



Standard operating protocol for mark and recapture monitoring of Brook Floater in streams

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A marked Brook Floater from the Nissitissit River, Massachusetts (photo by Peter Hazelton)

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The Brook Floater Working Group (BFWG) is a collection of managers and scientists from federal and state agencies and academic institutions, who specialize in mussel ecology and conservation and are specifically working on the conservation of Brook Floater (*Alasmidonta varicosa*) across its range. We thank all the past and present working group members for their contributions to the development of this protocol.

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I. Introduction

The Brook Floater (*Alasmodonta varicosa*) is a small (<100 mm) freshwater mussel (Family: Unionidae) found in streams of the eastern United States (U.S.) (Nedean 2008). While there has been limited effort to document the status of Brook Floater across its range, there is evidence of Brook Floater range contraction and declining local abundances over recent decades (Wicklow et al. 2017, NatureServe 2021). Brook Floater is a Species of Greatest Conservation Need (SGCN) in 15 states (94% of range); listed as endangered, threatened, or special concern in nearly every state and province where it still occurs; and has been extirpated from two states (Rhode Island and Delaware). Brook Floater was petitioned for Federal listing under the U.S. Endangered Species Act; however, the listing was determined not to be warranted (U.S. FWS 2019), although it remains a Regional SGCN of very high concern in U.S. Fish & Wildlife Service (U.S. FWS) Regions 5 (Terwilliger 2015) and 4 (SEAFWA-WDC 2019) and is an At-Risk Species in U.S. FWS Region 5.

A critical component of understanding population declines is site-specific information about population density and demographics (e.g., growth, age structure) to assess population viability. This information had previously only been collected for a few populations of Brook Floater (e.g., Massachusetts Division of Fisheries & Wildlife, North Carolina Wildlife Resources Commission) and methods to collect these data varied from state to state, thus limiting comparisons across the range. In 2016, a competitive State Wildlife Grant (SWG) was awarded to develop a standardized monitoring technique that will aid in understanding differences in population viability across its range and assess changes in populations through time. The protocol described in this report was subsequently developed and tested by Massachusetts and Maine (2 sites in each state) and revised based on field experiences. Data collected using this protocol will allow for state managers to make informed decisions about management actions for Brook Floater.

Monitoring approaches are ideally designed to meet management objectives. Management objectives are specific, quantifiable outcomes that reflect the values of the decision makers and relate directly to the management decisions (Conroy and Peterson 2013). Lack of well-defined objectives hinders success of conservation and management actions because there are undefined metrics to determine when the objectives have been met (Yoccoz et al. 2001, Nichols and Thompson 2006). While monitoring to understand a system (i.e., status and trends; Reynolds et al. 2016) provides baseline information for developing management recommendations in the future, Nichols and Thompson (2006) criticize status and trends monitoring because of time lags associated with conservation and the costs and resource availability needed for surveillance, among other reasons. State partners in the Brook Floater SWG have a variety of different monitoring objectives (e.g., abundance/density, survival, recruitment) that depend on the population sizes and demographics.

There are many approaches for estimating population parameters such as density, age structure, recruitment, and growth rates. For example, presence/absence (i.e., multi-state models), counts (i.e., multi-state models or Dail-Madsen model; Dail and Madsen 2011), and capture mark-recapture (CMR; e.g. Cormack-Jolly-Seber models; Lindberg and Rexstad 2002) are all approaches for assessing population status and viability.

CMR is among the most common methods to monitor population states and demography. Several research studies have used CMR for freshwater mussels in streams and rivers (e.g., Peterson et al. 2011, Wisniewski et al. 2013). For example, studies have been conducted to estimate survival and temporary immigration/emigration in large rivers (Meador et al. 2011) and to assess effects of stream flows on survival, recruitment, and immigration/emigration (Wisniewski et al. 2016). These studies, along with general documents describing approaches in animal populations (Williams et al. 2002), provide valuable information on numerous modeling approaches available to estimate population parameters if appropriate data have been collected in the field. We refer readers to these and other sources to understand when to use CMR most effectively and how to analyze CMR data.

Objective

The objective of this protocol is to develop a coordinated, standardized, monitoring approach for Brook Floater to estimate population status and trends and demographic rates that allow for comparisons among populations throughout its range. As part of the Brook Floater SWG, we aimed to collect data to compare demographic data among locations with different densities and habitat conditions and understand why there may be differences in demographic stability. As such, the protocol includes methods for collecting ancillary data for assessing detection and addressing questions about habitat. This protocol may also be adapted to address a variety of research questions that require population and demographic data (e.g., population size, emigration, survival, etc.) and supplemental habitat or water quality data that may be collected to address additional hypotheses about the species' sensitivity to environmental stressors. Furthermore, while this protocol was developed for Brook Floater, many aspects of this monitoring approach are likely applicable to mark and recapture of other stream-dwelling freshwater mussel species.

The protocol describes methods that can be used in a variety of habitats (e.g., small streams and large rivers), at sites with different population densities, and for a range of number of personnel with various levels of experience. We aim to be clear about where the protocols are flexible and may vary depending on resources, including the area sampled, the number of times sampling occurs per year, and the method for processing mussels. We also identify a few methods that are optional depending on the goals of the study. That said, there are many critical components of the protocol that are needed for meeting the assumptions of CMR (Appendix A), including visiting a site at least two times per year (for resampling of what is assumed to be a closed population), marking all individuals captured, and returning mussels to the same location (although mixing is allowed) (Williams et al. 2002, Powell and Gale 2015). Careful attention to what is required and what is flexible or optional is advised to ensure the data are useable.

II. Personnel Needs and Requirements

Personnel assigned to lead surveys for this monitoring project should have sufficient experience and knowledge of mussel ecology, identification, and field methods. In this protocol, we recommend that there are ≥ 3 observers on a survey, of which at least one observer has extensive experience (i.e., minimum of 5-10 years) sampling mussels and

specifically Brook Floater. Surveyor experience in identifying mussels will affect workflows (Appendix B); where there are surveyors who do not have experience identifying Brook Floater, mussels must be collected and identified by an experienced malacologist, whereas experienced surveyors may flag target mussels as they survey. Observers not familiar with mussel assemblages that overlap with Brook Floater should work closely with malacologists that have this experience and may more appropriately be used to help with data recording, measuring animals, and collecting habitat information. All observers should also be familiar with guidelines and safety procedures for safe care and handling of all mussels, particularly sensitive species like Brook Floater.

III. Sampling Design

Overview

To monitor population status and dynamics, this proposed sampling approach will employ visual and tactile surveys (i.e., surface searching with hands but no extensive excavation) using multiple observers at a specified number of sites across years. Mussel sampling is intended to use mask and snorkel, thus streams should be <1-m deep; however, this protocol could be adapted for deeper sites requiring SCUBA. Target species, including Brook Floater, will be measured, marked, and returned to the population. Certain site-level environmental covariates will be collected for each population. It is inevitable that variations in study design exist across the range of Brook Floater, which may create potential confounding factors when comparing population parameters. To account for variation in monitoring approaches across the range, auxiliary metadata covariates will be collected.

Study objectives

Prior to using this protocol, it is critical to define study objectives. A clearly defined problem or question with well-defined objectives that align with the need ensures that the approach is useful and the data collected will directly address the needs. The four typical uses of biological monitoring include: status and trends evaluation, threshold assessment, management effectiveness evaluation, and adaptive management (Reynolds et al. 2016). For example, a question assessing the effectiveness of a dam removal may have objectives of maximizing mussel recruitment and increasing the total population size. Here, information on age structure and sex ratios through time (before and after removal) is needed to address the objectives. If the goal is also to understand the mechanism of dam removal effects on mussels, then additional information may be needed to assess hypotheses (e.g., increased numbers of host fish). Specifying objectives is also useful to determine whether CMR is the best approach to address the questions and meet management goals. For example, questions related solely to population estimates may be better suited for other approaches that more efficiently estimate population densities.

Site selection

The location of monitoring sites will depend on several factors, including objectives for monitoring, prior knowledge of Brook Floater populations, and access to the site, among others. For example, if primarily interested in the population size within a given reach over time, the site should be placed where there are adequate mussels (e.g., >50) to

increase power in detecting a change in the population. If interested in the differences of population growth and survival across different habitats at multiple sites, then sites should be selected based on habitat and adequate replication will be needed in each habitat type (e.g., 3 sites in riffles, 3 sites in pools, 3 sites in runs). Multiple sites will be needed to compare differences in habitat characteristics and may require additional survey sites in multiple streams. If a site is selected based on prior knowledge and an interest in monitoring a particular population, then inferences of Brook Floater populations will be limited to that population.

Sampling area

A sampling area is the minimum area sampled to make inference about a population. The sampling area should be large enough to encompass enough animals to facilitate estimation (i.e., it is hard to estimate population parameters with <10 animals captured) while being small enough to allow for sampling the population in a day or a certain period of time in which the population can be considered closed (i.e., to immigration, emigration, mortality, and recruitment (e.g., <1 week)). There is no rule of thumb for how large a population should be (or what the recapture rate needs to be) to be included in monitoring for population demographics; however, we suggest that sampling areas have at minimum 50-100 individuals. Thus, the size of the sampling area will depend on the local density of Brook Floater, the stream size, and personnel/resources. High mussel densities (e.g., 1 mussel/m²) combined with limited resources may make CMR challenging in some situations. Most CMR approaches (e.g., Cormack-Jolly-Seber) require that the sampling area remains constant through time.

Frequency and timing of surveys

We recommend that long-term monitoring sites are visited a minimum of two times per year and for a minimum of three consecutive years. Two visits per year are necessary for resampling of what is assumed to be a closed population (Strayer and Smith 2003), and at least three years is needed to estimate population parameters (e.g., survival, abundance) using CMR. If time and resources allow, additional sampling within a year will increase the number of marked individuals and thereby reduce the number of years needed to get good population estimates; however, additional sampling must be balanced against concerns for disturbing the population. More years of sampling may be needed to reduce variability around population estimates (e.g., recruitment rates) if annual recapture rates are low (Strayer and Smith 2003). For monitoring and estimation, sampling during the same time period each year reduces some confounding factors, such as vulnerability to capture or seasonal differences in vertical movement and detection (Watters et al. 2001), which may affect estimates of mortality, immigration, and emigration of the Brook Floater population. Surveys should take place within the same three-month period each year (i.e., April-July for southeastern states; June-September for northeastern states), when possible, during periods with lowest flows and warmest temperatures which will maximize mussel detection (Clayton et al. 2015, MANHESP 2013).

Stream conditions and guidelines for sampling

Best judgement should be used for sampling populations during and following a large rain event. High discharge can both reduce the detection of mussels (i.e., increased

turbidity, higher depth, hard for surveyors to keep position on the bottom) and perhaps decrease the safety of mussels being returned to sediment following capture and processing. We suggest only attempting a survey if there is 1 m of visibility, which can be measured using a secchi disc or meter stick, and when depths are <1 m. Higher flow rates may further reduce an observer's ability to concentrate and detect mussels on the surface and should be avoided (Clayton et al. 2015), thus waiting a week following high flows will allow mussels to resurface. Sampling during consistent, low flow stream conditions also helps to standardize methods, including habitat data collection.

IV. Field Methods and Processing of Collected Materials

A. Sampling area delineation

Overview

Site selection and delineation will be reflective of monitoring objectives (see Section III); thus, specific guidance on site selection is not included in this protocol. Each sampling area will be surveyed using CMR through time and therefore each sampling area must be a permanent study site that is accessible and discoverable (i.e., marked and able to revisit) in the future. Any relevant details related to the persistence of the site as a sampling location (i.e., public or private access, large scale modifications such as dam removal) should be considered, anticipated, and noted.

Equipment needed:

- Flagging
- Measuring tape (100 m)
- Mallet
- Stake or other metal marker, painted neon for visibility
- Geographic positioning system (GPS) unit
- Digital camera

Procedure

The upper and lower bounds of the sampling area should be marked with flagging (for visual relocation) and metal stakes (for relocation with a metal detector). If the entire width of the stream is not sampled (e.g., pools or thalweg are too deep to sample efficiently, or would result in too large of a sample area), the distance from the banks should be marked. Marking with a metal stake may occur after you determine the extent of the mussel population you will sample. A detailed map and description of the study site and access locations is helpful. Additionally, a GPS point of the upper and lower bounds of the site should be taken for being able to return to the sampling plot if other markers fail.

A photo monitoring point should be established on the site as standardized point where a photo is taken at each visit. The photo monitoring point should be marked with a metal stake and placed in an appropriate location to photograph the entire sampling area.

The site should be sub-divided into 10-m “bands” for searching (Figure 1). Bands and lanes are helpful to ensure that the area is fully searched, and to keep track of where

mussels should be returned. These sub-divisions may be marked for consistency among sampling events or re-established at each sampling occasion.

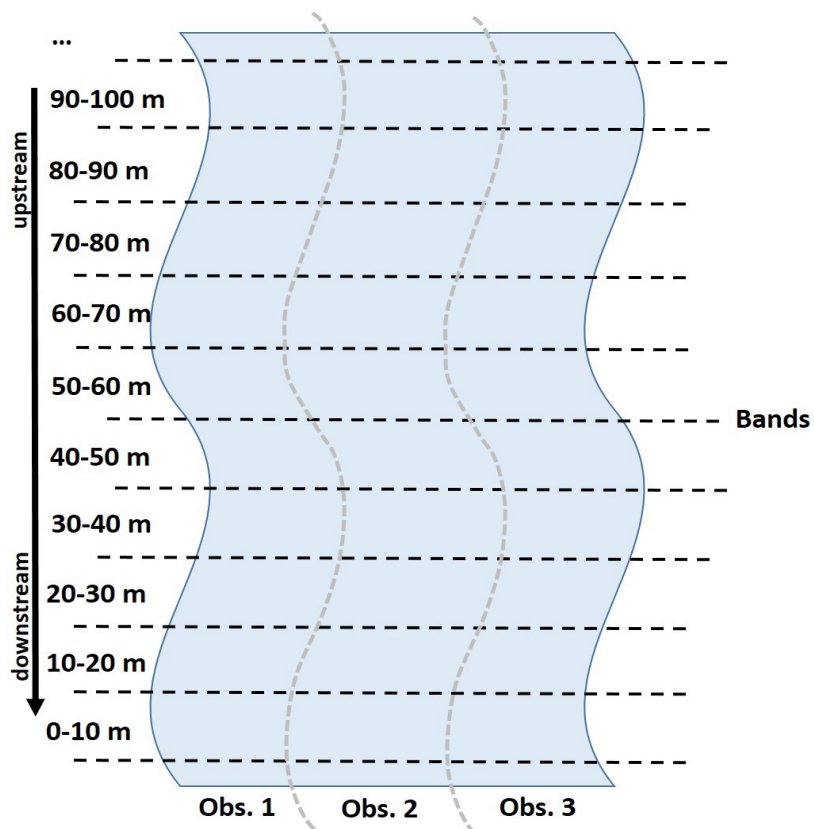


Figure 1. A diagram illustrating the set up for a Brook Floater long-term monitoring site, which is divided into 10-m bands and longitudinally into lanes which each observer searches. This hypothetical site is 100-m long.

B. Mussel survey, processing, and relic shell collection

Overview

Mussel surveys will consist of collecting, identifying, measuring, and marking newly found mussels, recording and measuring recaptured animals (for repeat visits), and collecting and documenting any relic shells of target species.

Equipment needed:

- Mask and snorkel for each observer
- Wetsuits or other clothing for snorkeling for each observer
- Mesh bags for each observer
- Belts (for holding bags) for each observer
- Bucket viewers (at least two)
- Device used to mark the location of individual mussels removed from substrate for marking. This could be pin flags, color-coated washers or washers with flagging attached.

- Geographic positioning system (GPS)
- Flow-through baskets to hold mussels instream
- Folding work table and chairs, if desired
- Small calipers (metric)
- Metric ruler (10 cm)
- Camera
- Waterproof data forms, clipboard and waterproof ink or pencils
- Towel or other tool for drying the shell
- Forceps
- Glue/epoxy
- Accelerator spray (optional)
- Tag of choice (conventional alpha-numeric or PIT tags)
- PIT reader (if using PIT tags; Appendix C)
- Ziploc bags
- Ethanol (95%)
- Sharpie
- Labels made of rite in the rain paper
- Field data sheets (see Appendices)

Mussel survey

Mussel surveys will consist of complete coverage, single pass surveys using snorkeling, viewer buckets, or SCUBA. Sites should be surveyed using multiple observers divided into appropriate sub-areas within the sampling unit. The allocation of lanes to surveyors should be randomized, especially if surveyor experience varies among individuals. Constraining the amount of space a surveyor searches ensures full coverage of the site, while maximizing detection probability. Surveyors will search stream sites following longitudinal (i.e., parallel with flow) lanes (Figure 1). Surveys should begin from the downstream extent of the site and move upstream to minimize turbidity in the area being searched. To ensure complete coverage, we recommend a maximum of 10-m wide lanes. Limiting the number of people in the stream during a survey should be considered to minimize disturbance to the site. While we leave it to the discretion of the team leader for exact methods, ultimately, surveyors should weave through their designated lane searching in all habitat types available to find any target mussel species. When a survey begins, the time should be noted and the team leader should designate a surveyor on the team that will set the pace for search, with the goal of a minimum search rate of 10 m²/min (Smith et al. 2001).

When a Brook Floater is found, a flag, coated washer, or flagging tape should be placed where the mussel was found to allow for returning to the location to collect habitat information and process the mussel (if they are not being bagged). Whether mussels are left in place or bagged for processing will depend on the size of the survey crew and the density of mussels. Flagging the mussel in place (Scenario A) allows a designated data collector to follow the survey crew and collect data and tag mussels independently. Bagging the mussels and enumerating/tagging after completion of mussel search surveys (Scenario B) may be preferred in large systems or where density is high. In the latter case, individual

mussels may not be returned to the exact location as collected, but effort should be made to return them to the same lane/band where they were found. Workflow scenarios for consideration are presented in Appendix B. If using flagging tape, we recommend a long flagging (e.g., 12-18 in) to maximize visibility. If feasible, the capture location of individual mussels should be noted with a GPS location and written description.

In all visits except the initial visit, a survey of an additional small section adjacent to the survey reach (e.g., 20 m upstream, downstream, and laterally, as appropriate) is useful to determine if marked mussels have made short-distance movements. Any marked individuals found can be used to inform the modeling in terms of determining whether the loss was mortality vs. emigration, but should not be directly included in the capture data. New mussels found in these locations should also not be included in the capture data.

Mussel processing

All Brook Floater should be measured for length and height (Appendix D), sexed (if possible), and the shell condition of each Brook Floater (and other target species) should be noted (Appendix E). While it is up to the discretion of the survey leader, we recommend also recording width of each mussel or a subset of all target mussels. While shell length is an important corollary for mussel age, shell wear may also aid in determination of age or environmental condition. Strayer (1993) showed that Brook Floater presence was significantly correlated with site calcium. As such, differences in shell wear across sites may be associated with pH and shell dissolution. If you are doing multiple surveys in a year, it is only necessary to measure an individual once annually. However, if interested in estimating individual growth rates, measurement of small individuals (e.g., < 40 mm) at multiple timepoints may allow these calculations. Any obvious shell anomalies & deformities should be noted and photographed (with a ruler as reference). After morphology data are collected, Brook Floater should be marked using one of the approaches below. For common species that are not targeted, species abundance should be recorded using abundance categories. Field sheets are provided for recording site metadata (Appendix F), mussel size and condition (Appendix G), mussel sampling effort (Appendix H), individual mussel locations (optional, Appendix I), and a site map (Appendix J).

Marking

All Brook Floater (and other target species of interest) of appropriate size (>20 mm) should be marked using conventional alpha-numeric tags (e.g., Hallprint™, FLOY® shellfish tags). Tags should be placed on both valves to estimate tag loss, thus addressing one of the major assumptions of CMR, which is that “marks are neither lost nor overlooked, and are recorded correctly” (Lindberg and Rexstad. 2002, Williams et al. 2002; Appendix A). The areas of the shell where the tags will be placed should be scrubbed to remove any exterior biofilms that would reduce the chances of the tag having a solid seal on the surface of the mussel. Tags should be properly attached using guidance from the adhesive brand (Table 1) and advice from literature supporting this method (Lemarie et al. 2000; Appendix C). Record the tag ID number for each valve on the data sheet (Appendix G) and make note of the tag type (i.e., conventional), manufacturer (e.g., Biomark®, Hallprint™), and adhesive brand (Table 1). In the case of highly eroded shells, it is possible to lay down adhesive on the shell to act as a base for the tag. Tag placement may be modified when a shell is eroded.

While the detection of small individuals (<20 mm) is low, alternative marking methods (nail polish, paint pens, glitter) or cut tags (half on each valve) may be used to mark these individuals. While growth is extremely important information for small animals, use best judgement when making the decision to tag a small mussel or one with heavy erosion to avoid harming the animal.

Table 1. Examples of commonly used adhesives for conventional marking of freshwater mussels.

Brand	Unit	Per Bottle Cost	Notes
Loctite® super glue gel	0.14 oz	\$2.50	
Loctite® flashcure 4307 Light cure	1 oz	\$55.00	Requires UV LED light for curing (~\$21)
Zapagap® Medium CA+	1 oz	\$8.00	May require additional flexy-tips for adhesive application

NOTE – Adhesives for conventional tags

Most adhesives are best used when the mussel is slightly wet and tagged mussels may be returned to their original location before the adhesive has completely cured.

Returning mussels to stream

After processing mussels, all individuals should be placed back into the stream in a natural, siphoning position with the posterior end of the shell pointing up, making note to record if you are 1) replacing individuals back at their exact locations, 2) mixing the population by returning individuals to approximate locations or 3) mixing individuals into the population by purposely returning mussels to locations other than their approximate collection location.

Resampling mussels

The approach to resampling a population will be similar to the initial survey. Following an initial sampling, the observers will repeat the monitoring procedure. Any targeted individuals found that are unmarked should be tagged and associated mussel, tag, and habitat information recorded. For already-marked individuals, record tag numbers and tag loss (if applicable), then replace lost tags and record new tag numbers.

NOTE - Sensitivity of species

Due to the sensitivity of Brook Floater to handling, every effort should be made to reduce handling time (handling, measuring, and marking time) and time out of water. Efforts should be made to ensure mussels are not out of the water for an extended period of time (i.e., >3 minutes) between capture, marking, and release.

Collecting relic shells or recently dead individuals of target species

Relic shells of potential target species that are in a condition that allows them to be identified should be noted, collected, and transported to a state agency with jurisdiction

over freshwater mussels. Any tissues from dead individuals may be useful for genetic or other biological tissue sampling that would otherwise be challenging to attain. If any shells or dead individuals include previously marked individuals, the marks and final measurements of shells should be recorded and the fate of the individual noted. Bags should be labelled with the date, time, site, and potential species of interest.

Optional: Passive Integrated Transponder (PIT) tags

An alternative marking technique is placing a conventional tag on one valve and a PIT tag on the other (Appendix A). PIT tags allow mussels to be located and identified without snorkeling, but require different equipment and expertise and are still time consuming (Appendix C). Furthermore, PIT tagging does not eliminate the need for snorkel surveys given the potential for tag loss and to get biological data (e.g., mussel size). PIT tags can be useful when populations are small and recapture using snorkeling is low (Kurth et al. 2007), but PIT tagging large aggregations of mussels may result in interference and compromise sampling effectiveness (Appendix C).

PIT tags should be placed on the right valve (or stay consistent within your program) using guidance from the adhesive and advice from literature supporting this method (Figure 2). After the adhesive is semi-dry, add another layer of cyanoacrylate glue on top of the tag and spray once with accelerator. If you choose to use an alternative tagging procedure, make sure to remain consistent and note the process. If methods for PIT tagging are not in place with your monitoring program, we recommend following suggestions from Ashton et al. (2017) for general procedure and adhesive type (Appendix A).

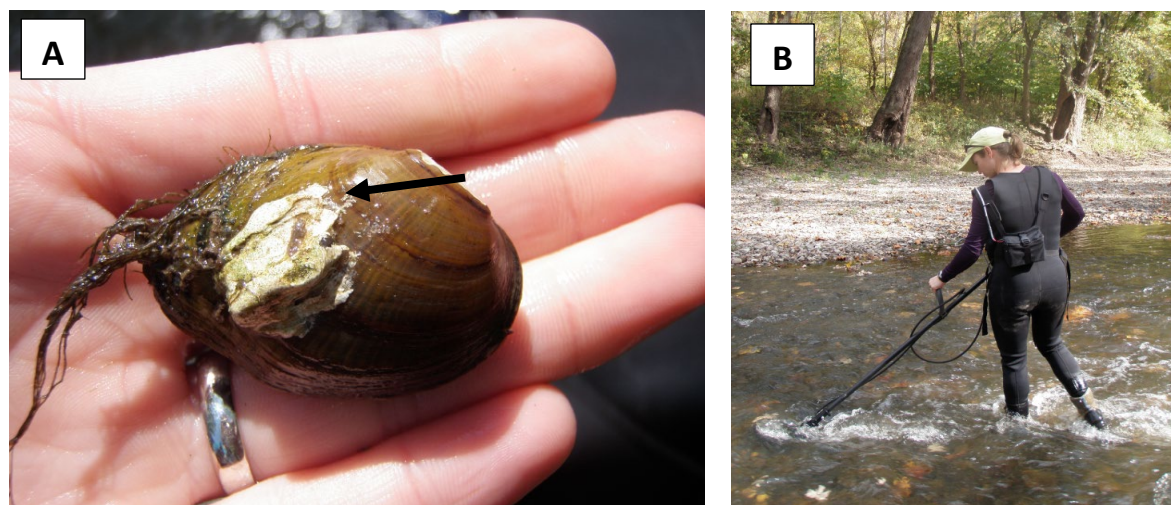


Figure 2. A PIT tagged mussel from Illinois (A - using Devcon marine grade epoxy (model 11800) and B - a demonstration of PIT tag sampling using an aquatic reader). The reader is swept in a systematic manner from a unique starting point (*sensu* Stodola et al. 2017; photos shared with permission from Alison Price-Stodola, Illinois Natural History Survey).

NOTE – When using both conventional and PIT tags

If you choose to use both types of tag, please note both tags on the datasheet for each individual mussel.

If you are using PIT tags, the approach to resampling a population will look different because there is an opportunity to resample existing tagged individuals with minimal disturbance and to resample while marking new individuals for resampling in the future. Initial tagging, resampling or additional marking of individuals will require a PIT tag reader (e.g., BioMark® FS2001F-ISO or BioMark® HPR Plus receivers; Portable BioMark® BP antenna), and resampling can be done by a single individual using existing protocols (Stodola et al. 2017). Record the reader type, detection distance, and resurvey approach. The re-sampling survey consists of an observer traversing the stream in a systematic direction while sweeping the antenna along the streambed in a repeatable fashion (Stodola et al. 2017). These surveys are continued until the entire survey area has been searched including 10 meters upstream and downstream of the site (Stodola et al. 2017). Additionally, because a PIT tag survey has an associated detection probability <1, a second observer may follow the same resampling procedure. If the mussel needs to be handled to check for tag retention and get mussel measurements, a box sieve may help to quickly find the animal if buried. In addition to resampling using a PIT tag reader, snorkel surveys should be employed at a minimum during the first sampling period of each field season to detect unmarked individuals within the population.

C. Habitat survey and processing

Overview

Habitat surveys will include three components: 1) visual habitat assessment, 2) reach-scale assessment, and 3) individual-scale assessment. Habitat should be assessed at each sampling occasion or annually, depending upon the variable (see Table 2). When combined with data across the range of Brook Floater, this habitat assessment will increase understanding of what habitat factors influence Brook Floater populations, as well as how survey conditions and habitat influence detection probability and occurrence probability for non-target mussels.

The visual habitat assessment can take place before or after mussel processing. Reach-scale and individual-scale assessments should take place after mussel surveys are completed, since several of the variables depend on the exact searched area. Because these are long-term monitoring sites and target species are sensitive, it is important to minimize in-stream disturbance to the site. Field data sheets are available for recording reach-scale and individual-scale habitat data in Appendices F and G, respectively.

Equipment needed:

- Tape measure (>100-m) or laser range finder
- Convex spherical densiometer modified for the 17-intersection method
- Meter stick
- Metric ruler
- Global position system (GPS) unit
- Flagging
- Flags or other tool for marking mussel locations
- Thermometer

- Waterproof data forms, clipboard and waterproof ink or pencils
- Digital camera
- Field data sheets (see Appendices)

Table 2. Frequency of collecting habitat data.

Type of Assessment	Habitat Variable	Frequency of Measurement
1. Visual habitat assessment	Habitat score (10 questions)	Each visit
2. Reach-scale assessment	Mesohabitat	Each visit
	Survey length	Annually, unless a survey is not completed to the set length.
	Wetted width	Each visit
	Canopy cover	Each visit
	Sky code	Each visit
	Water temperature	Each visit
	Optional: water chemistry	Each visit; or repeated (seasonally, monthly, etc.). Data should be downloaded and checked for quality control and assurance twice a year.
3. Individual-scale assessment	Optional: continuous temperature loggers	Continuous
	Dominant substrate	Each visit
	Depth	Each visit
	Vegetation	Each visit

1. Visual habitat assessment

The EPA rapid visual habitat assessment has 10 questions that characterize the macro-scale features of the stream reach (Barbour et al. 1999). The data sheet (Appendix K) describes each habitat parameter and scoring between optimal and poor. Use the high gradient sheet regardless of the actual slope of the stream reach and make a note if the habitat mostly resembles low gradient streams. Further details on the habitat parameters used to characterize stream reaches using the rapid visual assessment approach can be found in Section 5 of Barbour et al. (1999).

2. Reach-scale assessment

Mesohabitat description

The mesohabitat of the entire surveyed reach should be characterized visually by describing the amount of different mesohabitat types (i.e., shallow riffle, run, deep pool, etc.). Provide approximate proportional area of each mesohabitat as it relates to the entire area searched. This description should be taken independently at each sampling occasion as streams change through time.

Survey length

At the end of the mussel survey, a measurement should be taken of the entire stream length that was surveyed. Because this is a long-term monitoring site, the survey length should only need to be measured at the first sampling visit. If the length changes for any reason, record a new survey length.

Wetted width

The wetted width of the survey site should be taken at 3 representative locations along the searched survey length. If the stream can be waded safely, the measurement should be taken with a measuring tape. If the stream is too wide for safe wading, a range finder can be used to take the measurements. Range finders should be used with caution as many have lower and upper bounds for reliable measurement (i.e., <8 m for lower and 350 m for upper, for many models). If the stream is too wide to take a measurement with tape and there is an unreliable measurement from the range finder, an estimated measurement can be made. If the area searched does not cover the entire width of the stream, the width of the searched survey area (measured using a tape measure) should be recorded.

Canopy cover

Canopy cover should be estimated using a modified densiometer for the 17-intersection method (Figure 3; Fitzpatrick et al. 1998). Measurements should be taken at 3 representative longitudinal locations of the site in the center of the surveyed stream width (when possible). Holding the densiometer at waist height, count the intersections (of 17 total) that are covered by canopy. Repeat this for four cardinal directions (upstream (US), downstream (DS), river left (RL) and river right (RR)) at each location. Because canopy cover may change seasonally and may vary based on latitude, this measurement should be taken during each visit unless the visits are close together in the same season.

Sky code

The condition of the sky should be assessed using the sky code conditions. This measurement will contribute to understanding how daily conditions affect mussel detection. Surveys should not take place with heavy rain.

Water temperature at survey

A measurement of the water temperature should be taken using a calibrated thermometer at the time of the survey. This measure will contribute to understanding how temperature affects mussel detection.

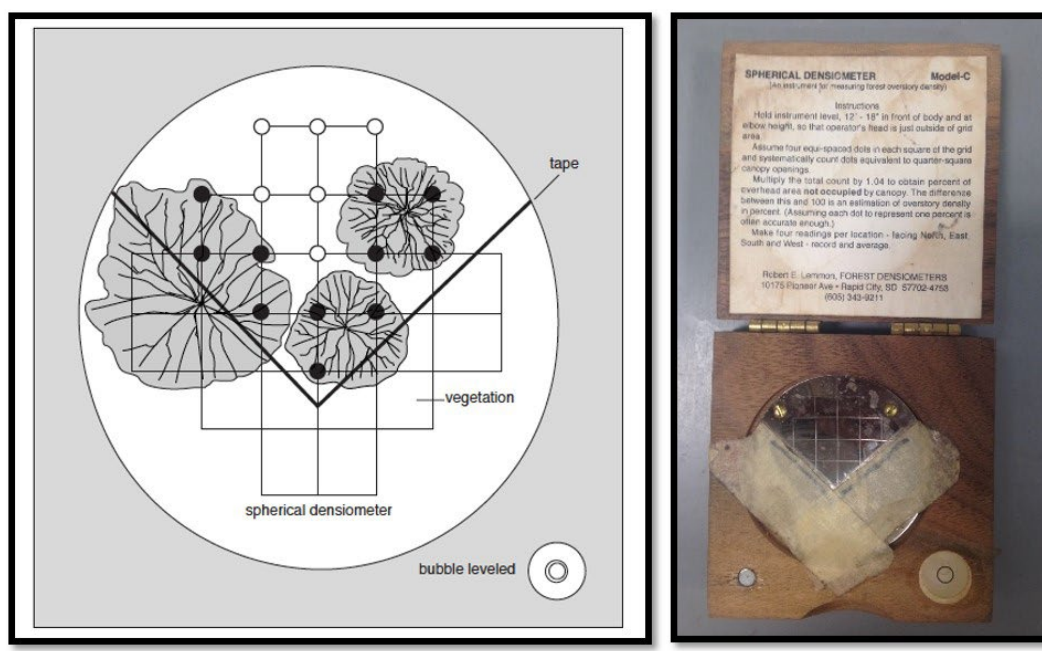


Figure 3. A modified concave spherical densiometer diagram (left) and photo (right) with bubble level, tape and 17 points of observation. A line of intersections at both open and closed circles is examined on the left. Closed circles represent line intersections counted in measurement of canopy closure (i.e., 11 out of 17 points; Fitzpatrick et al. 1998).

Optional: Water quality

The water chemistry of long-term monitoring sites may be characterized using an *in situ* multiprobe water quality meter (e.g., YSI). Measurements taken at a single point (i.e., during a mussel survey) provide the current conditions. Ideally, these measurements are taken at regular intervals seasonally and annually at each site. In addition to temperature, variables of interest for mussels include dissolved oxygen, pH, salinity, conductivity, and ammonia (NH₃).

Optional: Continuous temperature loggers

A continuous measurement of temperature at a site focused on Brook Floater may be valuable. A single waterproof temperature logger (e.g., Onset HOBO Water Temperature ProV2, Onset v2 TidbiT) can be deployed year-round at monitoring sites in a position in the stream that will collect data on how mussels experience water temperature (i.e., on the bottom of the stream where it will not become dewatered). Temperature loggers should be programmed to monitor temperature at 15 min intervals. Data logger housings should be constructed to ensure that the data loggers are not damaged due to stream dynamics. Further details on constructing data logger housing and deployment can be found in Dunham et al. (2005) and U.S. EPA (2014).

Optional: Flow and discharge

Flow information can be helpful for describing the stream reach at the time of sampling. A handheld flow meter (e.g., HACH® FH950) can be used to get flow

measurements for each habitat type, at random locations in the surveyed reach, or at the location of individual mussels (see #3 below), if desired. Stream discharge can be helpful for temporal comparisons, such as understanding potential reasons for low detection at a given sampling event. If a U.S. Geological Survey gage is on the stream (<https://waterdata.usgs.gov/nwis>), recording the gage height and discharge at the time of each sampling may be helpful. If there is no relevant existing gage, using a flow meter to measure discharge at the time of sampling may be worthwhile if completed during each sampling event. Protocols for measuring discharge and collecting continuous water level measurements in streams are in U.S EPA (2014).

3. Individual-level assessment

Understanding the instream conditions at the individual scale can help to understand characteristics of habitat where mussels are found for comparison among locations within sites, at the same sites through time, and across sites. These variables may also be helpful to evaluate detection probability (Meador et al. 2011, Wisniewski et al. 2013). At each individual mussel location, habitat should be rapidly assessed for depth, dominant substrate, and % vegetation. For depth, measure the distance from the streambed at the mussel location to the water surface. Dominant substrate and % vegetation should be assessed within a 0.5 m x 0.5 m area (0.25-m²) around the mussel. For this process, a quadrat is not necessary, but this approximate area should be used for assessing habitat at the individual level. For dominant substrate, assess which substrate size class (Table 2) is dominant in the area surrounding the mussel. For vegetation, estimate the % of vegetation surrounding the mussel.

Table 3. Substrate size class codes from the EPA National Wadeable Stream Assessment habitat form (U.S. EPA 2013).

Abbreviation	Size class
RS	Bedrock (smooth) - larger than a car
RR	Bedrock (Rough) - larger than a car
RC	Concrete/Asphalt
XB	Large boulder (1000-4000 mm) - meter stick to car
SB	Small boulder (250-1000 mm) - basketball to meter stick
CB	Cobble (64-250 mm) - tennis ball to basketball
GC	Coarse gravel (16-64 mm) - marble to tennis ball
GF	Fine gravel (2-16 mm) - ladybug to marble
SA	Sand (0.06-2 mm) - gritty - up to ladybug size
FN	Silt / clay / muck - not gritty
HP	Hardpan - firm, consolidated fine substrate
OT	Other

IV. Data Management and Reporting

All data (i.e., reach-scale habitat and mussel collection data) should be entered into a database that has fields corresponding to field data sheets. All data sheets should be photocopied for your records and a copy provided to the state malacologist.

V. References

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VI. Appendices

Appendix A. General assumptions of capture-mark-recapture (CMR) and example sample design

General assumptions of CMR approaches for closed populations (Lindberg and Rexstad 2002, Williams et al. 2002):

1. Parameters estimated for the marked population can be applied to the unmarked population of interest.
2. Marks do not affect the behavior or fate of the marked individuals.
3. Marks are not lost.
4. Every marked animal alive in the population at time i has the same probability of capture.
5. The fate of each marked animal is independent of the fate of other marked animals.
6. Resampling is instantaneous; that is, births, deaths, immigration, and emigration do not occur during the resampling process.

We recommend that long-term monitoring sites are visited a minimum of two times per year and for a minimum of three consecutive years.

Sampling design example:

1st and 2nd sampling periods: June – August 2018, X # of sites

3rd and 4th sampling periods: June – August 2019, X # of sites

5th and 6th sampling periods: June – August 2020, X # of sites

7th and 8th sampling period: June – August 2021, X # of sites

...

Example data from a single site using the above sampling design, where 1 = present and 0 = not found at different sampling periods (a, b) within a year:

Animal

<u>ID</u>	<u>2018a</u>	<u>2018b</u>	<u>2019a</u>	<u>2019b</u>	<u>2020a</u>	<u>2020b</u>	<u>2021a</u>	<u>2021b</u>
323	1	0	0	1	0	1	0	0	0	1
400	1	0	0	1	0	0	1	1	0	1
125	0	0	0	0	0	0	0	1	0	0
64	1	1	1	1	1	0	0	1	1	1

...

Potential modeling approaches (Williams et al. 2002):

- *Cormack-Jolly-Seber (CJS) models* can be used to estimate apparent survival, migration, movement, recapture probabilities, and abundance/density.
- *Pradel models* can estimate survival, growth rate, per capita recruitment, and population growth rate (λ).
- *Jolly-Seber superpopulation models* can estimate abundance.

Mark-Recapture References

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Appendix B. Workflow scenarios

Implementing this protocol will require developing your own mussel and habitat survey workflow and making adjustments to the workflow to maximize efficiency and effectiveness depending upon survey personnel, density of target mussels in the chosen stream, and stream conditions. Below are suggestions for adjusting workflow depending upon the situation (i.e., number of personnel, mussel density). Detection of mussels will vary by surveyor, but variation in habitat data and mussel measurements should be kept to a minimum by standardizing who on a survey team is collecting these data.

Scenario 1 (High personnel (4-7 people), Low mussel density (<50 mussels))

1. One team (i.e., 3 individuals) actively surveys 10-m bands for mussels, collects and bags or flags target species (that are not to be bagged but instead left in place).
2. One team (i.e., 1-2 individuals) moves behind the mussel surveyors and works up individual mussels and individual-scale habitat data at mussel locations as they complete a band. Mussels are returned to the stream.
3. One team (i.e., this could be a single individual) completes reach-scale habitat data collection on the stream – this may also be conducted at the end of the survey.

Scenario 2 (High personnel (4-7 people), High mussel density (50-100 mussels))

1. One team (i.e., 3 individuals) actively surveys 10-m bands for mussels, collects and bags mussels or flags individual target species (that are not to be bagged but instead left in place), and flags mussel locations.
 - a. For keeping track of all mussels in a high-density population, reducing the size of the bands that are surveyed (i.e., 10 to 5 m) may be appropriate.
 - b. In this case, it may be best for most of the personnel to focus on mussel surveys and associated individual-scale data collection until the majority of survey bands are completed.
2. As soon as the surveying team is completed with surveys, they can either assist with recording individual mussel data or returning mussels to the stream.
3. When the mussel surveys are nearing completion, one team can complete individual-scale and reach-wide habitat data collection.

Scenario 3 (Low personnel (<4 people), High mussel density (50-100 mussels))

1. Most personnel (3) survey for mussels, collect in bags or flag target species (that are not to be bagged but instead left in place).
2. As each band is completed, all surveying personnel record individual-scale mussel and habitat data as they go.
 - a. It may also be efficient to work through several bands of mussel surveys (i.e., marking the locations of mussels in 2-4 bands) and then processing mussels (i.e., measuring and marking) from those bands.
 - b. Alternative – If you record mussel data as you finish each 10-m band, it may be helpful if one person collects data as they go. Two people follow surveyors, process and tag mussels, collect individual habitat measurements, and then immediately return mussels to the stream.

3. One person collects reach-scale habitat data on the stream site. When they are finished, they can help in recording individual mussel data.

Mussels are returned to the stream.

Appendix C. Tradeoffs on the use of passive integrated transponders (PIT) and conventional tags

	Advantage	Disadvantage
Conventional tags (e.g., Hallprint®, Floy®)	<ul style="list-style-type: none"> • Lower expense (\$0.30-0.40/tag)¹ • Retention rates studied • Fast to attach 	<ul style="list-style-type: none"> • Low recapture rates in small populations • Requires at least 1 experienced mussel expert to resample
PIT tags	<ul style="list-style-type: none"> • Ability to resample without removing mussels from the stream • Higher recapture rates possible • Does not require experienced mussel expert to resample² 	<ul style="list-style-type: none"> • Higher expense (\$2-4/tag, plus cost of PIT tag reader and wand)¹ • Retention rates unknown • Detection rates unknown and may vary by reader (and other reasons) • May require two-step process for attachment • Potential influence on behavior • Potential interference in high density populations • Requires expertise using a PIT tag reader²

¹ Does not include the cost of adhesives or field time (for more information, see Ashton et al. 2017).

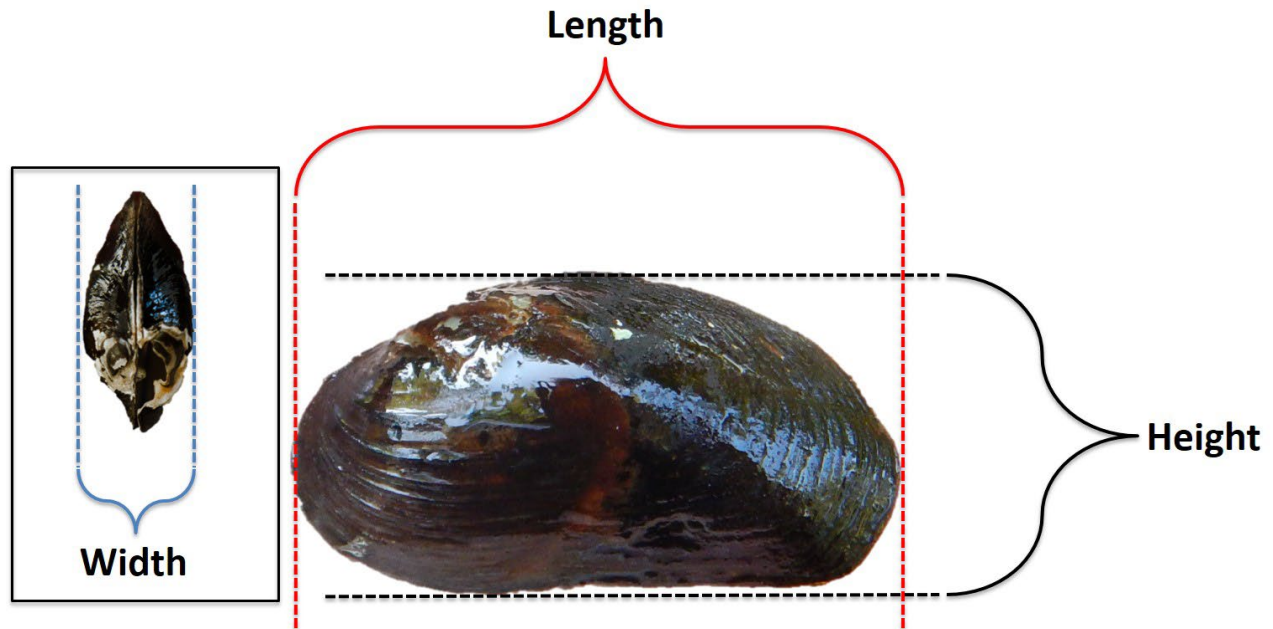
² When accompanied with snorkel surveys, as recommended, a mussel expert is also needed.

Mussel Tagging Resources

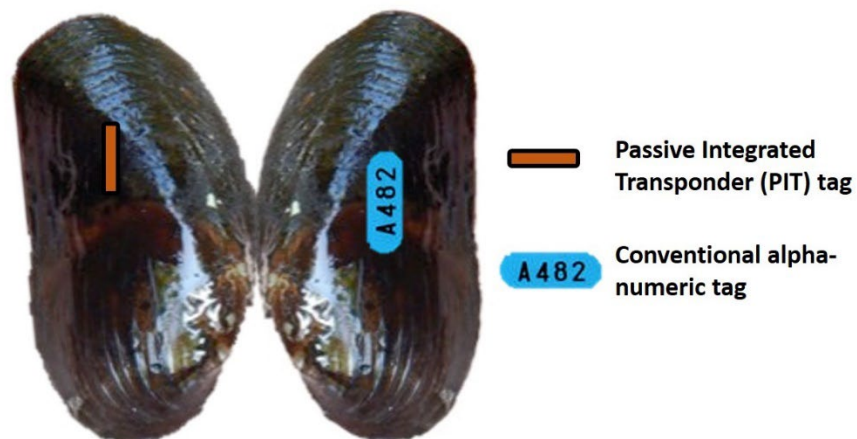
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Appendix D. Shell measurement and tag placement diagram

Measurements should be recorded for each target species mussel using calipers. For length, the measurement is the maximum distance between the posterior and anterior margins of the shell. For height, the measurement is taken as the maximum distance between the dorsal and ventral margins of the shell. Both measurements should be recorded in millimeters. Width is optional but should be measured at the widest point.








Proper placement of both conventional (e.g., Hallprint, Floy) tags and passive integrated transponder (PIT) tags. The tags should be placed on a portion of the shell that is most resistant to erosion. In the case of the PIT tag, the long axis of the tag should be oriented parallel to the long axis of the mussel to increase detection of the tag when surveying using an aquatic PIT tag reader.



Appendix E. Shell condition index

Shell condition provides an index of the general physical health of a mussel, considering erosion of the periostracum, which may reflect age or instream conditions (e.g., high flows, pH, etc.). The index for this protocol allows for giving an absolute score to a mussel based on the percent erosion of the periostracum (ranging from 1-5, little or no erosion to heavy erosion, respectively). The series of photos below shows a progression of shell degradation from light to heavy. Keep in mind that the value assigned to a mussel may be upgraded if there is obvious erosion of the shell through multiple layers of the shell or if there are multiple areas on the shell that are eroded.

Code	1	2	3	4	5
Description	Light	Light-Medium	Medium	Medium-Heavy	Heavy
% periostracum eroded	<10	<20	<30	<40	>40
					

Appendix F. Field data sheets – Metadata and Reach-Scale Habitat Data

State _____ County _____ Watershed _____ Site ID _____					Mussel Survey and Habitat Data Sheet pg. ____ of ____			
Date:			Observers (name and years of experience):			Time arrived at site		
Water Temp:	GPS point? Y N	X: Y:				Time mussel survey start		
Sky Code ¹ :	Search Method (by lane) ² :					Time mussel survey end		
		Time departing from site						
Stream Wetted Width (m)	Photo Monitoring Point? Y N		Canopy Cover (# out of 17 intersections)			Visibility > 1 m		Y N
1.			1	2	3	Mesohabitat: % of entire search area		
2.			US:	US:	US:	% Run	% Riffle	% Pool
3.	Mussel Return Method ³		DS:	DS:	DS:			
			RR:	RR:	RR:	Measured Area of Mussel Survey		
			RL:	RL:	RL:	Length (m):		Width (m):
Site Description					Tagging Methods: (circle)			
					Conventional PIT Both			
Notes					Tag manufacturer:			
					Tag specs/size:			
					Adhesive type:			
					Reader type:			
¹ Sky Code: Do not conduct surveys if sky codes are 6 or above. Code Sky Condition 0 Clear or few clouds (< 20% of sky) 1 Partly cloudy or variable (20-50% of sky) 2 Cloudy or overcast (> 50% of sky) 3 Fog 4 Mist 5 Showers or light rain 6 Heavy rain			² Search Method (by lane) S = Snorkel B = Bucket Viewer O = other or mix; explain in notes		³ Mussel Return Method? When you return individuals to the population, do you: 1. Replace individuals back at their exact locations, 2. Mixing the population by returning individuals to approximate locations.			

Appendix G. Field data sheets – Mussel and Individual-Scale Habitat Data

[illegible]


Appendix H. Effort and target mussel location datasheet (Note: Multiple copies of this sheet are needed so lines correspond to #Lanes X # Bands)

State _____ County _____ Watershed _____ Site ID _____				Date _____				Pg _____ of _____			
SURVEY EFFORT				TARGET MUSSEL COUNT FROM EACH BAND							
Obs.	Lane	Band	Effort (min) Start and End time	SPECIES							
<u>NOTES</u>											

Appendix I. Field data sheets - Individual mussel GPS locations (optional)

[illegible]

Appendix J. Site map

State _____ County _____ Watershed _____ Site ID _____	Date _____
Map of long-term site - This area can be used to illustrate or describe the site in general, or specifically show where mesohabitats are within each survey visit or where mussels are generally found within the site.	
	

Appendix K. EPA rapid visual habitat assessment (from Barbour et al. 1999)

HABITAT ASSESSMENT FIELD DATA SHEET—HIGH GRADIENT STREAMS (FRONT)

STREAM NAME _____		LOCATION _____	
STATION # _____ RIVERMILE _____		STREAM CLASS _____	
LAT _____ LONG _____		RIVER BASIN _____	
STORET # _____		AGENCY _____	
INVESTIGATORS _____			
FORM COMPLETED BY _____		DATE _____ TIME _____ AM PM	REASON FOR SURVEY _____

	Habitat Parameter	Condition Category			
		Optimal	Suboptimal	Marginal	Poor
Parameters to be evaluated in sampling reach	1. Epifaunal Substrate/ Available Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are <u>not</u> new fall and not transient).	40-70% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	2. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	3. Velocity/Depth Regime	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). (Slow is < 0.3 m/s, deep is > 0.5 m.)	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/depth regime (usually slow-deep).
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

HABITAT ASSESSMENT FIELD DATA SHEET—HIGH GRADIENT STREAMS (BACK)

Habitat Parameter	Condition Category																				
	Optimal					Suboptimal					Marginal					Poor					
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.					Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.					Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.					Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.					
SCORE	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
7. Frequency of Riffles (or bends)	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.					Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.					Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.					Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.					
SCORE	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
8. Bank Stability (score each bank)	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.					Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.					Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.					Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.					
Note: determine left or right side by facing downstream.																					
SCORE (LB)	Left Bank	10	9			8	7	6			5	4	3			2	1	0			
SCORE (RB)	Right Bank	10	9			8	7	6			5	4	3			2	1	0			
9. Vegetative Protection (score each bank)	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.					70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.					50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.					Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.					
SCORE (LB)	Left Bank	10	9			8	7	6			5	4	3			2	1	0			
SCORE (RB)	Right Bank	10	9			8	7	6			5	4	3			2	1	0			
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.					Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.					Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.					Width of riparian zone <6 meters; little or no riparian vegetation due to human activities.					
SCORE (LB)	Left Bank	10	9			8	7	6			5	4	3			2	1	0			
SCORE (RB)	Right Bank	10	9			8	7	6			5	4	3			2	1	0			

Total Score _____