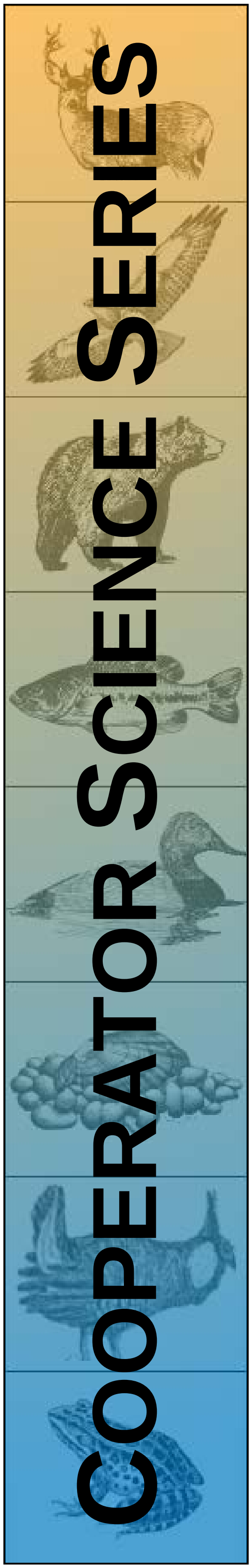

Brook Floater Rapid Assessment Monitoring Protocol

Sean Sterrett[1], Allison Roy[2], Peter Hazelton[3], Brian Watson[4], Beth Swartz[5], T.R. Russ[6], Lisa Holst[7], Mike Marchand[8], Jason Wisniewski[9], Matt Ashton[10], Barry Wicklow[11]

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Cooperator Science Series # 132-2018

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Contractual References:

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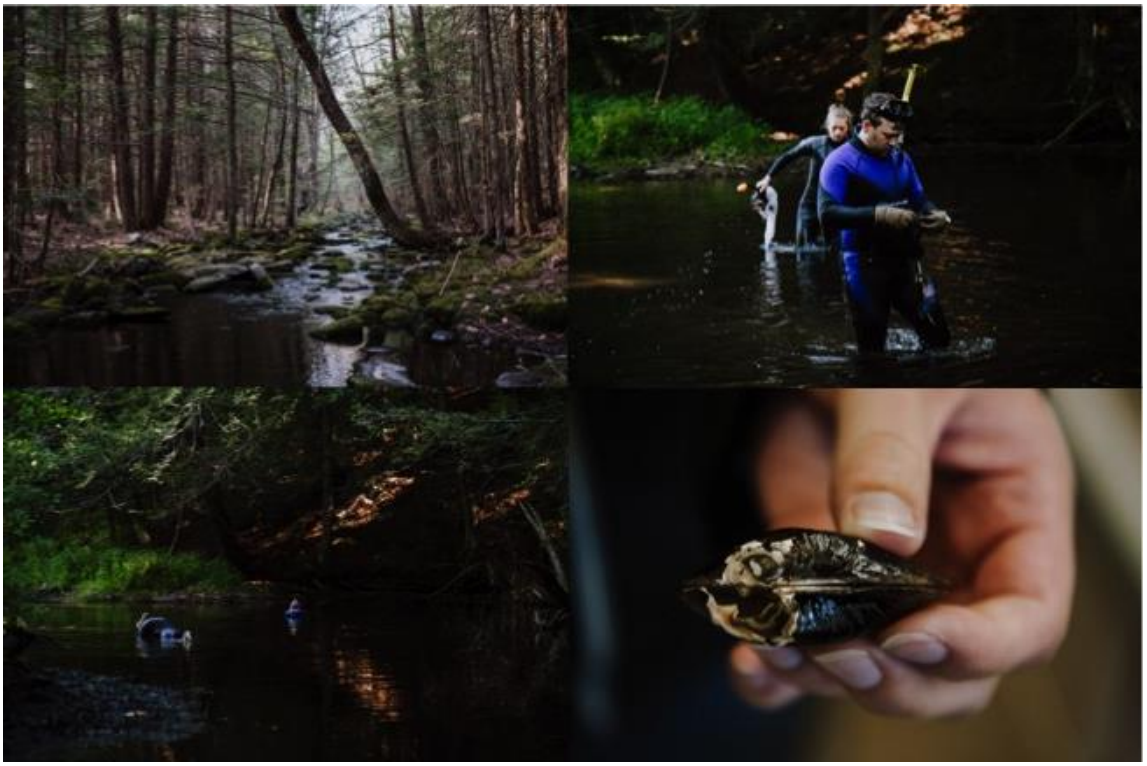
Brook Floater Rapid Assessment Monitoring Protocol



Photos by Ayla Skorupa

Prepared by:

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I. Introduction

Overview

The Brook Floater (*Alasmidonta varicosa*) is a small (<100 mm), stream dwelling freshwater mussel (Family: Unionidae) from Atlantic Slope drainages in the eastern U.S. (Nedeau 2008). Brook Floater have dramatically contracted in distribution over recent decades, and there is limited evidence of recruitment in most locations, despite minimal effort to document population status (Wicklow et al., 2017). Brook Floater is listed as a Species of Greatest Conservation Need (SGCN) throughout its range in the United States (state-listed as imperiled or critically imperiled in all 15 states), has been extirpated from two states (Rhode Island and Delaware) and was recently petitioned for Federal listing in 2011 (Wicklow et al. 2017). Currently, there is a U.S. Fish and Wildlife (USFWS) Species Status Assessment underway to determine if Federal listing under the Endangered Species Act is warranted. Brook Floater is also listed as a species of special concern in Canada, the northern extent of its range. In 2016, a state wildlife grant was awarded to MassWildlife and coordinating states to develop range-wide conservation initiatives and strategies, including the development of rapid assessment and long-term monitoring techniques and the development of conservation strategies to improve its probability of persistence in the future. The purpose of this protocol is to describe and facilitate a rapid approach to estimating Brook Floater occupancy to better understand the factors that influence Brook Floater distribution.

Occupancy estimation approaches allow for estimation of species occupancy (ψ; percent area occupied) within some scale of interest (for our purposes, the watershed), while simultaneously estimating species detection probability (p; 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 (i.e., $p<1$; MacKenzie et al. 2004, Shea et al. 2013, Wisniewski et al. 2013, Pandolfo et al. 2016, MacKenzie 2016). This approach does not rely on existing information about species presence or absence to select sites. Occupancy estimation operates on a robust probabilistic framework of randomly selected sites to infer what proportion of sites are occupied. Occupancy estimation also incorporates imperfect detection ($p<1$; i.e., animals are cryptic and elusive; observers have varying experience searching, etc.; MacKenzie et al. 2003). For example, two mussel species that occupy a site might have two very different detection histories, as determined by revisiting a site and using the same methods on repeated visits to find both species. See hypothetical results here:

	Visits				
	1	**2**	**3**	**4**	**5**
Mussel species A	1	1	1	0	1
Mussel species B	0	0	0	1	0

(1 = detected, 0=not detected)

Both of these mussel species occupy this site, yet Mussel A was detected in 4 out of 5 visits (high *p*) and Mussel B was detected in 1 out of 5 visits (low *p*) with the methods used to survey this site. Covariates may explain differences in detection between species or visits. Organisms may be: 1) present and not observed, 2) present and unavailable for capture (i.e., buried in sediment), or 3) not present at the site. Occupancy estimation uses "repeated visits" of randomly selected sites to build species detection histories (i.e., 1, 0, 1) to simultaneously estimate occupancy (ψ) and *p*. Typically, repeated visits are discrete sampling events and are more time consuming because each site requires >3 separate visits. In our rapid assessment protocol, we use multiple independent observers searching longitudinal lanes to estimate detection in a single site visit as opposed to multiple discrete visits. Below are hypothetical results of occupancy by observer:

	Independent Observers				
	1	**2**	**3**	**4**	**5**
Mussel species A	1	1	0	1	1
Mussel species B	1	0	0	1	0

(1 = detected, 0=not detected)

Objectives:
The objectives of this rapid assessment survey approach are to guide collection of data that can be used to:

A. Estimate the occupancy of Brook Floater within watersheds.
B. Estimate the effects of reach- and watershed-scale habitat features on Brook Floater occurrence.
C. Understand how survey covariates (e.g., surveyor experience) influence detection of Brook Floater.

While this protocol explicitly targets collection of Brook Floater, it is likely that the methods can be adapted for occupancy surveys of other stream-dwelling freshwater mussel species.

II. Personnel Requirements and Training

Overview
Personnel assigned to conduct surveys under this protocol should have sufficient experience and knowledge of mussel ecology, identification, and field methods to independently find and identify threatened and endangered mussel species in watersheds where Brook Floater occur. Observers not familiar with mussel assemblages that include Brook Floater should work closely with malacologists who have this experience. All observers should also be familiar with guidelines and safety procedures for safe care and handling of all mussel species. See safety notes below (section IV, part B).

Each survey requires a minimum of **<u>3 trained observers</u>**. The number of observers may vary across watersheds or sample events. If a large site (i.e., much greater stream width) is chosen, it may be necessary to increase the number of observers (section IV, part B).

III. Sampling Design

Overview

A random (or probabilistic) sampling design allows inference about species distributions and occupancy and maximizes efficiency. For this protocol, sites are randomly selected from all stream and road crossings (hereafter "crossings") within a targeted watershed (i.e., watersheds with known Brook Floater presence). Crossings will be identified in a Geographic Information System (GIS) and a subset of these sites will be randomly selected for sampling. The goal of this site selection process is to find a randomly selected location that is not influenced by the crossing itself.

A. Random site selection protocol for watershed inference

Sixty sites are randomly selected from crossing sites (DOT layer) within each targeted HUC10 watershed within ArcGIS, with the goal of ultimately sampling at least 30 sites within a watershed (see Appendix A). The 60 sites are split randomly into 2 sets of 30 sites: Priority A and Priority B sites.

<u>Priority A sites</u> should be visited and sampled within the watershed unless there is a condition that precludes sampling (see conditions below). Within this group, biologists should group the sites into clusters that can be visited in a single day.

<u>Priority B sites</u> are used to make up for any sites from Priority A list that are not able to be sampled or if time is available to continue sampling within the watershed. Ideally, these sites are added in numerical order; however, it is acceptable to prioritize based on logistics (i.e., proximity to other sites).

B. Excluding sites based on maps and field reconnaissance

It is inevitable that a proportion of randomly selected sites will be inappropriate for sampling given the conditions necessary for sampling (see below). Keep in mind that the lack of quality habitat for Brook Floater is <u>NOT</u> a reason for not sampling. If sites can be determined to be inappropriate for sampling with high certainty based on field maps or field reconnaissance, and based on our conditions for not sampling (see below), then it is justified to not visit a field site or attempt a survey.

Sites must meet the following conditions for sampling*:

1. Fit the criteria for the sampling within the protocol (i.e., <1 m deep in sampling area and ≥3 m in width to accommodate 3 observers)
2. Accessible from a bridge site
3. Safe to traverse (i.e., high flows in areas that need to be sampled or near a highway which may be unsafe to park near)

*NOTE: The lack of "quality habitat" for Brook Floater (i.e., habitat that you may not think is suitable) is NOT a reason for not sampling.

C. Variations for inference at different spatial scales

The sampling design outlined for this protocol provides inference at the watershed scale (i.e., "What is the percent area occupied by Brook Floater in this watershed?"). However, it is possible that, given the constraints of resources by a partner or the geographic range inhabited by Brook Floater, that variations on the scope of inference would change. For example, Brook Floater exists in a single river in Georgia (i.e., Chattooga River). In this case, if a partner is interested in understanding the percent area occupied within that river (and not its tributaries), then it would be appropriate to modify the random site selection of this protocol to randomly select sites within the river (rather than the watershed) to meet those objectives.

IV. Field Methods and Processing of Collected Materials

A. Identifying survey starting location

Overview
Sites should be selected from crossing sites as starting point and follow directions below to identify the downstream locations to start a mussel survey.

Equipment needed:
Tape measure

Procedures:
The starting point for a mussel survey will be based on:
1. An initial fixed upstream distance from the crossing (i.e., to move beyond the upstream scour pool of the crossing).
2. A random distance to the starting point.

Upon arriving at the randomly selected crossing, measure **100m** upstream of the crossing site. At the end of this fixed distance upstream, a random distance (0-100m; Appendix B) should be used to find the starting point (Fig. 1). If the end of the fixed distance is within a scour pool, use your professional judgement to move the fixed

distance end upstream of the scour pool before adding the random distance. The random distance should be recorded on the field data sheet (Appendix E).

Surveys can only take place in reaches with depths that facilitate surveys by mask and snorkel (i.e., <1 m of depth; however, view-buckets may be used in marginal habitats) with tactile and visual encounter surveys (i.e., not extremely shallow riffle or deep pool habitat). This means that following the 2 steps above may lead you to a starting point that is not suitable for surveys. If this is the case, select the closest suitable and surveyable habitat from the randomized location as a starting point. Account for your decision using comments on the data sheet, and include the distance up/downstream from the randomly chosen data point.

SPECIAL NOTE on adding on non-random search minutes
We understand that users may be interested in additionally searching habitat that is near to the randomly selected site. If suitable habitat has been identified near the study site, then additional minutes of non-random searching can take place. If this takes place, please note it on the data sheets and include the amount of effort for the search (i.e., person-hours and estimated area searched), so this effort can be easily separated from the random search effort that will be used to determine occupancy.

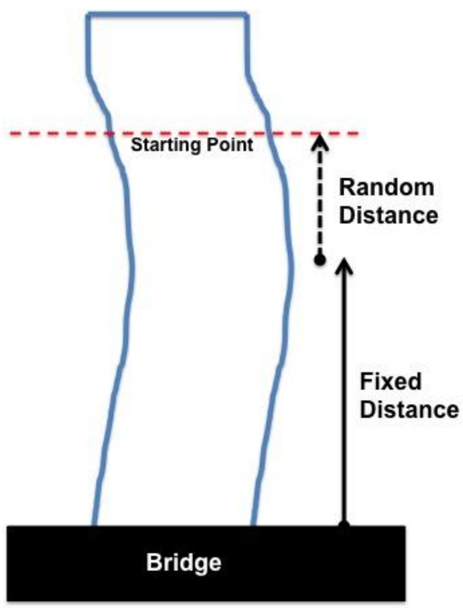

Figure 1. A diagram illustrating the refinement of randomly selected sites.

B. Mussel survey, processing and collection of relic shells

Overview

Mussel surveys consist of a timed effort search using snorkelling and multiple independent observers following longitudinal (i.e., parallel with flow) lanes.

Information on individual Brook Floater and target species (i.e., any species of interest to the group performing this protocol), including the collection of relevant relic shells, should be collected following protocol below.

Equipment needed:

- Mask and snorkel for each observer
- Wetsuits or other clothing for snorkelling
- View buckets (at least two)
- Mesh bag for each observer
- Small calipers (metric)
- Tape measure (100 m recommended)
- Metric ruler (10 cm)
- Camera
- Waterproof data forms, clipboard and waterproof ink or pencils
- Stopwatch or other timing device
- Geographic positioning system (GPS)
- Pin flags (~100, depending on expected density) to be used to replace rare/target mussels to capture location
- Ziploc bags
- Ethanol (95%)
- Sharpie
- Labels made of rite in the rain paper

Procedures: Mussel survey

The width of the stream and the number of observers will determine how to proceed with mussel surveys (Survey A or B). Survey A is the preferred survey type as long as the search lane width of each observer does not exceed 3 m. When a stream is wider than 3 m * number of observers, then Survey B should take place. For example, if there are 3 observers, the stream should be wider than 9 m to follow Survey B. Otherwise, follow instructions for Survey A.

> **<u>Full Width Survey (Survey A)</u>**: The stream site should be divided into lanes of equal size proportional to the number of observers performing the survey (Fig. 2). During each survey, the search lanes used by the observers should remain independent and not overlap (Fig. 2). Keep in mind that lanes may vary throughout through the site. If this occurs, the lanes may shrink or expand. Surveyors should be conscious of this and communicate to keep lanes independent.

> **<u>Random Bank Survey (Survey B)</u>**: If Survey A is impossible due to stream width or number of observers, a randomly selected bank should be used to refine the search area using a coin toss (heads=right descending bank). In a random bank survey, the width of the search lanes should remain at 3 m, but should be anchored to the randomly selected bank (i.e., all observers stay confined to their lanes on one bank; Fig. 2). During each survey, the search

lanes used by the observers should remain independent and not overlap (Fig. 2). As depths allow, this survey should proceed similar to Survey A but on the randomly selected bank.

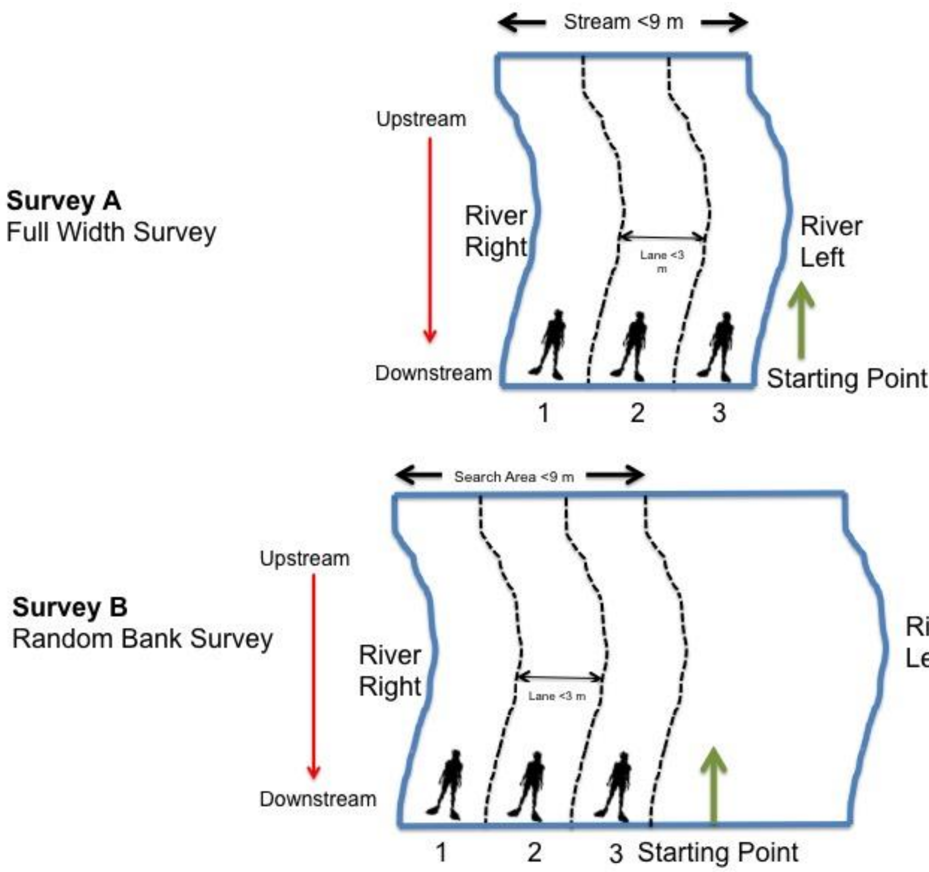

Figure 2. A diagram of a stream site with the sub-division of "lanes" appropriate for a survey group of three independent observers for Survey A - Full Width Survey and Survey B - Random Bank Survey.

All surveys should consist of **2 person hours** of searching time per site. For example, 3 observers would search a site for 40 min and 6 observers would search a site for 20 min. Search time does not include site selection or mussel processing time.

Before beginning each survey, observers should be randomly assigned to lanes. Randomly assign a lane to each observer by having each observer draw a number from 1-n (n=total number of observers). Order lanes from smallest to largest number from "river right" to "river left". Surveyors should start at the downstream starting point at the same time and one observer should note the starting time of the survey. Ideally, all surveys will consist of visual and tactile surveys using mask and snorkel. If the habitat is too shallow to effectively snorkel, then view-buckets (if shallow for the entire length) or visual scanning at the surface (for infrequent shallow areas) are acceptable. Areas that are too deep for snorkelling should be

avoided in this protocol. Record the survey gear used by each observer on the data sheet.

Observers should weave through their designated lane searching in all habitat types available to find any target mussel species. The target search rate for the survey should be 10 m^2/min (Smith et al. 2001, Pete Hazelton and Nevin Welte (Pennsylvania Natural Heritage Program), pers. comm.).

In softer sediments, dig gently into sediment with 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 readily observed visually. Snorkel surveys allow surveyors to float above habitat with only minor disturbance to instream habitat (i.e., gentle digging). When a Brook Floater is observed, an observer should place a pin flag in the exact position in which the mussel was found and place the mussel in a mesh bag to be processed at the end of the survey. Because turbidity created by one observer upstream may influence the detection of observers downstream, all observers should remain at a similar transverse position during the survey. At the end of the survey, record the time for when the survey finished and an exact length of stream that was surveyed.

Because of time constraints with processing target species other than Brook Floater, observers should categorize other target species (i.e., species of greatest conservation need by the state in which the surveys are conducted) into an abundance category (0-10, 10-25, 25-50, >50). Exact numbers and sizes of other target species may be recorded as long as the time needed to do so is not unreasonable (i.e. added time precludes you from finishing the survey). If any mussels are measured (i.e., included as individuals on a data sheet), be careful to not include these in the abundance category in which they may be double counted.

SPECIAL NOTE on disturbance of streams during surveys
Disturbance of potential Brook Floater (and other sensitive and imperiled stream species) habitat is an important consideration in performing these surveys. Snorkel surveys have been specifically chosen because they *reduce the amount of stream bottom disturbance* (i.e., as opposed to walking surveys with bucket viewers when it can be avoided). Please take this into consideration when moving through streams, and use other methods to limit disturbance (e.g., walk single file along bank to site start and end points)

In addition to target species, the abundance of common species (e.g., *Elliptio complanata*) should be categorically assessed for each lane following the same abundance categories: (0-10, 10-25, 25-50, >50). The presence or absence of any non-native species (e.g., *Corbicula fluminea* - Asiatic clam or *Dreissena polymorpha* - Zebra mussel) in the survey site (not lane-specific) should also be noted. An abundance category (above) may also be assigned to a non-native species.

Procedures: Mussel processing
At the end of the survey, all Brook Floater and target mussels should be identified to species, sexed (if possible), measured (see Appendix C) and the shell condition should be noted (see Appendix D). *Note: Brook Floaters are not dimorphic, but some sexes for some mussel species can be distinguished in the field.* Any obvious shell anomalies & deformities should be noted, and photographed (with a ruler as reference). The lane in which each mussel was captured should be recorded, with lanes consistently numbered from descending river right to river left (Fig. 2).

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.

<u>SPECIAL NOTE on species identification</u>
Mussels can be very challenging to identify, especially when similar looking species overlap within the same watershed. If there are doubts about the identification of a species, this is important to note. When in doubt, take representative photos (photos of both valves, and one dorsal view, including a ruler for reference and comparison) and record characteristics that may help identify it later by an independent malacologist. Please note whenever unconfident species identifications are made.

<u>SPECIAL NOTE on mussel safety during processing</u>
Special precaution should be taken for keeping mussels safe during the processing period. When mussels are being processed, minimize the time out of water and in direct sunlight. After returning mussels to their final position, check all mesh bags (i.e., turn them inside out) to ensure that animals are not being removed from the site.

Procedures: Documenting relic shells or recently dead individuals of target species

Identifiable relic shells and freshly dead shells of Brook Floater and any other target species at the survey site should be noted on the data sheet, collected, and transported to the state depository. Bags should be labelled with the date, time, site, and potential species of interest. If tissues are present on the shell, bags should be preserved or stored in a freezer as soon as possible.

C. Habitat survey

Overview
Reach-scale habitat should be rapidly assessed within **<u>each search lane</u>** (i.e., searched area by each observer; #1-5 below) and within **<u>each whole reach</u>** (the

entire searched area; #6-8 below). Both of these habitat assessments may provide insights about how survey conditions and habitat influence detection probability and occurrence probability for Brook Floater (and other mussels). A GPS point should be taken to allow for landscape-level features to be connected to each site. Habitat surveys should take place after mussel surveys are completed.

Equipment needed:

- Tape measure (>100m)
- Rangefinder
- Convex spherical densiometer modified for the 17-intersection method
- Meter stick (x3 or the number of observers)
- Global position system (GPS) instrument
- Flagging
- Thermometer
- Calipers
- Waterproof data forms

Procedures:

Lane-specific habitat variables

Following the completion of mussel surveys, the following habitat characteristics of ***each search lane*** should be assessed.

1. Bed texture

The substrate in each lane will be assessed using a particle measurement at 5 equidistant locations longitudinally along the reach (i.e., bottom, 25%, middle, 75%, top of the sampling reach). At each stop where the depth is measured, select the particle at the end of the meter stick and determine its substrate size class (Table 1; USEPA 2013).

Table 1. Substrate size class codes from the EPA National Wadeable Stream Assessment habitat form (USEPA 2013)

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

2. Vegetation
The emergent, submerged and algae should be visually assessed within each lane. For each searched lane, provide a % cover of each vegetation type, if they exist, for each lane.

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

4. Depth
The depth of each lane should be assessed by taking 3 equally spaced longitudinal measurements (i.e., at bottom, middle, and top of reach) with a meter stick. In addition, record the minimum and maximum measurement for each lane.

5. Stream velocity
The stream velocity should be assessed visually and categorically using descriptors from the EPA National Rivers and Streams Assessment (EPA 2013):

> **1**: Still water; low velocity; smooth, glassy surface; usually deep compared to other parts of the channel
>
> **2**: Water moving slowly, with smooth, unbroken surface; low turbulence
>
> **3**: Water moving, with small ripples, waves, and eddies; waves not breaking, and surface tension is not broken; "babbling" or "gurgling" sound.

It is understood that the stream velocity category should be reflective of the general stream velocity within the surveyed lane, which may include multiple mesohabitats.

Reach-scale habitat surveys
Following the completion of mussel surveys, the following habitat of the entire survey area should be assessed.

6. Water visibility
The visibility of the water should be assessed at 1 m distance using the meter stick. Record whether the bottom of the meter stick is visible or not at 1 m depth. *Note: If the bottom of the meter stick is not visible at 1 m depth, the surveyors should consider delaying this survey because the lack of visibility may influence detection of mussels.*

7. Water temperature
A measurement of the water temperature should be taken using a calibrated thermometer at the time of the survey in the center of the stream channel.

8. Sky code

The condition of the sky should be assessed using the sky code conditions listed on the field data sheet. Surveys should not take place with heavy rain.

9. Canopy cover

Canopy cover should be estimated using a modified densiometer for the 17-intersection method (Fig. 3; Fitzpatrick et al., 1998). Measurements should be taken in the center (i.e., longitudinally) of the reach where the water meets each bank. 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)) for each bank.

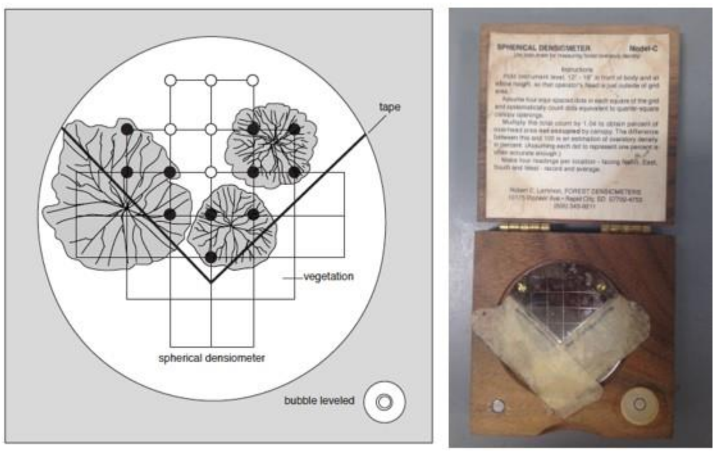

Figure 3. A modified concave spherical densiometer (e.g., a diagram (left) and photo (right)) with bubble level, tape and 17 points of observation. A line of intersections at both open and closed circles is examined on the left. Closed circles represent line intersections counted in measurement of canopy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

10. Wetted width

The wetted width (in meters) of the survey site should be taken at 3 representative locations along the searched survey area. If the stream is able to be waded safely, then the measurement should be taken with a measuring tape. If the stream is too wide for safe wading, then a range finder can be used to take the measurements. Range finders can be used with caution and many have a lower and upper bounds for reliable measurements (i.e., <8m for lower and 350m 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.

11. 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.

12. Measured survey length
At the end of the mussel survey, a measurement should be taken of the entire stream length that was surveyed.

Survey Site Diagram and Description – If desired, draw a diagram and description of the site that was surveyed. It may be helpful to label your drawing with upstream/downstream and include riffles, runs, pools and tributary junctions.

V. Data Management and Reporting

All data (i.e., macro data for site/survey, mussel collection and processing, habitat) should be entered into a corresponding database, which will correspond to field data sheets (Appendix E). All data sheets should be photo copied for your records and a copy provided to the state malacologist.

VI. References

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VII. Appendices

Appendix A. Random site selection protocol in GIS

This protocol provides the sources of necessary GIS layers and steps for creating randomly selected sites using ArcGIS tools (ESRI 20xx. ArcGIS Desktop: Release 10.5 Redlands, CA: Environmental Systems Research Institute).

Required GIS layers and sources:

Layer	Source
State Bridges (bridge-stream crossing sites)	State GIS repository
HUC10 watersheds	National Hydrography Dataset (NHD) watershed boundary dataset
State Brook Floater data	Contact your state malacologist

Overview

Through this process, we are randomly selecting sites from Brook Floater-present HUC10 watersheds in individual states, which are also priority watersheds for state partners. This selection process selects 60 random crossing sites from available crossing sites (i.e., DOT). It then separates those sites randomly into 2 sets of 30 sites, which are priority lists of sites to visit.

Priority A sites – These sites that should be visited and sampled within the watershed unless there is a condition (see below) that precludes sampling.

Priority B sites –These sites are used to make up for any sites from Priority A list that are not able to be sampled, or to supplement Priority A sites if time is available to continue sampling the watershed.

Workflow (GIS tools for ESRI ArcMap are denoted in brackets)

NOTE – We provide a workflow in ArcMap, but other GIS tools can be used for these operations (e.g., GRASS GIS, QGIS)

1. Prioritization of watersheds is made by the state partner (e.g., West Branch Farmington and Ware watersheds in MA for 2017).
2. The bridge layer is clipped **[Clip]** to the individual HUC10 watershed of interest.
3. From available bridges within the HUC10 watershed, 60 sites are randomly selected **[Create Random Points]**.
4. From these 60 random sites, 30 sites are selected to create the Priority A list. **[Create Random Points]**
5. Then "select by location" is used to switch selection to opposite 30 sites from the original 60 sites and exported to create Priority B list. **[Select by Location]**
6. After the priority lists have been randomly selected and exported, each has a new field added called "Priority" and assigned A or B. **[Export] [Add Field]**
7. The two lists are merged and X/Y coordinates are added as a field. **[Merge] [Add XY]**

8. The attribute table is exported to excel using “table to excel”. **[Table to Excel]**

GIS tools (denoted in brackets above)

Clip
Geoprocessing (top menu) -> Clip
Input Features: Bridges
Clip Features: HUC10 Watershed of interest
Output Features: Unique identifier

Create Random Points
[ArcMap Toolbox] Data Management Tools -> Sampling -> Create Random Points
Output Location: Define output location
Output Point Feature Class: Name output
Constraining Feature Class (optional): Define point file of crossing sites within HUC10 of interest. This constrains the random point generation to those points.
Number of Points [value or field] (optional) – long: = 60 (this will be the total number of sites given to the partner, broken into “Priority A” and “Priority B”

Select by Location
Selection method: “Select features from”
Target Layer(s): Choose the original layer created with 60 random points
Source Layer: Choose the layer in which you created random 30 points constrained by the original 60 random points
Spatial selection method for target layer feature(s): “Are identical to the source layer feature”

After you have selected by location, go into the random 60 layer and “switch selection” from the menu. This switches the selection the other 30 points. Export points as “random 30B”

Add Field
Open attribute table (and with editor turned off) –> Add Field -> Give it a unique name (i.e., Priority A or B) and select the data type (i.e., short integer)

Merge
Geoprocessing (top menu) -> Clip
Input Datasets: Add both Priority A and B sites
Output Dataset: Unique identifier

Add XY data
[ArcMap Toolbox] Data Management Tools -> Features -> Add XY Coordinates
Input Features: Define which point file you want to add XY data to

Export table as excel file
[ArcMap Toolbox] Conversion Tools -> Table to Excel
Input Features: Name your file

Converting Datum Projection

In some cases, data from state databases (i.e., bridges) will be projected in a different projection than what you are working in. If this is the case, then use the "Project" tool from the toolbox (not "define projection") to project the data to your existing map.

Products Resulting from this workflow

1) List of Priority A and Priority B sites
2) Zoomed in maps of groups of Priority A and B sites
3) Spreadsheet of site numbers with latitude and longitude of all sites
4) A kml file of the ArcMap layer which can be uploaded in Google Maps and/or ArcMap

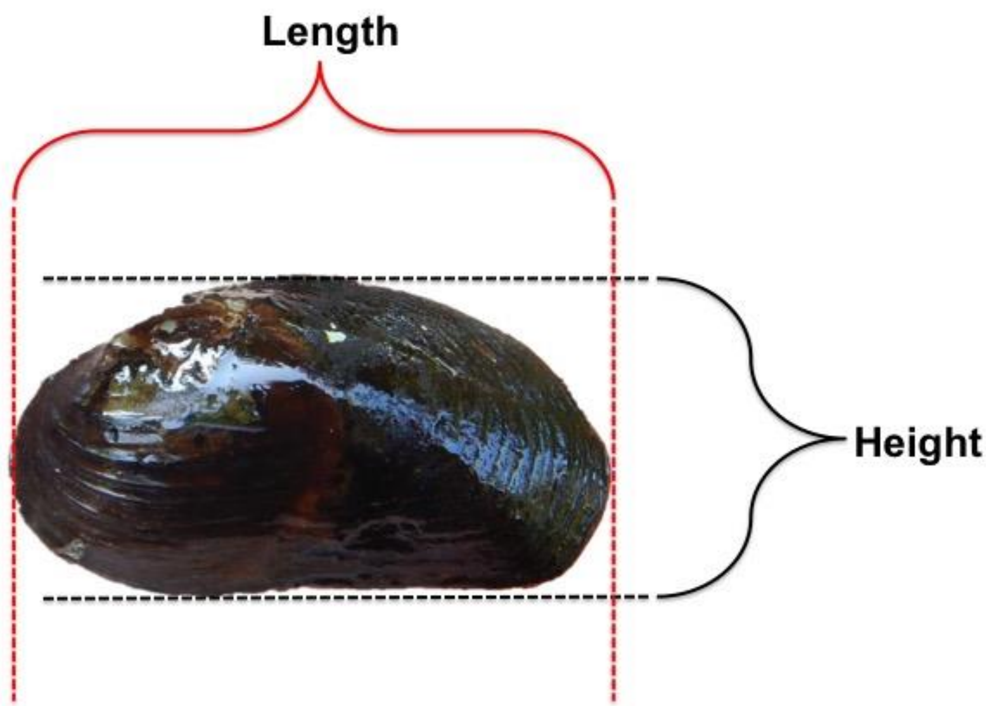
Appendix B. Random distance to find starting point at site

Random distances can be assigned in any way that allows for the process to be random and to be constrained from 0-100m (i.e., smart phone, GPS, other). The sets of numbers below are random values (0-100) that can be used to assign a random starting point for each site. The value here is the number of meters added onto the initial fixed distance upstream of the crossing (i.e., 100m). Each value below represents a single random value and should not be used twice. For example, at the first site, you may add 60 m onto the initial fixed distance. Once a value is used, you may move horizontally or vertically to the next value.

60	3	71	1
89	11	12	1
90	35	0	19
99	38	17	88
16	22	98	92
87	24	34	52
2	45	20	38
84	67	72	40
84	63	22	59
22	57	83	54
96	82	39	45
88	40	28	7
94	89	7	44
100	79	2	39
77	94	3	34
85	73	56	18
52	92	91	30
52	57	1	5
37	82	87	26
13	90	0	79
47	97	66	30

Appendix C. Shell measurement diagram

This diagram shows the measurements that should be recorded for length and height of 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 mm.



Appendix D. Shell condition index

Shell condition provides an index of the general physical health of a mussel, taking into account 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 (for Brook Floater, *Alasmidonta varicosa*) shows a progression of shell degradation from light to heavy for Brook Floater. 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. Photos are provided by Ethan Nedeau.

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