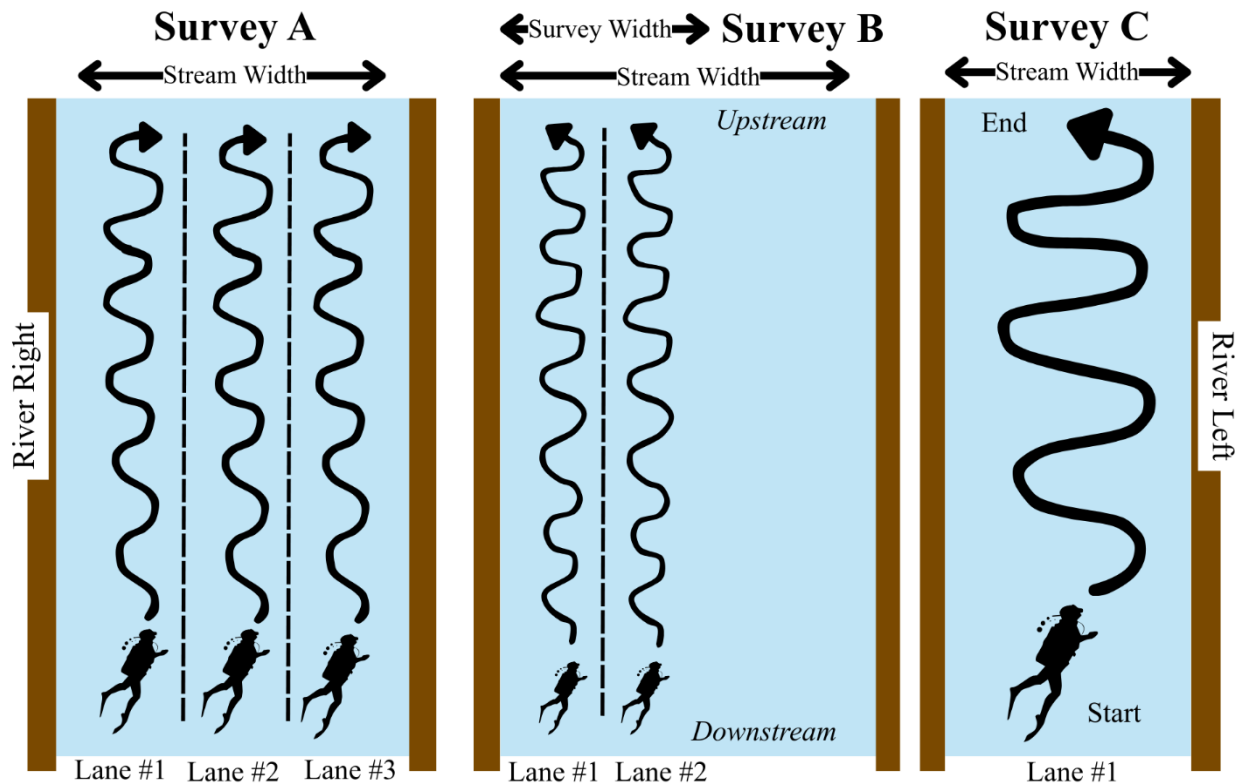


Figure 2. Diagrams of a stream site with the sub-division of “lanes” for covering the full stream width with multiple observers (Survey A), covering only a portion of the stream width and pinned to the bank with multiple observers (Survey B), and for a single observer (Survey C). Dashed lines indicate the outline of each lane. The curved arrows in each lane show the path of each observer within each assigned lane.

Survey effort is an important measure to help standardize effort across sites and can be incorporated into recorded species counts to calculate CPUE metrics. Survey effort is recorded in person hours (e.g., the number of observers multiplied by the duration of the survey). The total amount of survey effort is dependent on study objectives and only includes search time and does not include site selection or mussel processing time. All surveys should consist of a minimum of 2 person hours of search time per site. For example, 3 observers would search a site for 40 minutes and 6 observers would search a site for 20 min. The minimum survey effort is based on the desire to be “rapid” balanced against the time needed to find rare species and personnel cost. Surveys could be longer if desired for other purposes (see Section 3.6).

Surveyors start at the downstream starting point simultaneously while one observer notes the starting time or starts a timer. Observers weave through their designated lane searching all habitat types. Aim for a target search rate of $< 10 \text{ m}^2/\text{min}$ (Sterrett et al. 2018). Search rates may slow when habitats are complex, tactile methods are being used, and mussel densities are high. Because turbidity created by one observer upstream may influence the visual detection of observers downstream, all observers should remain at a similar transverse position during the survey (e.g., designated by an experienced leader keeping pace).

In softer sediments or systems with high turbidity, observers can feel into the sediment with their fingers to a depth up to 10 cm. In larger, harder sediments (e.g., cobble or boulder), use fingers to feel around and under larger stones for mussels that are not observed visually. At the end of the survey, record the time or survey time elapsed, the surveyed stream length and width, survey approach (A, B, or C), and survey gear used by each observer. The sampled width may not equal the stream channel width based on the survey approach taken (e.g., Survey B). If the study objectives are to target a specific mussel species, the detected mussel species of interest can be collected into a mesh bag or left in place and marked using a flag inserted into the substrate for subsequent processing. Collection and marking of mussel locations can vary based on survey objectives and species sensitivity to habitat conditions. If there is more than one target species, consider using different colored flags by species if collecting in mesh bags.

Record the counts by species for each lane. If a species is observed in large numbers, observers can roughly estimate their counts or provide an estimated range (e.g., 500-1000). Although a single count is preferable over an estimated range for subsequent cross-site analyses, precise counts of high-abundant species will significantly slow survey time. Note the presence or absence of any non-native species (e.g., *Corbicula fluminea* – Basket clam or *Dreissena polymorpha* - Zebra mussel) in the survey site (not lane-specific).

3.5 Mussel Processing

At the end of the survey, record the appropriate data for the survey objective. Any obvious shell anomalies and deformities should be noted and photographed (with a ruler as a reference). If Survey A or Survey B were followed, then the lane in which each mussel was captured should be recorded. If targeting specific species, individuals of target species should be sexed (if possible), measured for length (Figure 3), and have the shell condition recorded (Figure 3; Table 3). After processing mussels, all individuals should be returned to one of the flags along the respective lanes and “seeded” into the substrate in a natural, siphoning position with the posterior end of the shell pointing up (see Section 2.4). Be particularly careful with species of concern.

Identifiable shells and freshly dead mussels (i.e., soft tissue present) should be noted on the data sheet. If warranted, shells can be collected and appropriately cataloged in a reference collection. Label bags with the lead malacologist name, date, time, site coordinates, and species identification (if known). We ultimately recommend depositing specimens in a well-curated and funded natural history museum, state, or university collection. Note permits may be required to collect, retain, and deposit state- and federally listed species (see Section 2.4).

SPECIAL NOTE on species identification

Mussels can be challenging to identify, especially when similar-looking species overlap within the same watershed (Shea et al. 2011). Multiple morphological characteristics (i.e., shell

morphology, siphon appearance, foot color, etc.) should be used when identifying a species to ensure correct species identification. Make a note whenever unsure of species identifications. When unsure about identification, take representative photos (e.g., photos of both valves, and one dorsal view including a ruler for reference, see Figure 3) and record characteristics that may help identify it later by a malacologist. Note that a quality picture of the beak sculpture (umbo) can be critical for identification in some locations. Only sacrifice a mussel for identification if there is proper permitting and no shell material is available. Genetic swabs may be an additional tool to validate species identification but check with your local malacologist on feasibility and accessibility to genetic markers. If more help is needed for mussel identification, these resources can be used: American Museum of Natural History (n.d.) to learn about mussel features, Blais (n.d.) for Canadian species, Blakely (2020) for tips on identifying species, iNaturalist (2023) for artificial intelligence species identification, and U.S. Fish and Wildlife Service (2020) for understanding shell morphology. Many states have additional field guides; contact state agencies to see about available mussel identification resources.

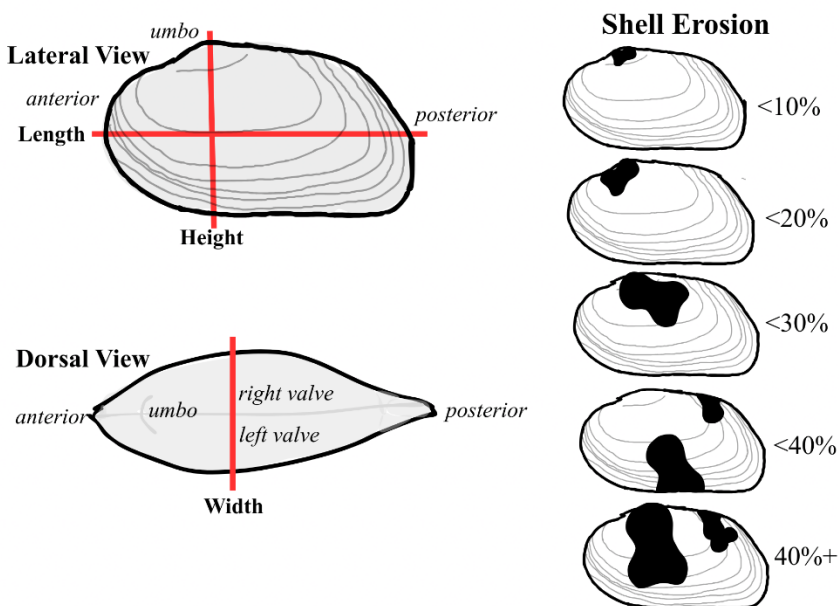


Figure 3. Measurements for length, height, and width of a freshwater mussel. Length is the maximum distance between the posterior and anterior margins of the shell. Height is the maximum distance between the dorsal and ventral margins of the shell. Width is the distance between the left and right valve aligned at the thickest part of the mussel. Record all measurements in mm. See Appendix D of Sterrett et al. (2018) for more information on shell condition.

Table 3. Live mussel shell condition scoring system ranging from good condition (1) to poor condition (5) based on percent erosion of the periostracum and depth of erosion (i.e., periostracum only, into prismatic layer or exposure of the nacre), which may reflect age or instream conditions (e.g., substrate roughness, pH, etc.). See Figure 3 for more information on shell erosion. Note: some areas of the shell (e.g., beak/umbo) may erode more rapidly than other locations on the shell. Table adapted from Sterrett et al. (2018).

Code	1	2	3	4	5
Erosion Description	Light	Light-Medium	Medium	Medium-Heavy	Heavy
Percent Periostracum Eroded	<10%	10–20%	20–30%	30–40%	40+%
Depth of Erosion	1 layer	1 layer	1–2 layers	1–2 layers	2+ layers

3.6 Mussel Survey Modifications

Modifications can be made to mussel surveys and processing methods to better suit project objectives. If a goal is to sample the entire stream width within a site, but the number of observers is less than stream width (~3 m lane width) consider increasing the lane width to >3 m or perform parallel surveys at 3 m lane widths until the entire width is sampled. Increasing observer lane widths may reduce the sample quality because of the need for an observer to search more habitat and consequently miss mussels. Another approach is to repeat surveys in additional parallel lanes with the same observer across the stream width (which is similar to performing Survey B iteratively across the entire stream width). This approach is less likely to reduce sampling quality but may take more time as opposed to increasing lane widths.

If a goal of the survey/project is to increase detection of seemingly low abundant/elusive species, more survey time can be added beyond 2 person-hours, and/or sites can be revisited. Depending on the study objectives and survey conditions, increasing survey effort may substantially increase the chances of species detection and capture an accurate representation of species composition at a site (Metcalf-Smith et al. 2000; Tiemann et al. 2009; Huang et al. 2011; Reid 2016). Additional survey time allows observers to sample more area and potentially detect a target species. This might be particularly useful when sampling wider streams (e.g., > 12 m) as 2 person-hours may only allow sampling over a relatively short length of stream (e.g., 10–20 m). Repeated surveys within a sampling season or sampling adjacent sections at a site may provide appropriate for additional modeling efforts (Appendix A).

Mussel processing can be expanded to include additional mussel measurements to meet survey objectives. In addition to shell dimensions (Figure 3), other measurements could include wet weight of the entire individual, age via external growth rings, and genetic information (Appendix A, B). When high numbers of mussels are resulting in long processing times, consider subsampling a random set of individuals up to a fixed number (e.g., 50 individuals).

4. Habitat Survey

4.1 Overview

Basic site conditions and habitat information may be helpful for interpreting mussel data, for example, quality/conditions during the survey may affect detection, while physical habitat and temperature may offer insights into why species were observed. Within this protocol, we describe physical habitat data that can be rapidly assessed within each search lane (lane-scale) and within each whole stream reach (reach-scale) to supplement the mussel survey (Table 4). We provide “core” habitat variables that are completed alongside mussel surveys as part of this protocol. In Appendix E, we provide habitat variables that could supplement the core habitat variables. Because habitat data collection takes additional time, careful consideration of the study scope, feasibility, and resources required can be taken and balanced against the desire for a rapid survey. Habitat surveys should take place after mussel surveys to avoid disturbing mussels prior to sampling.

Table 4. Rationale of core habitat variables collected in the rapid sampling protocol for wadeable streams.

Variable	Rationale
Photo & Sketch	Photos and sketches of the study site can be an efficient long-term documentation tool for sites. Make sure photos are labeled with coordinates and indicate whether facing upstream or downstream.
Survey Length	Survey length can be used to estimate survey effort and search rate.
Survey Wetted Width	Wetted width can help classify the size of the system. This description can provide insights on mussel habitat preferences, especially between large river and small river species.
Water Visibility	Turbid water can impair visibility when detecting mussels.
Weather	Cloudy, rainy weather can create dark conditions or increase the turbidity of water which can reduce mussel detection.
Percent Mesohabitat	Mussels may be more prominent in specific mesohabitats (Skorupa et al. 2024), and particular mesohabitats may also increase challenges for detecting mussels.
Riparian Buffer Zone	A larger natural buffer zone can help mitigate environmental land use stressors and bank erosion, protecting existing freshwater mussel habitat (Lu et al. 2024).
Canopy Cover	More canopy cover can cool streams in low-gradient systems (Coats & Jackson 2020), which may influence mussel occurrence given species-specific thermal preferences (Pandolfo et al. 2010; Ganser et al. 2015).
Substrate Type	Coarse substrates may inhibit mussel anchoring due to large and unstable interstitial spaces, whereas fine sediments may be unstable and reduce the mussels ability to maintain position (Haag 2012).
Aquatic Vegetation	More submergent and emergent aquatic vegetation can impair visibility during surveys while also serving as habitat for host fishes.
Algal Cover	The quantity of algal (periphyton) cover can modify the nutrient availability for mussels (Vaughn et al. 2008) while macroalgal cover may reduce visibility and alter local habitat (e.g., modifying flow) potentially affecting detectability and occurrence.
Water Depth	Deeper water can make snorkel surveys challenging and impart visibility.
Large Wood	Large wood may provide habitat refugia by modifying substrate and protecting mussels and host fishes from high flows.

4.2 Equipment

We outline the equipment needed to collect the core habitat variables in addition to the mussel survey (Table 5).

Table 5. Additional supplies needed for sampling habitat to supplement mussel surveys (Table 2).

Item	Quantity	Purpose
Measuring tape (100 m) or rangefinder	1	Measure survey length and wetted width
Meterstick or gravelometer	1	Size of substrate if needed
Meterstick or Secchi disk	1 per observer	Measure water depth and gauge visibility
Densiometer	1	Measure canopy cover
Camera or phone	1	Photo of site

4.3 Procedures

This section describes the procedure for the core habitat variables described in this protocol. Reach-scale variables consider the entire extent of the survey and lane-scale variables are specific for each observer lane. When collecting habitat data, aim to minimize stream disturbance as described in Section 2.

Reach-Scale:

1. Survey Site Diagram/Description/Pictures:

Record the site coordinates, photo orientation and date when a photo is captured. If desired, draw a diagram and write a description of the underwater conditions that cannot be captured with a photo. In the diagram, you can label where the photos were taken, and the ordination of the river, including mesohabitat, tributary junctions, large wood, and manmade structures.

2. Survey Length:

To measure survey length, use a measuring tape to record the total distance traveled in meters. Make sure the stream length accounts for stream meanders to the best of your ability. A rangefinder or aerial imagery may also be used but see note below (under Wetted Width).

3. Wetted Width:

The wetted width (m) of the survey site should be taken at a minimum of three representative locations along the searched survey area (e.g., start, middle, and end of survey). The wetted width is the extent of the river covered by water, perpendicular to the stream bank. If only part of the river width is surveyed (see section 3.4), take a measurement of survey width to determine total area surveyed in addition to the wetted width. If the stream is too wide for safe wading, then a range finder can be used to take measurements. *Note: be cautious when using rangefinders because many have lower and upper bounds for reliable measurements (e.g., < 8 m for lower and 350 m for upper, for many models). Make sure to verify the accuracy of range finders periodically throughout*

the field season. If the stream is too wide to take a measurement with tape and there are unreliable measurements from the range finder, an estimated measurement can be made (be sure to note if this is estimated in the datasheet) or the stream width could be measured with aerial imagery.

4. Water Visibility:

The visibility of the water should be assessed using the meter stick (if < 1 m) or a Secchi disk (if > 1 m) to assess survey visibility. If the bottom of the meter stick is not visible at 1 m depth, the surveyors may want to consider tactile methods or delaying this survey (if a system typically has low turbidity) because of the lack of visibility since it may influence detection of mussels.

5. Weather:

Record the weather (e.g., air temperature, cloud conditions, precipitation) at the time of the survey. Also note any major weather events (such as heavy rain) that occurred within 24 hours prior to the survey. In systems with usually low turbidity, heavy rainfall prior to a sampling event at the site or upstream of the site may increase the turbidity of the system. If there are rain and cloudy conditions at the time of the survey, the surveyors may choose to delay the survey due to lack of visibility or opt for a more tactile survey.

6. Percent of Mesohabitat:

The mesohabitat of the entire surveyed reach can be characterized visually by describing the percentage of each mesohabitat type (i.e., riffle, run, pool). Provide the approximate proportional area of each mesohabitat as it relates to the entire area searched. Mesohabitat description can also be supplemented with a drawing of the site to better describe the mesohabitat arrangement within the reach. When a species of concern is detected, document the mesohabitat it was present in. This can be completed at the end of the survey when processing the mussels that are marked with pin flags.

7. Riparian Buffer Zone:

The natural or forested riparian buffer zone of the entire surveyed reach may be assessed visually with the table below (Table 6). Record the riparian buffer zone measurements for both riverbanks in the area surveyed.

Table 6. Riparian buffer zone code index (from Barbour et al. 1999).

Code	Description
1	Width of riparian zone < 6 m and there is little to no riparian vegetation due to human activities
2	Width of riparian zone is 6–12 m and human activities have impacted zone a great deal
3	Width of riparian zone is 12–18 m and human activities have impacted zone only minimally
4	Width of riparian zone is > 18 m and human activities have not impacted zone

8. Water Temperature

A measurement of the water temperature may be taken using a calibrated thermometer at the time of the survey in the center of the stream channel. Make sure to note if the measurement was taken in Fahrenheit or Celsius.

9. Canopy Cover:

Canopy cover can be estimated with a modified densiometer using the 17-intersection method (Figure. 4; Fitzpatrick et al. 1998). Measurements should be taken in the center of the river channel in line with the start and finish locations of the mussel survey. If the system is wide, more measurements may be needed at the banks to better represent general canopy cover. Holding the densiometer at waist height, count the intersections (of 17 total) covered by canopy. Repeat this for four cardinal directions (upstream, downstream, river left and river right) for each location. If a densiometer is not available, satellite imagery may be used to estimate canopy cover; however, satellite imagery may not be as accurate as on-the-ground measurements.

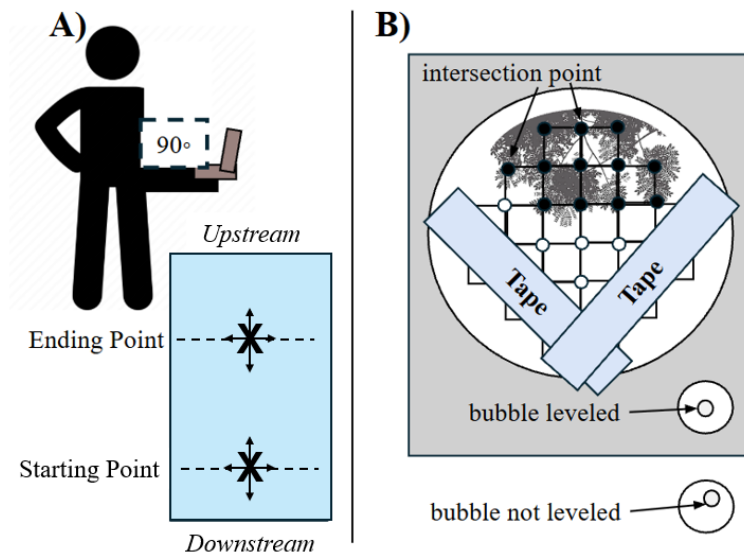


Figure 4. A modified concave spherical densiometer A) position for reading and stream location and B) diagram with bubble level and tape with 17 points of observation. Closed circles represent line intersections counted in measurement of canopy closure (i.e., 12 out of 17 points are covered; Fitzpatrick et al. 1998). Photo adapted from Sterrett et al. (2018).

Lane-Scale:

10. Substrate Type (Bed Texture):

Record the bed texture in each lane at five equidistant locations longitudinally along the reach or at each location of the mussel species of interest. In addition, record the dominant and subdominant particle size in the observer lane (Table 7; U.S. EPA 2013a). If interested in a more quantitative approach, the percentage of each substrate type can be estimated across the lanes or stream reach. If interested in the microhabitat use of a particular species of interest, consider recording the bed texture surrounding the mussel.

Table 7. Substrate code index. Extracted from Sterrett et al. (2018) and Wolman (1954).

Substrate Code	Size (mm)	Size Description
Smooth Bedrock (RS)	4000 +	Larger than a car
Rough Bedrock (RR)	4000 +	Larger than a car
Concrete (RC)	4000 +	Larger than a car
Large Boulder (XB)	1000–4000	Meter stick to car
Small Boulder (SB)	250–1000	Basketball to meter stick
Cobble (CB)	64–250	Tennis ball to basketball
Coarse Gravel (GC)	16–64	Marble to tennis ball
Fine Gravel (GF)	2–16	Ladybug to marble
Sand (SA)	0.06–2	Gritty - up to ladybug
Fines (FN)	silt/clay	Not gritty - up to ladybug
Hardpan (HP)		Firm and consolidated to fine substrate
Other (OT)		Define texture type in notes

11. Aquatic Vegetation and Algal Cover

Visually assess the percent of emergent vegetation, submerged vegetation and algal cover. For each searched lane, provide a percent cover of each vegetation type (emergent and submerged vegetation). When reporting algae, specify the algal type (e.g., macroalgae, diatoms, biofilm).

12. Water Depth

Measure the water depth of each lane at a minimum of five equidistant locations longitudinally along the reach with a meter stick or/and at the mussel's location.

13. Large Wood

Count the number of pieces of large wood (>10 cm diameter and >1.5 m in length). When large wood is found in clumps (i.e., root wad or large wood pile or snag), then these can be noted as the number of clumps per lane.

5. Data Management

5.1 Overview

Data management is an important step in scientific transparency and knowledge advancement. Proper data management can facilitate reproducibility and help ensure consistency across positional and project timelines. When striving for reproducibility in ecological research, there are multiple components that are addressed. These key components include 1) long-term data

storage, 2) understanding coding language, 3) avoiding jargon and undefined terms to facilitate reproducibility across state, federal, and academia collaborators, and 4) using data science tools to communicate findings. Here, we highlight data management practices that can help support these key components.

5.2 The Data Life Cycle

The data life cycle is composed of the preparation and the storage of data for a specific project (Rüegg et al. 2014). Data preparation transitions to data storage sometimes during a project or after all data collection. To ensure long-term preservation of data, all aspects of the life cycle should be considered (Figure 5). Several data preparation steps may only need to be completed one time for a project, but it is important to allocate effort to preserve the collected data at least once per field season.

Data preparation steps:

1. Plan: Prior to starting a project, plan where data will be stored and who will be the curator of the data. Identify if the curator will be an individual or an organization. Make sure the organization style is identified prior to any data collection. Planning ensures the researcher is prepared for any data management issues (i.e., how to label, organize, store).
2. Create: Aim to digitize all data collected immediately following data collection. Digitization of data includes taking a photo of the site, typing in entries on a computer and scanning/photographing the datasheet for backup. Consider quality control (i.e., checking data entries and photo labels by a second individual) in this step.
3. Process: A suitable subsequent analysis may be performed (see section 1.2 for applications of data collected in this protocol). All manipulated data should be in a copied file of the raw data - never manipulate raw data files.
4. Document: Explain metadata (description of data), data manipulations, and the context of the study (i.e., project aims) in this step. Additional notes may include reasons for missing data and explanation of code.

Data storage steps:

5. Preserve: Securely preserve all data and documentation to prevent loss. Likewise, data should routinely be backed up and stored in at least two locations (physical and electronic). These locations can include Microsoft (i.e., OneDrive, Dropbox) or Google (i.e., Google Drive) applications.
6. Share: Making data and code accessible can help preserve data but also improve the learning barrier to data science tools (see section 5.4). *There may be restrictions on data sharing for rare and endangered species. Make sure to check with your state malacologist prior to any data and code releases.*

7. Reuse: The goal of data management is to improve reproducibility, which can be achieved by following all aspects of the data life cycle (Wilson et al. 2017).

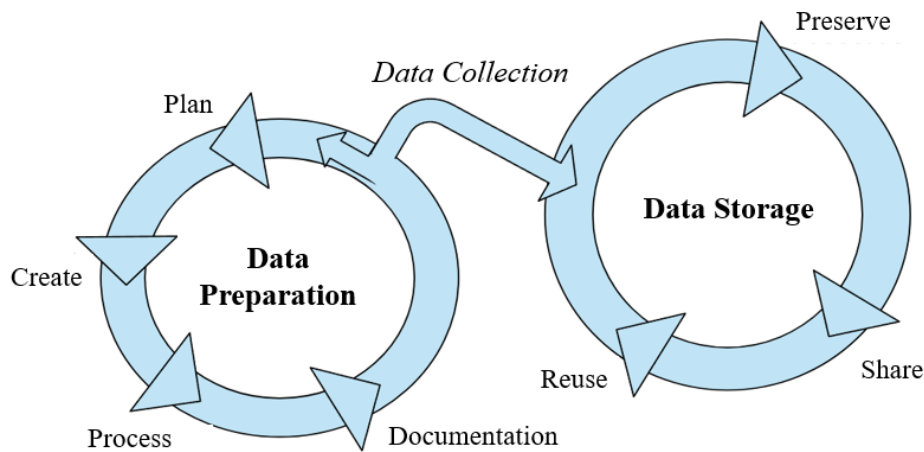


Figure 5. Diagram of data life cycle. Data preparation begins at ‘plan’ and moves in a counterclockwise rotation, repeating until the data is ready to be preserved which transitions to the data management cycle. Data management moves clockwise and repeats until more data is collected. Figure adapted from Rüegg et al. (2014).

5.3 Data Entry and Documentation

To promote scientific transparency and replication, it is important that data is entered and documented clearly. When entering data, there are few strategies that can help promote consistency and minimize entry errors. Consider having one person enter data and a second person check the data entry (or have the same person check their entry at a different time) to ensure accuracy of the data entries. Digitizing your dataset (i.e., taking a photo of the datasheet or entering the datasheet in a spreadsheet) immediately following sampling can minimize chance of data loss. Always report the sampling date, metadata and the contact person for the datasheet.

5.4 Coding Languages and Practice

There are several options for selecting an appropriate statistical software (e.g., Microsoft Excel, Python, ArcPro, etc.), but R is the most common and accessible free software for statistical models in ecology. Program R has the capability to run on a wide variety of UNIX platforms, Windows, and MacOS (R Core Team 2024). Program R works with RStudio to create a user-friendly coding environment. For example, if thinking about a computer, program R is the central processing unit (not many people are qualified to construct and edit this) and RStudio is the user interface (many people are capable of using a computer) (Posit Team 2025). Both programs can be downloaded from <https://www.r-project.org/>; and are needed to run complex analyses. Within R, users can download packages online to run more advanced analyses beyond base R’s computational power. These packages can be thought of as applications on the

computer. There are many great online resources that detail further coding practices (e.g., Sandve et al. 2013; Wilson et al. 2017; Turner et al. 2022; Pruim et al. 2023).

5.5 Data Science Tools and Artificial Intelligence (AI)

Code documentation is where all data manipulation is recorded, so the analysis can be reproducible and properly stored for future use. Here, we highlight a few data science tools to help improve project reproducibility and storage.

Git: Git and GitHub are compatible tools that can store code either publicly or privately. Git is a program that will track changes to code and allows researchers to work collaboratively. Git has a version control system that manages and keeps track of code, so that all manipulations can be tracked. GitHub is a place where researchers can build code together (if you work with GitHub, then you are working with Git in the background) and manage the Git repositories/folders (similar to a shared folder on Google Drive). Both tools are free to download (GitHub Inc 2026).

Bitbucket: Bitbucket is an additional code hosting and collaboration software built for teams. Any collaborative work is automatically stored on the cloud. An advantage of this software is its ability to incorporate AI; however, some of the tools provided by this program are behind a paywall (Bitbucket 2026).

RShiny: Shiny is an R tool used to build interactive web applications (apps) straight from R. Examples of Shiny Apps are interactive plots and maps that can be used to effectively share research findings in a digestible manner. RShiny can have a steep learning curve, so it is important to first identify what would be the most effective product for your findings (Chang et al. 2026).

AI (e.g., ChatGPT and Claude): It is important to understand the code that is being used in any statistical analysis; however, sometimes there is a learning barrier to coding. Claude and ChatGPT are free open AI softwares that can assist with coding. These chatbots can help to troubleshoot error messages or to reformat code (OpenAI 2026). While a potentially useful tool, there are several practical, ethical, and environmental issues one may consider prior to deploying AI. Large language models like ChatGPT are text generation systems, and do not actually understand your input: they can falsify information (Emsley 2023), fabricate or misrepresent references (Athaluri et al. 2023; Bhattacharyya et al. 2023; Salvagno et al. 2023), or “hallucinate” data (Park & Lee 2024). AI models are trained on existing data, often without the knowledge or consent of the owners (Gordijenko 2025), and there is little existing infrastructure for AI transparency, accountability, privacy, security, and trustworthiness (Reinhardt 2023; Gosh 2025). Due in part to the specialized hardware required for natural language processing and the growing size of training data sets (Li et al. 2016), energy demands for development of new AI models have been estimated as high as 626,155 pounds of CO₂ emissions (Strubell et al. 2019).

Water demands, for cooling data centers, are also substantial at nearly two billion liters/day in the United States alone (Mytton 2021; George et al. 2023). If you choose to use AI, tools like Code Carbon (Courty et al. 2025), Green Algorithms (Lannelongue et al. 2021), or ML CO2 Impact (Lacoste et al. 2019) can estimate the energy consumption and carbon footprint of a given AI model, which can help in evaluating costs and benefits (Ligozat et al. 2021).

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Freshwater Mussel Rapid Assessment Sampling Protocol for Wadeable Streams

Prepared by: Alexa M. Hershberger¹, Allison H. Roy², Peter D. Hazelton³, Ayla J. Skorupa⁴, Jason Carmignani⁵, Michael A. Baker³, Stefanie Farrington^{1,6}, and Sean Sterrett⁷

Affiliations:

¹ Organismic and Evolutionary Biology Program, Massachusetts Cooperative Fish and Wildlife Research Unit, University of Massachusetts - Amherst

² U.S. Geological Survey, Massachusetts Cooperative Fish and Wildlife Research Unit, Department of Environmental Conservation, University of Massachusetts - Amherst

³ Warnell School of Forestry and Natural Resources, University of Georgia

⁴ Illinois Natural History Survey, University of Illinois at Urbana-Champaign

⁵ Natural Heritage and Endangered Species Program, Massachusetts Division of Fisheries and Wildlife

⁶ U.S. Fish and Wildlife Service Richard Cronin Aquatic Resource Center

⁷ Biology Department, Monmouth University

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Appendix

Appendix A. Applications for Different Study Designs.

Overview

The protocol described in this document can be used for a wide variety of purposes, with each requiring unique sampling approaches, data collection, and analysis methods. Here, we provide descriptions of five potential applications to guide researchers in data collection following this protocol. Applications include: habitat assessment for mussel stocking, field scouting, species occurrence, species abundance, and site validation for eDNA. These applications typically apply to un-surveyed streams or streams with unknown current population statuses to provide information on the mussel community and habitat at a given site. This is not an all-inclusive list, and adaptations to these applications may be desired. Some of these applications (e.g., occupancy estimation, abundance using N-mixture models) require random site selection and spatial or temporal replicates; information is provided below to guide the reader since they are not required in this protocol. By following this protocol, this can provide standardized sampling effort across a larger geographic range.

Resident Mussel and Habitat Assessment for Stocking or Translocation

An initial assessment of stream habitat can be useful for identifying areas for mussel stocking and translocation. This protocol offers a rapid assessment of habitat that can be used for these purposes. For this application, follow the habitat survey methods (Section 4) and pair with a future in-depth mussel survey. It is important to confirm mussel status prior to stocking. Habitat variables at the site- and lane-level can be identified by the state malacologist or informed researcher. However, determining how to rank sites or define what is ‘high quality’ habitat for a species may require additional information.

Field Scouting

Field scouting (also referred to as site reconnaissance) can be used to identify sites with mussel populations of interest or suitable habitat for future sampling efforts. This study design could be paired with additional sampling (e.g., for assessing population abundance). The study area can expand from a single site to an entire state. Sites can either be randomly selected or intentionally selected within the study area. Sites not selected randomly can incorporate some aspects of randomness in future sampling efforts to avoid sampling biases in the analysis. Situations where field scouting may be advantageous include: 1) when conducting surveys prior to an anticipated disturbance, 2) when attempting to gain a better understanding of possible populations of mussels within historically unsampled streams and 3) when trying to determine if randomly selected sites should be surveyed prior to visiting with a larger crew.

When using this protocol for field scouting, the starting point for a mussel survey can be based on: 1) site accessibility, 2) study objectives, and 3) suitable habitat (if using non-random site selection). Surveys can follow Survey A, B, or C (see Section 3.4); depending on the number of observers. Survey effort is usually dependent on the area you want to cover. If multiple observers are used for the survey, observers may be randomly assigned to longitudinal lanes (parallel to stream bank) to minimize observer bias. Habitat does not need to be collected during this survey, since it is usually paired with a more in-depth future survey.

Species Occurrence (Presence/Absence)

Occupancy approaches allow for estimation of species occurrence within a spatial constraint while simultaneously estimating species detection probability (the probability of finding an organism if present). Occupancy estimation has been used with many wildlife taxa and is essential for understanding the presence or absence of wildlife in a particular area while accounting for imperfect detection (species is present but not detected, which is common for elusive species) (MacKenzie et al. 2002). Occupancy estimation operates on a robust probabilistic framework of randomly selected sites (see Appendix B) to infer what proportion of sites are occupied (Bailey et al. 2014; Sterrett et al. 2018). Sites need to be randomly selected within a spatial constraint, but the size of the spatial constraint can vary (e.g., watershed, stretch of river, etc.). Occupancy models may use temporal replicates (repeat through time) or spatial replicates (e.g., each observer lane; Charbonnel et al. 2014; Sterrett et al. 2018) to estimate detection. If using a single-season single-species occupancy model, data needs to be collected in one field season (other models are available for multi-season occupancy). Situations where occupancy modeling could be advantageous include: 1) estimating occurrence probability of a mussel species within a spatial constraint, 2) estimating the effects of reach- and landscape-scale habitat features on mussel occurrence, 3) understanding how habitat influences the detection of mussel species, and 4) estimating spread of non-native freshwater mussels. See Sterrett et al. (2018) for more detailed information on an occupancy approach to this methodology. If you are not able to have spatial or temporal replicates, naïve occupancy can be estimated within a geographic area without accounting for imperfect detection (while acknowledging that the estimate may be lower than true occupancy probability).

Another approach to characterize species occurrence without accounting for imperfect detection is species distribution modeling. These types of models estimate occurrence probability or habitat suitability, rather than detection probability. As such, they do not require multiple observer lanes and Survey C can be used (see Section 3.4). Site selection should be random or representative of the study area, to avoid overconfidence in the statistical model.

Species Abundance While Incorporating Detection

Predicting species abundance is an important tool for ecological and management planning. Incorporating environmental variables can be advantageous for estimating abundances across unsampled locations within a species distribution and better understanding spatial and temporal trends (Joseph et al. 2009). Count data is required when estimating species abundance. The decision to use this more intensive sampling approach can be weighed on 1) sampling time-constraints, 2) number of observers available, and 3) estimated detection of a species at a site. Abundance models, such as the N-mixture model, can account for imperfect detection and abundance simultaneously. By incorporating imperfect detection through spatial or temporal replicates, estimates can become stronger; however, it is at the expense of time and effort (Royle 2005). Additionally, the N-mixture model can also accommodate zero-inflated data (a common issue with rare species) for count data (Joseph et al. 2009). Although spatial replication using a minimum of three observers and Survey A or B (see Section 3.3) may provide an alternative way to obtain detection estimates, that would assume abundance is homogenous across spatial replicates which is not typical for patchily distributed mussels. Additionally, N-mixture models require random site selection if the goal is to estimate average abundance across the study area. If interested in estimating the effect of habitat on relative abundance without accounting for imperfect detection, regression-based models may be an appropriate approach.

Site Validation for eDNA

Environmental DNA (eDNA) has been used for rapid detection surveys for specific freshwater mussels species (i.e., single species eDNA assays) and community assemblages (i.e., metabarcoding) (Coghlan et al. 2021). If eDNA sampling (see Appendix B) results in detecting a species of interest, this protocol may be used as a tool to survey sites. In that case, the goal of this survey would be to 1) confirm species' presence, 2) estimate species densities, 3) locate specific mussel occurrences, and 4) collect other biological mussel data (e.g., buccal swabs, mantle clippings, shell-size frequency, etc.).

Since sites are already selected (eDNA was already collected at the site), observers can target suitable habitat to increase detection probability rather than using random site selection. Mussel snorkel surveys can be conducted at multiple points surrounding each eDNA hit to increase detection (as opposed to one long continuous survey fixed at 2 person hours). Surveys can include one observer or multiple observers depending on available personnel and site characteristics. If site validation is the goal of the survey, observers may choose not to incorporate habitat measurements (Section 4).

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Appendix B. Environmental DNA (eDNA) Considerations

Overview

Environmental DNA (eDNA) refers to the trace DNA found in environmental samples (water, soil, air, etc.). eDNA has been used for rapid detection surveys for specific freshwater mussel species (i.e., species-specific quantitative PCR assays) and mussel assemblages (i.e., metabarcoding or amplicon sequencing). Surveys collecting eDNA can be attractive as they have lower habitat disturbance, require less field effort, require fewer people, and may detect species not observed during visual mussel surveys. Here, we provide information on decisions related to eDNA sampling and processing methods.

qPCR vs Metabarcoding

Selection of eDNA methods may depend on survey objectives, existing data, and available resources. qPCR is generally faster and less expensive than metabarcoding but is limited in number of targets; up to four target taxa can be simultaneously assessed via multiplexing. Metabarcoding can include many species and taxa, which may be particularly beneficial for mussel population assessments as suitable host fishes could be included (e.g., Dokai et al., 2023, Mason et al., 2025); however, metabarcoding entails DNA sequencing. Most universities have laboratory equipment suitable for DNA extraction and may be able to provide qPCR and sequencing services; these services are also provided by a number of private companies.

Primers and Probes

Primers and probes are the genetic "fingerprints" that link the unknown DNA in a sample to a known species. Genetic sequence data of reference mussels may be needed if primers and probes have not already been developed for your species and gene region of interest (qPCR, e.g., Rodgers et al., 2020, Serb et al. 2003) or if the species of interest are not represented in the National Center for Biotechnology Information (NCBI) GenBank serbdatabase (metabarcoding; <https://www.ncbi.nlm.nih.gov/genbank/>). Developing and testing primers may require collection and sequencing of genetic samples (tissue clips, buccal swabs, or whole individuals) prior to primer development or bioinformatic analysis. Additionally, sequences may vary geographically, so incorporating reference samples from the target watershed or region may improve specificity. For qPCR, existing primers for unionids include CO1 (Currier et al. 2018; Deiner and Altermatt, 2014; LeBlanc et al. 2021; Preece et al. 2021; Rodgers et al. 2020; Schill and Galbraith 2019) and 16S gene regions (Stoeckle et al. 2016). For metabarcoding, primers exist for the CO1 (Cho et al. 2016; Dokai et al. 2023; Klymus et al. 2021), ND1 (Marshall et al. 2018; Klymus et al. 2021) and 16S gene regions (Coghlan et al. 2021; Prié et al. 2021).

Sample Collection and Processing

Collect water samples from approximately 10 cm above the substrate (Currier et al. 2018), being careful not to disturb the bottom or collect any sediment (Sakata et al. 2020). If collecting at multiple points within a reach, move from downstream to upstream to avoid disturbing or contaminating sites. Consider the size of HDPE bottles to use: larger bottles will increase likelihood of detection (Hunter et al. 2019) but will also increase space and filtration time needed. Generally, 2-3 replicates per site are recommended to increase probability of detection (Dickie et al. 2018). Field and lab blanks (i.e. bottled or tap water with minimal eDNA present) are collected and processed in concert with samples to identify areas of potential contamination. Samples must be stored on ice for transport from the field and kept frozen at 4°C

(Currier et al. 2018) or stored in 95% ethanol (Hinlo et al. 2017) until DNA filtration. It is important to clean all equipment with a 10% bleach solution between sites to avoid contamination (Coghlan et al. 2021).

Water Filtration

DNA is isolated from water samples by filtering through a filtration manifold. Water may be filtered in the field using a filtration backpack or in the lab; timing of filtration will affect the volume of samples and the time spent in the field. Clean the filtration apparatuses with an RNase/DNase decontaminant between samples. Running deionized water through the filtration system before and after filtering samples will reduce clogging. Following filtration, samples can be stored at -80°C for sample preservation until DNA extraction.

eDNA Limitations

Environmental DNA surveys are limited in their capability to detect densities or relative abundance, may provide only limited information on population genetics, provide no biological data (measurements, age, etc.), and do not indicate specific locations of individual occurrences (Cho et al., 2016; Ferreira-Rodríguez et al., 2019; Schill & Galbraith, 2019; Rodgers et al., 2020; Prié et al., 2021). There are also limitations to the methodology that need to be considered. For example, there may be a lack of eDNA primers for a particular species or interest, which would result in high costs to develop the appropriate primers. These primers may also be geographically limited and not account for species hybridization. The quantity of water filtered for eDNA samples may also be a limitation as more water may be needed to detect rarer species or species in larger systems and increased sample volume may introduce more ‘noise’ in samples (Hunter et al. 2019).

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Appendix C. Random Site Selection

Overview

Random site selection is often included in study designs to draw inferences about the entire study area. Specifically, this approach has benefits related to the following:

1. Statistical generalization/inference: By randomly selecting sites, measures of variability (e.g., variance or confidence intervals) can be appropriately computed.
2. Species distribution: Randomly selecting sites within a study area (for example in a watershed) captures the range of environmental conditions allows the researcher to understand the extent of a species' distribution and informs where the species is and is not observed.
3. Reduced selection bias: When biologists have a preconceived idea of where a species is present or the types of habitat it uses, populations can go unnoticed. Random site selection allows for the discovery of new populations by selecting areas biologists might never have chosen.
4. Occupancy/Abundance estimation: Similar to above, random site selection allows for inference about the proportion of the study area being occupied by the species or the average number of animals across sites. Detection probability can also be estimated during this process.
5. Habitat correlates: By randomly selecting sites, researchers can understand what habitat variables are affecting species presence (or potentially abundance/density) and can evaluate habitat suitability. Without random site selection, only habitat conditions where the species is present are generally assessed. This means limited inference can be drawn related to habitat suitability since there is no comparison made between the habitat where the species is present vs the habitat that is available but not occupied within the study area.
6. Risk metrics: Random site selection allows for the estimation of extinction risk at the scale of the study area as well as estimating site level extirpation/colonization rates.

Methods to obtain random sites

Several approaches can be used to obtain random sites within a study area. In some instances, pseudo-random site selection (e.g., limited to road-stream crossings) may be conducted for efficiency. Sterrett et al. (2018) describes the process to select bridge crossings by overlaying road and stream map layers. After compiling a list of all available crossings in the study area, a subset can be randomly selected for sampling. To prevent bias due to altered habitat conditions near the bridge, researchers can travel a fixed distance upstream from the bridge followed by an additional random distance (Sterrett et al. 2018). Since bridge crossings are generally not randomly distributed within a watershed, using them as starting locations may produce results that are not entirely representative of the study area.

Other approaches to random site selection can be employed that allow for study area-wide inference. For example, if the entirety of the stream system is easily accessible (due to property access or the availability of kayaks/canoes), sites could be randomly selected within the entire bounds of the stream system rather than using bridge crossings. If interested in a set number of sites per river mile, then number of sites (x) per river mile (y) can be randomly selected using a stratified sampling approach. This process could be done using available tools in a GIS program to delineate the entire stream system and randomly select points along the stream. A National

Hydrography Dataset (NHD) layer could be used to limit selection to unique stream segments or buffers could be placed around sites to prevent sites from being too close to one another. In cases where there is prior knowledge of heterogeneity in habitat across the study area and the researcher is interested in guaranteeing that sampling is conducted across habitat types, stratified random sampling could be used. To stratify sites, the stream system can be broken up into multiple blocks/strata (e.g. habitat types), and a set number of sites can be randomly selected from among each strata. Lastly, systematic sampling using a random start point is useful especially in linear systems containing environmental gradients (Strayer and Smith 2003). If particularly interested in habitat associations, an initial visit to classify habitat over a fixed area can be then categorized and subsets of habitat types can be randomly selected to survey for mussels.

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Appendix D. Additional Habitat Variables

Habitat variables that could supplement the rapid assessment protocol, including example methods and associated references. Relative effort reflects the resources required to collect the variable using the methods with the least effort: low (visual in the field or desktop analysis), moderate (minimal field equipment or handheld instrumentation), and high (sample collection requiring laboratory processing). Methods are listed in order from least effort (1) to most effort in the method column.

Characteristic	Variable	Purpose	Least Relative Effort	Method (reference)
Landscape Context	Proximity to anthropogenic impact	Human disturbance proximity	Low	1a. Distance measurement using Google Maps (Google Maps distance tool) 1b. Visual identification using Google Earth imagery (Google Earth Pro ruler tool) 2. Distance measurement using GIS (ArcGIS Pro spatial analysis tools)
Channel Morphology	Stream slope	Channel gradient controlling flow	Low	1. GIS slope calculation from digital elevation models (U.S. EPA 2023) 2. Clinometer and measured reach length (James et al. 2023) 3. Reach slope calculation from upstream/downstream elevations (Harrelson et al. 1994)
Channel Morphology	Bank slope	Bank stability and erosion potential	Moderate	1. Handheld clinometer measurement (Harrelson et al. 1994) 2. Stadia rod and auto level survey (USGS 2019) 3. Total station or differential GPS survey (Harrelson et al. 1994)

Channel Morphology	Bed texture	Substrate grain size composition	Low	1. Visual classification of percentage or dominant/subdominant substrate type (Lamberti & Hauer 2017) 2. Wolman pebble count (Wolman 1954) 3. Sediment core or grab samples for grain-size analysis (USGS 2019)
Channel Morphology	Rugosity	Streambed surface complexity	Moderate	1a. Chain-and-tape rugosity measurement (McCormick 1994) 1b. Cross-sectional bed profile survey (Harrelson et al. 1994)
Sediment Conditions	Sediment porosity / penetrability	Interstitial habitat availability	Moderate	1. Substrate penetration resistance (Geist & Auerswald 2007) 2. Sediment core sampling with laboratory porosity calculation (Lamberti & Hauer 2017)
Sediment Conditions	Suspended solids	Suspended particles affecting filtration	High	1. Water grab samples analyzed for total suspended solids (TSS) (U.S. EPA 1999)
Sediment Conditions	Sediment chemical analysis	Sediment contaminant exposure	High	1a. Sediment core collection with porewater extraction (U.S. EPA 2001) 1b. Sediment grab samples analyzed for nutrients or metals measurements (USGS 2019) 1c. In-situ porewater sampling (Lamberti & Hauer 2017)

Hydrology	Velocity	Local flow conditions	Moderate	1. Float method over known distance (Harrelson et al. 1994) 2. Handheld flow meter measurements (USGS 2019)
Hydrology	Discharge	Stream flow volume	Low	1. Discharge records from nearby stream Gauge (USGS n.d.) 2. Velocity–area method using flow meter (USGS 2019) 3. Stage–discharge rating curves (Harrelson et al. 1994)
Hydrology	Drainage area	To estimate the size of the watershed (note HUC level)	Low	1. Shapefiles can be downloaded from state or federal repositories (USGS n.d.)
Water Chemistry	Water quality (pH, conductivity)	Physicochemical water conditions	Moderate	1. Handheld field meters (U.S. EPA 1983) 2. Multiparameter water quality sonde (USGS 2019) 3. Laboratory analysis (American Public Health Association 2026)
Water Chemistry	Water quality (chemicals)	Dissolved contaminant exposure	High	1. Grab samples analyzed for metals and ions (Shelton 1994) 2. Organic contaminant analysis (American Public Health Association 2026)
Water Chemistry	Water quality (nutrients)	Suspended food resources	High	1. Nutrient analysis (nitrate, ammonia, phosphate) (Hach 2006)
Water Chemistry	Dissolved contaminant exposure	Dissolved organic matter indicator	Low	1. Visual comparison using color standards (USDA 2009) 2. Colorimeter measurement (U.S. EPA 1983)

Food Availability	Chlorophyll a	Algal food availability	Moderate	1. In-situ fluorometer probe (see specific equipment manual (e.g., YSI)) 2. Grab water samples filtered and analyzed via laboratory extraction (U.S. EPA 2013b) 3. Periphyton scraping from substrate and chlorophyll extraction (Lamberti & Hauer 2017)
Food Availability	Fine particulate organic matter	Suspended organic food resources	High	1. Water grab samples filtered and analyzed for ash-free dry mass (Lamberti & Hauer 2017)
Rapid Habitat Assessment	EPA Rapid Bioassessment	Qualitative stream habitat assessment	Low	1. Visual habitat scoring following RBP Protocol (Barbour et al. 1999)
Climate Variables	Precipitation	Estimate total rainfall 24 hours prior to survey	Low	1. Local weather application 2. WorldClim Database (Fick and Hijmans 2017)
Climate Variables	Land use	Percentage of impervious cover, agriculture, etc.	Low	1. State ArcGIS repositories 2. WorldClim Database (Fick and Hijmans 2017)

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Site ID:		State:		Waterbody Name:												
Date		Site Latitude		Riparian Buffer Zone										Time <u>mussel</u> survey start:		
Water Visibility:		<i>Upstream:</i>		<i>Left bank</i> 1 2 3 4										Time <u>mussel</u> survey end:		
		<i>Downstream:</i>		<i>Right bank</i> 1 2 3 4												
Weather		Site Longitude		Notes										Personnel (years of experience)		
		<i>Upstream:</i>														
		<i>Downstream:</i>														
Wetted Width (m)		Canopy Cover (17 = max)		Mesohabitat (%)										Measured Length of Survey (m)		
1.		<i>Upstream</i>		<i>Downstream</i>		Riffle		Run		Pool						
2.		US:		US:												
3.		DS:		DS:												
Air Temp (F or C)		Water Temp (F or C)		RR:		RR:		Notes								
				RL:		RL:										
Photo ID																
Lane #	Observer	Search Method	Water Depth (cm)					Substrate					Submergent Vegetation (%)	Emergent Vegetation (%)	Algal Cover (%)	Large Wood (count)
			1	2	3	4	5	1	2	3	4	5				
1																
2																
3																
4																
5																
6																
7																
Search Method (by lane):			Substrate Code:										Riparian Buffer Code (Barbour et al. 1999):			
S = Snorkel			RS = Rough bedrock (4000 mm+)										1 = Width of riparian zone < 6m and there is little to no riparian vegetation due to human activities 2 = Width of riparian zone is 6-12m and human activities have impacted zone a great deal 3 = Width of riparian zone is 12-18m and human activities have impacted zone only minimally 4 = Width of riparian zone is > 18m and human activities have not impacted zone)			
B = View bucket			RR = Smooth bedrock (4000 mm+)													
S/B = Both Methods			RC = Concrete (4000 mm+)													
V = Visual (no snorkel or view bucket)			XB = Large boulder (1000-4000 mm)													
O = Other (explain in notes)			SB = Small boulder (250 – 1000 mm)													
			CB = Cobble (64 – 250 mm)													
			GC = Coarse gravel (16-64 mm)													
			GF = Fine gravel (2 – 16 mm)													
			SA = Sand (0.06 – 2 mm)													
			FN = Silt/clay (not gritty)													
			HP = Hardpan													
			OT = Other (explain in notes)													

Mussel Survey and Habitat Data Sheet pg. 2 of ____

Site ID:	State:	Waterbody Name:
Site Description (Specify if near a USGS gauge – record gauge height and discharge)		
Site Diagram (This is a space to draw a diagram of the stream that illustrates features that may be important, not appear in photos, or is noteworthy for mussels. It may be helpful to label upstream/downstream, mesohabitat, roads/bridges, and tributary junctions.)		

