

**STUDY PLAN FOR
FRESHWATER MUSSEL INJURY DETERMINATION,
POPULATION ASSESSMENT AND POTENTIAL
FUNCTIONAL ROLES
OF NATIVE MUSSELS
IN MULTIPLE SECTIONS OF THE UPPER HUDSON
RIVER:
2015 REMEDIAL INJURY STUDY
AMENDMENT 2**

HUDSON RIVER NATURAL RESOURCE TRUSTEES

STATE OF NEW YORK

U.S. DEPARTMENT OF COMMERCE

U.S. DEPARTMENT OF THE INTERIOR

PUBLIC RELEASE VERSION*

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**Names of certain individuals and affiliations have been removed to maintain confidentiality.*

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1.0 INTRODUCTION

Past and continuing discharges of polychlorinated biphenyls (PCBs) have contaminated the natural resources of the Hudson River. The Hudson River Natural Resource Trustees—New York State, the U.S. Department of Commerce, and the U.S. Department of the Interior—are conducting a natural resource damage assessment (NRDA) to assess and restore those natural resources injured by PCBs.

The Hudson River PCBs Superfund Site (the “Site”) extends about 200 miles between Hudson Falls and the Battery in New York City. A 40-mile stretch of the freshwater non-tidal Upper Hudson River, from Fort Edward to the Federal Dam at Troy, NY, is the site of an extensive PCB federal Superfund remediation project being conducted by the General Electric Company pursuant to the Record of Decision issued by EPA in 2002. Dredging to remove PCBs, followed by capping or backfilling of dredged areas, was implemented between 2009 and 2015 with habitat reconstruction continuing into 2016 (Parsons 2016).

The Hudson River Natural Resource Trustees have been assessing PCB contamination and injuries to natural resources in the Hudson River. As part of the NRDA, the Trustees conducted a pilot freshwater mussel survey in the Fort Miller and Stillwater Pools in 2013 (HRNRT 2014a). Based on the 2013 preliminary investigations, the Trustees then determined it was appropriate: (a) to conduct a freshwater mussel survey in 2014 in additional pools of the Upper Hudson River where remediation had yet to commence, (b) to revisit pools following remediation, and (c) to sample upstream reference pools.

The Draft Study Plan for Mussel Injury Investigation for the Hudson River was released for public review and comment on June 2, 2014 (HRNRT 2014b). On August 15, 2014, following the 30-day comment period, the Study Plan for Freshwater Mussel Injury Determination, Population Assessment and Potential Functional Roles of Native Mussels in Multiple Sections of the Upper Hudson River: 2014 Remedial Injury Study (Study Plan) was finalized (HRNRT 2014c). The Responsiveness Summary for the Study Plan was issued September 2, 2014 (HRNRT 2014d). A May 26, 2015 amendment to the Study Plan (HRNRT 2015) informed the public that the survey would be implemented in 2015 rather than 2014. That amendment recognized that a pool might be surveyed after dredging such that results would constitute mussel surveys in “unremediated”¹ and “remediated areas” of the pool rather than in “unremediated” and “to be remediated areas”. The results of the 2013 and 2015 studies will be used to assess potential injuries to these freshwater mussel resources and will also be used to help determine whether future studies will be performed, and if so, to help in their design.

2.0 Methods

This document constitutes a second amendment to the 2014 Study Plan. The survey work conducted in 2015 is consistent with the work set forth in the 2014 Study Plan and the 2015 Amendment with the following modifications.

2.1 Data Collection and Sample Processing

1. The Trustees planned to survey “unremediated” areas, “to be remediated” areas, and “remediated” areas during 2015. The Lower Mechanicville Pool was targeted because remediation had not occurred in prior years but was scheduled for 2015. Our goal was to survey this pool (Reach 4) in advance of the dredges, but remediation commenced in May, before the start of the mussel survey, and work continued in the pool until November (Parsons 2016). Due to safety concerns, the sampling was shifted to the Upper Mechanicville Pool, which was dredged

¹ Synonymous with “non-remediated” areas

(Certification Units 91-93) the prior year (Parsons 2015). The Upper Mechanicville survey was limited to “unremediated areas”. This allowed for completion of mussel surveys in other larger upstream pools affected by more extensive targeted dredging but allowed for the assessment of mussels in the Upper Mechanicville Pool relative to the other surveyed pools.

2. It was anticipated that around 100 sites would be surveyed per strata per pool, but the number of sites sampled per pool varied based on pool and strata size and sampling constraints (e.g., water level shifted sample location from submerged to upland condition, presence of dense beds of water chestnut).
3. Reasonable distances upstream and downstream of dams were excluded prior to sample selection because of known safety concerns. This exclusion zone included the area between the dam and the safety wire and beyond.
4. The start point for sampling within a pool was randomly selected from the list of sampling locations, but the block of daily sampling sites was not random due to logistical constraints. Each targeted pool and stratum was completed, so bias was not introduced by this modification.
5. Mussels whose dry weight (Strayer *et al.* 1994) was used in the length-weight regressions were stored in a refrigerator at 5°C prior to processing.

2.2 Thin Sectioning

1. Thin-section analysis will be used to internally age a representative cross section of shell sizes of each abundant species of mussels following the Standard Operating Procedures and Methods for Determination of Mussel Age. Data will be recorded on the Mussel Aging Data Sheet. These Standard Operating Procedures and data sheet are provided in Appendix 1.
2. We set a goal of sending 100 shells from each strata present within the impacted pools surveyed in 2013 and 2015 for thin-sectioning, although some shells shipped for sectioning may turn out to be unsuitable for aging by this method. Shells from some pools or strata may not be thin-sectioned, e.g., limited number of mussels collected in a given pool or strata, shell length <11 mm. If the total number of shells collected in a given strata is fewer than a count of 100, some or all may be thin-sectioned.
3. Strata sampled in 2013 consisted of areas within a pool that were targeted for remediation but had not been dredged yet (“to be remediated”, also termed “before remediation”) and areas not targeted for remediation (“non-remediated” areas). Strata sampled in 2015 had already been dredged (“remediated”, also termed “after remediation”) or were not targeted for cleanup (“unremediated” areas, also termed “non-remediated”).
4. Ninety mussels were randomly selected and binned into 5 mm lengths, e.g., 45-49 mm, 50-54 mm. These shells were used in the age length histograms developed for each pool. Ten additional mussel shells were non-randomly selected to increase the number of mussels in a given 5 mm size class that were under-represented or lacked any representation. Shells within those size classes were then randomly selected. The random and non-random samples are used together to develop an age-length von Bertalanffy growth curve (von Bertalanffy 1938) for each pool.
5. During the 2013 survey, most of the surveyed mussels were retained while in 2015 all surveyed mussels were retained. To avoid introducing bias into the 2013 shells selected for aging, the length of shells retained and therefore available for aging were compared to the full complement of surveyed shells. These size frequency distributions were binned into 5 mm lengths and examined. Differences in size class structure of the retained vs. surveyed mussels were addressed during the selection of shells for aging to ensure that the population subsampled for aging was representative of the proportional distribution and frequency of size classes surveyed per pool and strata. This adjustment was performed for the Fort Miller Pool to randomly exclude or select individuals within each length bin to restructure a size frequency distribution from the retained shells similar to the surveyed samples.

6. A pilot mark and recapture study (e.g., Haag and Commens-Carson 2008) was implemented in two pools of the Upper Hudson River during summer 2018 following the Standard Operating Procedures for Freshwater Mussel Mark and Recapture to Confirm Annual Growth Ring Deposition (see Appendix 2). Approximately 50 mussels representing various size classes of abundant species were collected, marked, and placed within a 2-3 m² plot per pool. Marked mussels will be collected about a year later in the summer of 2019. The expectation is that only a subset of marked mussels will be found. The recaptured mussels will be thin-sectioned to demonstrate deposition of a single internal growth ring (annulus) over the approximate one-year time frame. The field data will be recorded on the Freshwater Mussel Mark and Recapture Data Sheet (included in Appendix 2). Thin sectioning will follow the Standard Operating Procedures and Methods for Determination of Mussel Age (Appendix 1) used for aging mussel shells collected in 2013 and 2015 with modifications described in the Standard Operating Procedures for Mussel Mark and Recapture to Confirm Annual Growth Ring Deposition (Appendix 2). Laboratory derived data associated with thin-sectioning will be recorded on the Mussel Aging Data Sheet (included in Appendix 1).

2.3 Schedule

The shifted schedule for surveys and thin sectioning resulted in the final project report being delayed. A final project report on mussel surveys and ecosystem services is anticipated in 2019, with the analysis of aging by thin-sectioning included in a separate report around the same time frame. Results of the mark and recapture study are anticipated in late 2019 to mid-2020.

3.0 REFERENCES

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- von Bertalanffy, L. 1938. A quantitative theory of organic growth (inquires on growth laws. II). *Human Biology* 10(2):181-213, https://www.jstor.org/stable/pdf/41447359.pdf?seq=1#page_scan_tab_contents.

APPENDICES

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APPENDIX 1: Standard Operating Procedures and Methods for Determination of Mussel Age

Version created April 23, 2018; revised March 14, 2019

The purpose of this standard operating procedure is to describe methods and analytical techniques for thin-sectioning mussel shells, aging mussels from thin-sections, and analysis of length and age using a von Bertalanffy growth curve.

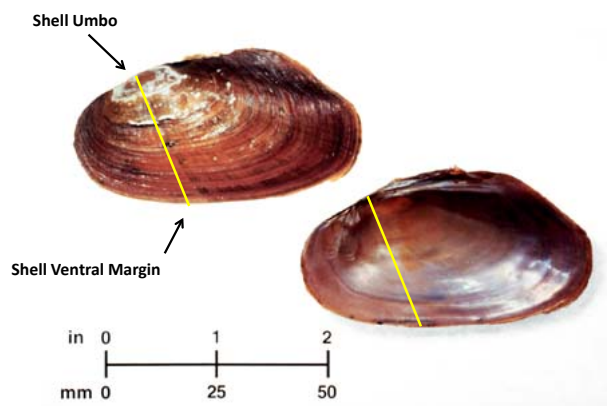
All shells, shell thin sections, and data sheets will be stored in a secured office at the facility conducting the thin-sectioning. These products will be shipped to the New York State Museum in 2019.

External Aging¹

1. Count the number of external annuli of each, record on the Mussel Aging Data Sheet.
2. If the periostracum is badly weathered such that annuli cannot be easily visualized, do not age the individual but make a note why this mussel was not aged.

Thin-Sectioning

1. Thin-sections of shells will be prepared following procedures described by Clark (1980) and Neves and Moyer (1988), using a Buehler Isomet low-speed saw unit with a diamond-impregnated blade (Buehler, Evanston, Illinois). Shells will be cut from the center of the umbo to the ventral margin. The yellow line in the following diagram illustrates the approximate location of where the shell will be cut and thin-sectioned.



2. Prior to gluing, the cut shell edges will be sanded with 320 and then 1000 grit waterproof sandpaper (Norton/Saint-Gobain Abrasives, Worcester, MA) on a glass plate.
3. Cut valves will be glued (2-Ton Clear Epoxy) to petrographic microslides (27 × 46 mm), vacuum-sealed into a petrographic chuck, attached to the cutting arm of the saw, and sectioned at a thickness of 280 μm (Neves and Moyer, 1988).
4. Thin-sections of shells will be examined using dissecting microscope at 40X magnification. Internal growth lines will be considered true annuli if they are continuous from the umbonal region to the outer surface of the shell. It will be assumed, based on previous shell-aging of mussels, that one annulus is formed each year. The assumption of annual shell ring deposition in freshwater mussels has been validated in more than

¹ External rings will be counted if metric not previously recorded.



a dozen species in North America (Veinott and Cornett, 1996; Haag and Commens, 2008). In the following diagram (Haag and Commens 2008), an example of a true annulus can be seen in a shell thin section of the freshwater mussel *Obliquaria reflexa* from the Sipsy River, Mississippi.

5. The thin-section slide will be read at least twice by at least two different people.
6. The age for each individual mussel will be recorded. The first reading will be considered the first read and score of the specimen. A second read and score of each individual thin-section will serve as the QA/QC for all aged specimens. Ages from each of the two readings should not be consistently higher or lower from each other. The two final sets of ages will be averaged together to represent the reported age.
7. A subset or more of shells with two independently recorded ages may be read a third time by a third party when there are discrepancies between the two reads that should be rechecked. If the third party reading is the final determination, it will represent one of the two readings of age for a given shell in calculating the reported age, as described in #6, above.
8. All data associated with internally aging each mussel will be recorded on the Mussel Aging Data Sheet and then uploaded to an Excel spreadsheet.

Shell Height and Length¹

1. Calipers will be used to measure shell length and shell height for each specimen internal aged by the thin-sectioning technique.
2. Shell length is defined as the projected straight line distance from the anterior most point of the mussel shell to the most posterior point, where length is measured with the shell sample held on its edge.
3. Shell height is defined as the maximum projected straight line distance between the dorsal and ventral edges on a valve (one half of a complete shell). Height includes the projection of the beak, the dorsal protuberance on the shell. It also includes the umbo if it is raised above the shell.
4. Shell length and shell height will be recorded on the Mussel Aging Data Sheet.
5. Lengths for 0 to 3 year-old individuals, if not included in specimens for internal aging, will be obtained by back-calculating length-at-age based on internal annuli of 5-10 shells >10 years of age because shells ≤3 years old are difficult to collect from the river. On adult mussels the first external annulus will be considered Age 0 annulus.

¹ Definitions per the American Museum of Natural History.

Growth Curves

1. Using internal aging and other results, von Bertalanffy growth curves will be developed. A von Bertalanffy growth curve (von Bertalanffy, 1938) is:

$$L_t = L_\infty (1 - e^{-K(t-t_0)})$$

where, L_t is mussel length at time t (age), L_∞ (L -infinity) is a theoretical average maximum (asymptotic) length, K is a growth coefficient indicating how quickly L_∞ is approached, t is time or age in years, t_0 is the time in years when length would theoretically be equal to zero, and e is the natural log exponent.

2. Parameters of the von Bertalanffy growth equation and associated significance tests will be estimated using the Fisheries Stock Assessment program (FSA-package) developed by Dr. Derek Ogle (2016) at Northland College, Wisconsin. The FSA-package will be implemented in the program R (R Development Core Team 2006).
3. The growth curves will be copied into an Excel spreadsheet containing the data associated with thin-section aging data.

Document and Shell Management

1. Shells with unique identification numbers will be received at the thin-sectioning facility from the field collector. The slide number will match the unique identification numbers on the shell. No new identification numbers will be added during the aging process.
2. Chain of custody sheets will be reviewed and signed after shells are received by the facility conducting the thin-sectioning and again upon shipment to the New York State Museum where shells will be archived.
3. All shells, shell thin sections, and data sheets will be stored in a secured office at the facility conducting the thin-sectioning. A second party will verify that the Excel data was correctly entered.
4. All shells, shell thin sections, and data sheets will be shipped to the New York State Museum, who will serve as a repository, after completion of the analyses.

Products: Age data of all mussel shells thin-sectioned, growth curves, statistical analyses of any site specific growth differences, and final report.

References

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MUSSEL AGING DATA SHEET

Date: _____ Project Name: _____ Site Location: _____

Species: _____ First Recorders Name: _____ Second Recorders Name: _____

First Recorders Signature: _____ Second Recorders Signature: _____

Shell Identification #	Slide #	Shell Length (mm)	Shell Height (mm)	External Annuli #	Internal Annuli # (1 st Read)	Date of 1 st Read	Internal Annuli # (2 nd Read)	Date of 2 nd Read

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APPENDIX 2: Standard Operating Procedure for Freshwater Mussel Mark and Recapture to Confirm Annual Growth Ring Deposition

Version created July 20, 2018; revised March 12, 2019

The purpose of this standard operating procedure is to describe methods and analytical techniques for marking and recapturing freshwater mussels and quantifying the shell growth patterns, i.e., number of growth rings deposited between time of initial marking and recapture approximately one year later.

Field Activities Mark and Recapture

1. The mark and recapture protocol is adapted from Haag and Commens-Carson (2008).
2. Approximately 50 freshwater mussels representing various size classes will be collected by wading, scuba or snorkeling from each of two Upper Hudson River pools (between Ft Edward and the Federal Dam at Troy, NY) during the summer of 2018. The start date of the study will allow for deposition of new shell material (i.e., shell growth) following handling and tagging and prior to cessation of growth in the winter. The study will focus on abundant species only. A GPS coordinate will be taken to represent the collection location area and will be recorded on the Freshwater Mussel Mark and Recapture Data Sheet.
3. To minimize handling effects, freshwater mussels will remain submerged in river water except during measurement, notching and attachment of a unique identification tag.
4. Each individual mussel shell will be notched 1-2 mm in the ventral margin (see image below under the Tagging subsection) of the shell using a triangular file. This notch will serve as a marker or reference point on both the shell exterior and interior. The pseudo-disturbance ring resulting from handling will also be used as a reference point.
5. Each mussel will be individually tagged (see Tagging section, below) on its external shell. Tags are typically placed on the posterior-dorsal margin away from the anterior where the mussel burrows to prevent abrasion to the tag during burrowing. Vinyl Hallprint FPN glue-on shellfish tags will be attached using superglue. The preprinted unique identification number is alphanumeric and will be recorded on the Freshwater Mussel Mark and Recapture Data Sheet.
6. The length of each mussel will be recorded at time of initial collection on the Freshwater Mussel Mark and Recapture Data Sheet following the procedure specified in the Standard Operating Procedures and Methods for Determination of Mussel Age and the 2014 Study Plan (HRNRT 2014). The data sheet will be checked and verified before putting notched and measured mussels into a labeled mesh bag in order to catch any possible ID or measurement transcription errors or illegibility.
7. Marked mussels will be placed in a 2-3 m² plot. The four corners of the plot will be demarked with Rebar hammered into the sediment except for a small length that will display distinctive marking (e.g., flagging, colored caps, paint) to aid in the recapture of the tagged mussels.
8. A GPS coordinate will be taken directly above the plot to aid in the recapture of the tagged mussels. The coordinates will be recorded on the Freshwater Mussel Mark and Recapture Data Sheet.
9. Marked mussels will be placed partially in the substrate with the anterior end oriented downward.
10. Efforts to recapture the marked mussels will occur in summer of 2019. Based on results of others (Haag and Commens-Carson 2008, Neves and Moyer 1988, Villella *et al.* 2004), a 100% recapture rate is not anticipated.
11. The length of each recaptured mussel will be determined in the field following the procedures used at time of initial collection and recorded on the Freshwater Mussel Mark and Recapture Data Sheet.

12. Recaptured mussels will be placed in labelled zip lock bags and placed in an ice-filled cooler for transport back to the laboratory where they will be frozen in a chest freezer at -20°C. The frozen mussels will be shipped to an external facility for thin sectioning and confirmation of annual ring deposition (described below).
13. The Museum collection number will be assigned to the shells and thin-sections upon receipt by the New York State Museum.
14. All data will be entered into an Excel spreadsheet.

Tagging

1. Keep the collected mussels in a mesh bag, cage or other holding container in the river near the tagging station. Mussels can even hang off the boat or a boat dock. Ideally, mussels should be kept in flowing water in the shade.
2. Have two pans of water at the tagging station, one with mussels “to be tagged” and one with freshly “tagged” mussels. Change the water regularly to keep it cool and oxygenated; the change frequency will depend on air temperature but will likely occur when a group of mussels have been tagged and the next round of tagging begins.
3. Grab five mussels from the holding container and put them in the “to be tagged” pan.



4. Use a green scrubby pad to clean a small spot of the shell. The best spot is on a flat spot away from the anterior end and away from the eroded umbo. See the placement of the gray letter/number tag in the image above. The glob of clear in the photo is a PIT Tag and should not be confused with the Hallprint tag being used in this study.
5. Put on gloves to protect hands from superglue. Place a drop of superglue on the cleaned spot. Drying of the shell prior to placement of glue is not necessary.
6. Grab the unique Hallprint tag with tweezers, and lay it on the drop of superglue and gently press down. Do not use fingers as this superglue will bond skin together quickly. Use acetone to remove glue on skin.
7. Place the freshly tagged mussel into the “tagged” pan of water. Allow glue to water-cure for several minutes in the pan.
8. Place tagged and notched mussels back in the river in a second mesh bag or other holding container until divers can place the marked mussels in the mark and recapture plot.

Thin-Sectioning

Thin sectioning of recaptured mussels will follow the thin-sectioning procedures specified in the Standard Operating Procedures and Methods for Determination of Mussel Age (Appendix 1) with the following modifications:

1. Mussels will be kept frozen at -20°C at the thin-sectioning lab until they are shucked.
2. Shucked shells will be stored at room temperature until they are externally aged¹, measured (length) and thin-sectioned.
3. The thin-section cut will be made adjacent to the notch so that the filed notch is intact and can be used as a reference point for determining the number of growth rings deposited after the notch was filed in the summer of 2018.
4. The shell margin at time of notching will be established for each thin section.
5. A mark will be made on the thin section slide corresponding to the filed notch on the other half of shell.
6. Disturbance rings alone are not indicative of annual ring deposition but can serve as a reference point similar to the notch, for quantifying the number of growth rings accumulated since the start of the study.
7. The number of shell rings deposited after notching are recorded by at least two independent readers.
8. All data will be recorded on the Freshwater Mussel Aging Data Sheet (included in Appendix 1) and entered into an Excel spreadsheet.

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¹ External rings will be counted if metric not previously recorded.

version created July 20, 2018

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Freshwater Mussel Mark and Recapture Data Sheet Collection and Marking Data

Date: _____ Hudson River Pool: _____

Field Personnel: _____

Collection Location(s) Coordinates: _____

Holding Coral Location Coordinates: _____

Mussel Species: _____

Initial Collection or Recapture (circle one)

Mussel Count	External Tag ID	Mussel Length (mm)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		

Mussel Count	External Tag ID	Mussel Length (mm)
26		
27		
28		
29		
30		
31		
32		
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37		
38		
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Notes:

Date entered _____ ()

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