ILLINOIS
Illinois Natural History Survey
PRAIRIE RESEARCH INSTITUTE

# Northern Riffleshell and Clubshell Reintroduction Project
# Summary of Activities from 2014-2018

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**Illinois Natural History Survey Technical Report 2019(06)**
22 February 2019

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# EXECUTIVE SUMMARY

Staff from the Illinois Natural History Survey participated in a joint project with the Illinois Department of Natural Resources and the U.S. Fish and Wildlife Service to reintroduce the federally-endangered Northern Riffleshell (*Epioblasma rangiana*) and Clubshell (*Pleurobema clava*) to Illinois. A salvage project in the Allegheny River, Pennsylvania provided an opportunity for the translocation of both species. Since the first translocation in 2010, a total of 3,699 Northern Riffleshell and 4,166 Clubshell were collected from the Allegheny River at the U.S. Highway 62 (=Hunter Station) Bridge, Tionesta Township, Forest County, Pennsylvania, and translocated to suitable habitat at eight sites in the Vermilion River basin (Wabash River drainage) in Champaign and Vermilion counties, Illinois.

Our monitoring data suggest the Clubshell translocation has been more successful than the Northern Riffleshell translocation. We estimate that Clubshell have approximately 5 times greater survival rates compared to Northern Riffleshell. As a result, very few Northern Riffleshell likely remain. Survival also varied among translocation sites, likely a result of local-scale differences such as substrate or gradient. Regardless, high discharge events posed the greatest threat for the long-term success of this project, as survival was significantly decreased following periods of abnormally high flows. Consequently, we believe that repeated translocations of individuals over a period of several years across several sites could be implemented to reduce the overall risk of failure due to high discharge events or local-scale differences.

This translocation project is being funded, in part, by a natural resource damage assessment settlement (Hegeler Zinc—Lyondell Basell Companies) to the U.S. Fish and Wildlife Service and to the State of Illinois.

# INTRODUCTION AND BACKGROUND

The Northern Riffleshell (*Epioblasma rangiana*) and Clubshell (*Pleurobema clava*) were once widespread throughout the Ohio River basin (Figure 1) but both have experienced significant range reductions during the last century as a result of reduced habitat and water quality. Because of this range reduction, the U.S. Fish and Wildlife Service (USFWS) listed both species as federally-endangered in 1994. The USFWS' Northern Riffleshell and Clubshell Recovery Plan, approved in 1994, identified a recovery objective of establishing viable populations in ten separate river drainages via augmentations and reintroductions (USFWS 1994). Beginning in 2006, the Illinois Department of Natural Resources (IDNR) and the Illinois Natural History Survey (INHS), partnered with USFWS and state agencies in Ohio, Pennsylvania, and West Virginia and began implementing portions of the federal recovery plan by reintroducing both the Northern Riffleshell and Clubshell into the Vermilion River basin (Wabash River drainage) in Illinois.

A salvage project in the Allegheny River at the U.S. Highway 62 (=Hunter Station) Bridge, Tionesta Township, Forest County, Pennsylvania River (Figure 2) provided an opportunity to translocate Northern Riffleshell and Clubshell. Between 2010 and 2016, a total of 3,699 Northern Riffleshell and 4,166 Clubshell were translocated to eight sites in the Vermilion River (Wabash) basin in Champaign and Vermilion counties, Illinois (Figure 3; Appendix 1). Of those animals, 2,099 Northern Riffleshell (1,196 males and 903 females) and 1,766 Clubshell were PIT (passive integrated transponder) tagged to allow monitoring to determine success of the project (Figure 4; Figure 5).

Specifically, during the contract duration (2014 – 2018), 750 Northern Riffleshell and 808 Clubshell were collected at the U.S. Highway 62 bridge construction project in Allegheny River, Forest County, Pennsylvania on 25 August 2014. Animals were quarantined for four days at the INHS Aquatic Research Pond Facilities in Champaign-Urbana. While in quarantine, all individuals were affixed with a unique PIT tag and a plastic shellfish tag. After the quarantine period, the animals were divided and translocated to four locations. In addition, EnviroSciences, Inc., facilitated the shipping of 1,640 Northern Riffleshell and 2,360 Clubshell in 2016; doing so expedited the release and eliminated the need to travel to Pennsylvania. These individuals were translocated to existing sites as well as new sites within IDNR properties.

The tasks of this contract, as described by the grant agreement between IDNR and INHS, were to translocate mussels when opportunities arose, monitor translocated mussels based upon a robust design sampling protocol, and submit a comprehensive report summarizing project results. Historical yearly data during contract duration were summarized by Tiemann (2014, 2015; Appendix 1) and Tiemann et al. (2015, 2016, 2017; Appendix 1). The robust design data analyses were used to determine both detection (the probability of finding an animal given that it is still present at a site) and survival (the probability an animal is still present at a site) of translocated mussels. Results from the robust design data analysis were published by Stodola et al. (2017; Appendix 2). This report summarizes the 2018 project activities, as well as consolidates all the findings from the contract duration into a comprehensive document.

# 2018 PROJECT ACTIVITIES

As with 2017 (Tiemann et al. 2017), there was not an opportunity in 2018 to translocate additional individuals from Pennsylvania[1]; thus, the only activity that occurred in 2018 was monitoring. Stodola et al. (2017) suggested monitoring translocated Northern Riffleshell and Clubshell in autumn because of greater detection rates. Therefore, we only monitored each site once during 2018 (Table 1). When monitoring PIT-tagged mussels, at least two samplers used a PIT tag reader to slowly sweep over the streambed in a systematic manner (Tiemann 2014). Detected tag data were stored in the PIT reader and later downloaded into a database after the sampling period ended. At least one additional sampler snorkeled (or used view buckets) if temperature and flow levels permitted and visually examined the streambed for tagged mussels. If a tagged mussel was directly observed, a note was made in the database whether that individual was alive or dead. The region sampled included the area stocked plus a 20-meter buffer to account for mussels moving or washing downstream.

Table 1. Raw-data observations by species for each translocation site (with stream name) for the 2018 calendar year for PIT-tagged mussels using the hand-held wands (NRS = Northern Riffleshell and CS = Clubshell; Figure 5). Data are number observed / number of individuals originally translocated in the stream at a site. Site information can be found in Appendix 1 and Figure 3. "NS" = not sampled.

| Species | *Richter (Salt)* | *Smith (Salt)* | *Donut (Salt)* | *MFNP (Middle)* | *Ford (Middle)* | *Horse (Middle)* | *Kennekuk (Middle)* | *Beaver (Middle)* |
|---|---|---|---|---|---|---|---|---|
| NRS | 10/236 | 61/549 | 81/420 | NS | 20/250 | NS | NS | NS |
| CS | 70/363 | 248/340 | 227/427 | NS | 53/285 | NS | NS | NS |

In addition to monitoring using hand-held wands, there was an opportunity to borrow a BioMark Inflatable Floating Antenna (Figure 6) from the USFWS – Marion Office. The floating antenna was approximately 10 feet x 4 feet and capable of detecting a PIT tag within ~3 foot radius of that. We were able to pull this antenna behind a canoe and traverse long sections of both the Middle Fork and Salt Fork with the hopes of detecting lost tags (e.g., individuals that may have moved or been washed outside of our translocation areas). We completed a ~6-mile float on the Middle Fork from "Ford" (e.g., our upstream most translocation area), through "Horse" and "Kennekuk" to the canoe launch at Kennekuk County Park (Figure 3). We also were able to float the Salt Fork from our Richter translocation area, downstream to the "Singing Bridge" (=Vermilion County Road 850E), just south of Oakwood (distance of ~4 river-miles, Figure 3). During these surveys, 96 individuals were encountered by the floating antenna and 23 of these were found downstream from their translocation site (Table 2). Distance traveled by the displaced mussels observed with the floating antenna ranged from 20 yards to over 2 miles! These data, along with the robust analysis data (Stodola et al. 2017, Appendix 2),

[1] The U.S. Highway 62 (=Hunter Station) Bridge over the Allegheny River (Forest County, Pennsylvania) mentioned in Tiemann (2014, 2015) and Tiemann et al. (2015, 2016) was imploded on 4 October 2017. Therefore, any additional translocations from this site seem unlikely as the majority of the instream work has been completed.

demonstrate that translocated mussels can become dislodged and washed downstream. Because of logistical constrains (e.g., water levels and time constraints), attempts were not made to assess survivorship of these dislodged individuals.

# PROJECT SUMMARY

Since the initial 2010 translocation, 3,866 (87%) of the 4,360 tagged individuals[2] have been observed at least one-time during monitoring efforts – 1,914 (82%) of the 2,347 placed Northern Riffleshell and 1,947 (97%) of the 2,006 placed Clubshell (there were 7 individuals that the identification did not get recorded during the 2012 tagging event; of those, 5 were later detected via a PIT tag reader during subsequent monitoring events). The robust design estimation procedure analyzed a subset of the observation data (2012 to 2016) to determine survival and detection rate for the translocated mussels. Detection of individual mussels was approximately 0.78 across both species, but was variable among sites and sampling periods (Stodola et al. 2017). However, average detection during the autumn period was 1.25 times greater than for spring or summer, suggesting that future monitoring efforts would be most efficient during this period. Annual survival of translocated Clubshell was approximately 5 times greater than for Northern Riffleshell, and on average Clubshell annual survival was 0.79 compared to 0.30 for Northern Riffleshell. Finally, survival for both species appeared to increase with time, with survival being greatest during the fourth year following translocation. However, major flood events were a significant predictor of survival, and potentially troublesome for long-term success of the reintroduction program. For more information see Stodola et al. (2017) in Appendix 2.

It is too early to determine if the translocations are successful because recruitment has not been assessed. We continue to collect raw observation data during monitoring efforts to update survival rate estimation. Our monitoring data and subsequent publication (Stodola et al. 2017) suggest that the Clubshell translocation is more successful than Northern Riffleshell because Clubshell have survived at a much greater rate. Across all sites, Clubshell were five times more likely to survive than Northern Riffleshell. We also observed that survival varied among sites, likely a result of local-scale differences such as substrate or gradient, among our translocation areas. Regardless, high discharge events seem to pose the most looming threat for success of this project. Repeated translocations of individuals over a period of several years could be implemented to reduce the overall risk of failure due to these stochastic events.

# ACKNOWLEDGMENTS

This project is a collaborative effort among the U.S. Fish and Wildlife Service (USFWS), Pennsylvania Fish and Boat Commission, Pennsylvania Department of Transportation, Illinois Department of Natural Resources (including the Illinois Nature Preserves Commission and the Illinois Endangered Species Protection Board), Illinois Natural History Survey (INHS), University

[2] Note: not all of the 3,699 Northern Riffleshell and 4,166 Clubshell translocated since 2010 were affixed with a PIT tag. Some of the individuals translocated in 2016 were marked with glitter tags.

of Illinois, Indiana Department of Natural Resources, the Ohio State University, West Virginia Department of Natural Resources, Kentucky Department of Fish and Wildlife, BioMark, and EnviroSciences, Inc. We'd like to single out EnviroSciences, Inc., especially Greg Zimmerman, Ryan Schwegman, and Phil Mathias, for facilitating the shipping of mussels in 2016, and expediting the translocation process. Rachel Vinsel (INHS) assisted in data management and field support. Donovan Henry (USFWS) allowed use of the BioMark floating antenna. Matthew Mangan (USFWS), Collin Moratz (USFWS), and INHS staff Sara Andree, William Nixon, Josh Sherwood, Allison Sieja, Andrew Stites, and Rachel Vinsel assisted in the field in between 2014-2018. The Illinois Department of Natural Resources (through the Natural Resource Damage Assessment settlement: Hegeler Zinc—Lyondell Basell Companies --- Reference Document #OREP1402 & #OREP1504), as well as the USFWS's Ohio River Basin Fish Habitat Partnership (Award #F14AC00538) providing funding for this project.

# LITERATURE CITED

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Stodola, K.W., A.P. Stodola, and J.S. Tiemann. 2017. Survival of translocated Clubshell and Northern Riffleshell in Illinois. Freshwater Mollusk Biology and Conservation 20:89-102.

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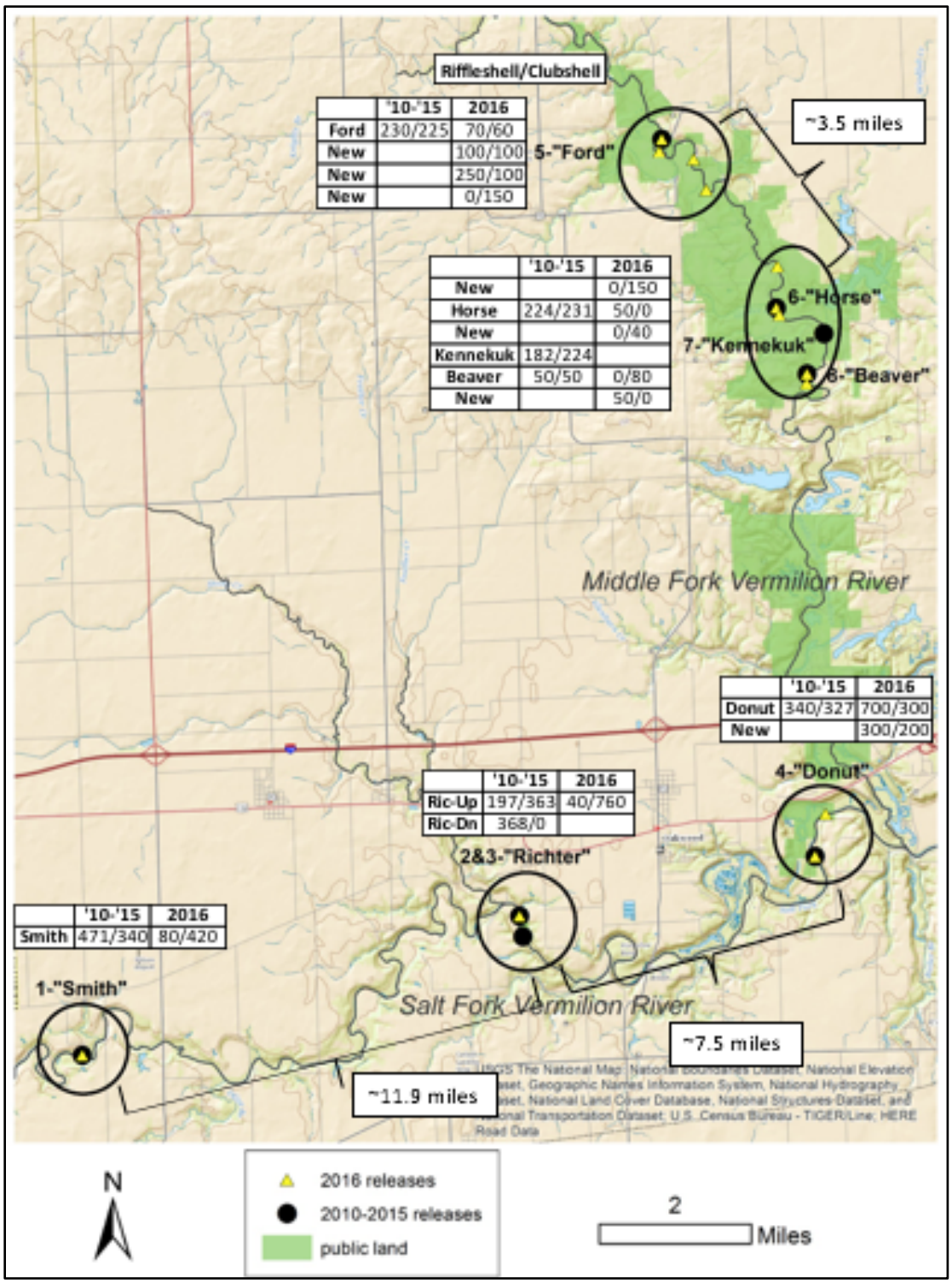
Figure 1. The Northern Riffleshell *Epioblasma rangiana* (lower left) and Clubshell *Pleurobema clava* (upper right).

Figure 2. Allegheny River at the former U.S. Highway 62 (=Hunter Station) Bridge, Forest County, Pennsylvania, about 3 river miles downstream of the town of Tionesta (approximate coordinates of bridge were 41.47235°N, 79.49992°W). Photo courtesy of Kevin Cummings (INHS).




| | '10-'15 | 2016 |
|---|---|---|
| Ford | 230/225 | 70/60 |
| New | | 100/100 |
| New | | 250/100 |
| New | | 0/150 |

| | '10-'15 | 2016 |
|---|---|---|
| New | | 0/150 |
| Horse | 224/231 | 50/0 |
| New | | 0/40 |
| Kennekuk | 182/224 | |
| Beaver | 50/50 | 0/80 |
| New | | 50/0 |

| | '10-'15 | 2016 |
|---|---|---|
| Donut | 340/327 | 700/300 |
| New | | 300/200 |

| | '10-'15 | 2016 |
|---|---|---|
| Ric-Up | 197/363 | 40/760 |
| Ric-Dn | 368/0 | |

| | '10-'15 | 2016 |
|---|---|---|
| Smith | 471/340 | 80/420 |

Figure 3. Translocation sites for Northern Riffleshell and Clubshell reintroduction project in the Vermilion River basin (Wabash River drainage). Not shown is Site "MFNP" located in Champaign County; this site was deemed unsuitable prior to 2014, and no additional translocations have occurred here since 2010 (Tiemann2014).

Figure 4. Northern Riffleshell with encased PIT tags (top two mussels) and plastic shellfish tags (bottom three mussels). Both tags were affixed with a “super glue” product and the PIT tags were later covered with marine grade epoxy. Each freshwater mussel had a PIT tag transponder on the right valve and a plastic shellfish tag on the left valve. Each PIT tag has a unique alphanumeric code (up to 13 digits), whereas each shellfish tag is unique when factoring in shape, color, and numeric code (up to four digits). All tags have been databased and contain the following information: PIT tag number and type, plastic shellfish tag number and type, species, sex, length at capture, collection year, origin of mussel, quarantine information, release date and site information, and detection history. Photo courtesy of Robert Szafoni (IDNR).

Figure 5. Researchers monitoring PIT-tagged freshwater mussels. When surveying for PIT-tagged mussels, a person carrying a PIT tag receiver wand slowly sweeps the streambed while walking the stream in a systematic manner. Detected tags are stored in the PIT reader, and data are later downloaded into a database after the sampling period ends. At least one additional sampler snorkels (or uses view buckets) if conditions are favorable and visually examines the streambed for tagged mussels. If an animal is seen, a note is made whether it was alive or dead. Photo courtesy of Rob Kanter (U of I).

Figure 6. Researchers using a BioMark floating antenna to monitor PIT-tagged freshwater mussels. Pulling it behind canoe allowed INHS staff to cover vast distances not normally monitored, thus allowing researches to encounter some translocated individuals >2 mi from where they were originally placed.

# APPENDIX 1. SUMMARY OF PROJECT ACTIVITIES 2014-2017

Summary of Northern Riffleshell and Clubshell reintroduction project activities from 2014-2017. Re-introduction site information, organized from upstream to downstream within a stream (Figure 3) is below; additional information can be found in Tiemann (2014).

**Middle Fork Vermilion River**

- Site "MFNP" is located within the Champaign County Forest Preserve District's Middle Fork River County Forest Preserve property. This site was deemed unsuitable prior to 2014, and no additional translocations have occurred here since 2010 (Tiemann2014).
- Site "Ford" is located within IDNR's Middle Fork State Fish and Wildlife Area.
- Site "Horse" is located within IDNR's Middle Fork State Fish and Wildlife Area.
- Site "Kennekuk" is located within IDNR's Middle Fork State Fish and Wildlife Area.
- Site "Beaver" is located within IDNR's Middle Fork State Fish and Wildlife Area.

**Salt Fork Vermilion River**

- Site "Richter Tract" is owned by the University of Illinois as a Research Natural Area and is managed by the U of I Committee on Natural Areas (CNA).
- Site "Smith Property" is a privately-owned property enrolled in the IDNR's Nature Preserve program (Edgewood Farm Land and Water Reserve).
- Site "Donut" – is located within IDNR's Kickapoo State Park.

## 2014 Project Activities (see Tiemann 2015)

A total of 750 Northern Riffleshell and 808 Clubshell were collected at the U.S. Highway 62 bridge construction project in Allegheny River, Forest County, Pennsylvania on 25 August 2014. After a 5-day quarantine period, the animals were divided and translocated to four sites in the Vermilion River basin, Vermilion County, Illinois. Sites were monitored during four sampling events – winter, spring, summer, and autumn (Table 3, Table 4).

Table 3. Raw data observation rates for Northern Riffleshell by site (with stream name per sampling period for the 2014 calendar year for PIT-tagged mussels (NRS = Northern Riffleshell and CS = Clubshell). Data are number detected / maximum number of individuals in the stream at a site at that period. "NS" = not sampled and "-" = sites were not yet established. *Only half of the Richter site was sampled.

| | *Richter (Salt)* | *Smith (Salt)* | *Donut (Salt)* | *MFNP (Middle)* | *Ford (Middle)* | *Horse (Middle)* | *Kennekuk (Middle)* | *Beaver (Middle)* |
|---|---|---|---|---|---|---|---|---|
| Winter 2014 | NS | NS | 17/50 | NS | 35/50 | NS | 11/49 | NS |
| Spring 2014 | NS | NS | NS | NS | 29/50 | 19/50 | 11/49 | 9/50 |
| Summer 2014 | 20/546 | 116/446 | 20/50 | NS | 25/50 | 19/50 | 9/49 | 11/50 |
| Autumn 2014* | 6/196 | NS | 275/336 | NS | 171/227 | 149/217 | 96/169 | 8/50 |

Table 4. Raw data observation rates for Clubshell by site (with stream name per sampling period for the 2014 calendar year for PIT-tagged mussels (NRS = Northern Riffleshell and CS = Clubshell). Data are number detected / maximum number of individuals in the stream at a site at that period. "NS" = not sampled and "-" = sites were not yet established. *Only half of the Richter site was sampled.

| | *Richter (Salt)* | *Smith (Salt)* | *Donut (Salt)* | *MFNP (Middle)* | *Ford (Middle)* | *Horse (Middle)* | *Kennekuk (Middle)* | *Beaver (Middle)* |
|---|---|---|---|---|---|---|---|---|
| Winter 2014 | NS | NS | 41/50 | - | 44/50 | NS | 20/50 | NS |
| Spring 2014 | NS | NS | NS | - | 47/50 | 32/50 | 27/50 | 43/50 |
| Summer 2014 | 190/367 | 286/338 | 42/50 | - | 47/50 | 31/50 | 20/50 | 41/50 |
| Autumn 2014* | 202/363 | NS | 296/328 | - | 194/225 | 197/231 | 173/224 | 41/50 |

## 2015 Project Activities(see Tiemann et al. 2015)

No mussels were translocated from Pennsylvania to Illinois during the 2015 calendar year. However, Sites were monitored during three sampling events – spring, summer, and autumn (Table 5, Table 6).

Table 5. Raw data observation rates for Northern Riffleshell by site (with stream name per sampling period for the 2015 calendar year for PIT-tagged mussels (NRS = Northern Riffleshell and CS = Clubshell). Data are number detected / maximum number of individuals in the stream at a site at that period. "NS" = not sampled and "-" = sites were not yet established.

| | *Richter (Salt)* | *Smith (Salt)* | *Donut (Salt)* | *MFNP (Middle)* | *Ford (Middle)* | *Horse (Middle)* | *Kennekuk (Middle)* | *Beaver (Middle)* |
|---|---|---|---|---|---|---|---|---|
| Spring 2015 | NS | NS | 193/336 | NS | 151/227 | 80/217 | 89/169 | NS |
| Summer 2015 | 16/546 | 96/446 | 188/336 | NS | 20/227 | 25/217 | 10/169 | NS |
| Autumn 2015 | 34/546 | 99/446 | 181/335 | NS | 19/227 | 9/217 | 11/169 | 5/50 |

Table 6. Raw data observation rates for Clubshell by site (with stream name) per sampling period for the 2015 calendar year for PIT-tagged mussels (NRS = Northern Riffleshell and CS = Clubshell). Data are number detected / maximum number of individuals in the stream at a site at that period. "NS" = not sampled and "-" = sites were not yet established (e.g., no Clubshell are placed at MFNP).

| | *Richter (Salt)* | *Smith (Salt)* | *Donut (Salt)* | *MFNP (Middle)* | *Ford (Middle)* | *Horse (Middle)* | *Kennekuk (Middle)* | *Beaver (Middle)* |
|---|---|---|---|---|---|---|---|---|
| Spring 2015 | NS | NS | 263/327 | - | 173/225 | 187/231 | 165/224 | NS |
| Summer 2015 | 116/363 | 288/338 | 240/327 | - | 107/225 | 96/231 | 21/224 | NS |
| Autumn 2015 | 154/363 | 300/338 | 240/327 | - | 102/225 | 25/231 | 24/224 | 38/50 |

It is important to note, a manure spill in the Stony Creek basin, a tributary to the Salt Fork, occurred on 11 September 2015. Over 100,000 gallons of liquid manure were discharged into Conkey Branch (=Stony Creek), resulting in nearly 100,000 dead fishes over a course of 10 stream miles, including portions of the Salt Fork that included the "Richter" translocation area. INHS and USFWS staff visited the relocation site on 17 September 2015 and detected 26 tagged animals. These animals were excavated and included one fresh-dead (=in situ without flesh but hinge line still pliable) Northern Riffleshell, 21 live Clubshell, three fresh-dead Clubshell, and one additional dead Clubshell that appears to have been dead prior to the spill based upon algae on shell. Six other freshwater mussel species, including the state-endangered Wavyrayed Lampmussel (*Lampsilis fasciola*) and state-threatened Purple Wartyback (*Cyclonaias tuberculata*), were encountered alive at the relocation site after the manure spill. With the approval of both USFWS and IDNR, we were able to excavate a larger portion of detected individuals at Smith and Donut in Autumn 2015 to compare survival of known fate individuals among sites in the Salt Fork post manure spill. Of the 20 individuals excavated at Smith, 17 were live Clubshell, one was a live Northern Riffleshell, and one Clubshell and one Northern Riffleshell were found dead in an exposed gravel bar. Of the 11 individuals excavated at Donut, six were live Clubshell, and five were live Northern Riffleshell. It was difficult to decipher what, if any, effects the spill had on the relocated mussel population.

## 2016 Project Activities(see Tiemann et al. 2016)

In 2016, EnviroSciences, Inc., facilitated the shipping of 4,000 mussels from Pennsylvania to Illinois. These individuals were released among 13 sites, which included existing sites (Ford, Horse, Beaver, Smith, Richter, Donut) as well as new sites within IDNR properties (Table 7). The new areas were sampled in 2013 as part of the initial site selection process and were deemed suitable as reintroduction sites, but were not initially selected because they were not feasible for monitoring with PIT tag readers due to lack of easy access. However, it was approved by USFWS and IDNR that any new sites established in 2016 in the Middle Fork and Salt Fork with suitable habitat did not require monitoring via PIT tags; therefore, we released glitter tagged mussels that did not need to be monitored. Additionally, we placed glitter-tagged mussels immediately upstream or downstream of existing sites (e.g., outside of the footprint that we monitor with a PIT tag reader) but still within the same riffle-run segment and within the property boundary. Richter, for example, has suitable habitat of over a quarter mile that stretches between two riffles. Sites were monitored during two events (Table 8) – spring (pre-2016 release) and autumn (post-2016 release).

Table 7. Locations and numbers where mussels were released during the 2016 calendar year. Site information can be found in Tiemann (2014) and Figure 3.

| Site | River | Property | PIT Clubshell | Glitter Clubshell | PIT riffleshell | Glitter riffleshell | Totals |
|---|---|---|---|---|---|---|---|
| Ford | Middle Fork | Middle Fork Fish & Wildlife Area | 60 | | 20 | 50 | **130** |
| downstream of Ford | Middle Fork | Middle Fork Fish & Wildlife Area | | 100 | | 100 | **200** |
| new area (sampled 2013) | Middle Fork | Middle Fork Fish & Wildlife Area | | 100 | | 250 | **350** |
| new area (sampled 2013) | Middle Fork | Middle Fork Fish & Wildlife Area | | 150 | | | **150** |
| new area (sampled 2013) | Middle Fork | Middle Fork Fish & Wildlife Area | | 150 | | | **150** |
| downstream of Horse | Middle Fork | Middle Fork Fish & Wildlife Area | | 40 | | | **40** |
| Horse | Middle Fork | Middle Fork Fish & Wildlife Area | | | | 50 | **50** |
| Beaver | Middle Fork | Middle Fork Fish & Wildlife Area | 80 | | | | **80** |
| downstream of Beaver | Middle Fork | Middle Fork Fish & Wildlife Area | | | | 50 | **50** |
| Richter (and near) | Salt Fork | University of Illinois | | 760 | 40 | | **800** |
| new area (sampled 2013) | Salt Fork | Kickapoo State Park | | 200 | | 300 | **500** |
| Donut (and near) | Salt Fork | Kickapoo State Park | 100 | 200 | 80 | 620 | **1000** |
| Smith (and near) | Salt Fork | Private conservation land | | 420 | 80 | | **500** |
| | | | **240** | **2120** | **220** | **1420** | **4000** |

Table 8. Raw data observation rates by species by site (with stream name) per sampling period for the 2016 calendar year for PIT-tagged mussels (NRS = Northern Riffleshell and CS = Clubshell). Data are number detected / maximum number of individuals in the stream at a site at that period. "NS" = not sampled. *Only half of the Richter site was sampled during both periods. ±One individual was placed at Horse and discovered at Beaver.

| Species | Sampling period | *Richter** *(Salt)* | *Smith (Salt)* | *Donut (Salt)* | *MFNP (Middle)* | *Ford (Middle)* | *Horse (Middle)* | *Kennekuk (Middle)* | *Beaver (Middle)* |
|---|---|---|---|---|---|---|---|---|---|
| NRS | Spring | 2/182 | 78/444 | 149/335 | NS | 16/227 | NS | 5/169 | 1/50 |
| NRS | Autumn | 26/221 | 144/522 | 212/416 | NS | 30/247 | NS | NS | 2/50± |
| CS | Spring | 95/351 | 277/337 | 209/327 | NS | 75/225 | NS | 10/223 | 33/50 |
| CS | Autumn | 106/351 | 258/337 | 304/427 | NS | 123/285 | NS | NS | 100/130 |

## 2017 Project Activities(see Tiemann et al. 2017)

There was not an opportunity in 2017 to translocate additional individuals from Pennsylvania because the U.S. Highway 62 (=Hunter Station) Bridge over the Allegheny River (Forest County, Pennsylvania) was imploded on 4 October 2017. Thus, the only activities that occurred in 2017 was monitoring. Stodola et al. (2017) suggested monitoring translocated Northern Riffleshell and Clubshell in autumn because of greater detection rates. Therefore, we only monitored once during 2017 (Table 9).

Table 9. Raw data observation rates by species by site (with stream name) for the 2017 calendar year for PIT-tagged mussels (NRS = Northern Riffleshell and CS = Clubshell). Data are number detected / maximum number of individuals in the stream at a site at that period. "NS" = not sampled.

| Species | *Richter (Salt)* | *Smith (Salt)* | *Donut (Salt)* | *MFNP (Middle)* | *Ford (Middle)* | *Horse (Middle)* | *Kennekuk (Middle)* | *Beaver (Middle)* |
|---|---|---|---|---|---|---|---|---|
| NRS | 19/236 | 125/549 | 178/420 | NS | 22/250 | 12/224 | 2/182 | 4/50 |
| CS | 104/363 | 288/340 | 300/427 | NS | 112/285 | 78/231 | 1/224 | 102/130 |

Also, three manuscripts using data from this project were published in 2017 – Ashton et al. (2017), Robinson et al. (2017), and Stodola et al. (2017) (Appendix 2).

# APPENDIX 2.
# MANUSCRIPTS THAT RESULTED FROM THIS PROJECT

*Freshwater Mollusk Biology and Conservation* 20:114–122, 2017
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REGULAR ARTICLE

# EVALUATION OF COSTS ASSOCIATED WITH EXTERNALLY AFFIXING PIT TAGS TO FRESHWATER MUSSELS USING THREE COMMONLY EMPLOYED ADHESIVES

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*ABSTRACT*

**Despite the increasing use of passive integrated transponder (PIT) tags in freshwater mussel research and conservation, there has been no evaluation of the trade-offs in cost and effort between commonly used adhesive types. These factors could be important to consider if tag retention rates do not vary by adhesive, the effects of handling are large, or resources are limited. We modeled and evaluated how material costs and effort function over a range of sample sizes by using field data from the relocation of 3,749 PIT-tagged Clubshell (*Pleurobema clava*) and Northern Riffleshell (*Epioblasma rangiana*) in Illinois, 261 Eastern Elliptio (*Elliptio complanata*) in Maryland, and the release of 99 Cumberland Combshell (*Epioblasma brevidens*) in Virginia. Each study used externally affixed 12.5-mm, 134.2-kHz PIT tags, but used a different adhesive to encapsulate tags (Illinois, underwater epoxy resin; Maryland, surface-insensitive gel cyanoacrylate; and Virginia, dental cement). We determined the total cost-per-tag-effort (CPTE) after parameterizing cost, quantity required, application time, and time for each adhesive. After accounting for standardized costs of staff time and adhesive, cyanoacrylate was the least costly adhesive to affix, encapsulate, and cure PIT tags on a per mussel basis. Differences in CPTE were small when the number of mussels tagged was low, but they increased by US$2–6 mussel$^{-1}$. A primary goal in mussel projects is reduced stress from aerial exposure. Using underwater epoxy, which requires time above water to cure, can negate this goal and increase costs as it requires more handling effort than cyanoacrylate or dental cement. Nevertheless, more resource-intensive adhesives may still be an appropriate choice when the number of study animals is low. Further study is warranted to understand how our model may vary by adhesive brand, application rate, staffing level, and environmental factors.**

***KEY WORDS:*** relocation, translocation, tagging, mark–recapture, monitoring, sensors

## INTRODUCTION

Relocation and reintroduction is a common conservation strategy to address the national decline in populations of freshwater mussels (Haag and Williams 2014; FMCS 2016). Understanding survival and demographic rates of mussel populations is imperative to assess conservation and management actions, which necessitates tracking a sufficient number of individual animals or cohorts over time. Studies that seek to monitor and assess the success of freshwater mussel conservation actions (e.g., translocation, relocation, and reintroduction) typically use sampling designs that require individually marked animals (e.g., capture–recapture, Villela

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et al. 2004). The resulting models of demographics and vital rates are based on the probability of detecting a marked animal in subsequent surveys (Burnham et al. 1987). Although mostly sessile, mussels exhibit imperfect detection that can vary by species, size, environmental factors, sampling design, survey method, and observer (Metcalfe-Smith et al. 2000; Meador et al. 2011; Stodola et al. 2017). Consequently, evaluating mussel conservation actions has been hampered by low rates of recapture (Cope and Waller 1995; Cope et al. 2003), leaving the fate of many mussels unknown. An inability to recapture a sufficient number of marked animals may cause data to be deficient, imprecise, or possibly even biased and has implications for conservation (Wisniewski et al. 2013; Hua et al. 2015).

Passive integrated transponder (PIT) tags are relatively inexpensive means of uniquely marking animals that has been widely used to track populations of large and small terrestrial vertebrates (Gibbons and Andrews 2004). As PIT tag technology has advanced, the reduced size of microchips and waterproof tag readers have allowed them to be used with small-bodied aquatic vertebrates and invertebrates, including fishes (Roussel et al. 2000; Cooke et al. 2011; Pennock et al. 2016), crayfishes (Black et al. 2010), and bivalve mollusks (Kurth et al. 2007; Hamilton and Connel 2009; Hale et al. 2012). More recently, this technology has been used to study freshwater mussel movement and behavior (Peck et al. 2007; Gough et al. 2012; Newton et al. 2015) and the survival of released endangered species (wild, Fernandez 2013; hatchery produced, Hua et al. 2015). In the first evaluation of PIT tag use for mussel translocation monitoring, Kurth et al. (2007) observed recapture rates were twice as high as rates observed using visual surveys. Hua et al. (2015) found near complete detection of hatchery-stocked mussels during seven monitoring events over a 2-yr period. Tiemann et al. (2016) recovered 83% of PIT-tagged mussels during 17 monitoring events over 3 yr following a short-distance relocation.

The PIT tags are located subcutaneously in vertebrates and larger invertebrates because their body mass is large relative to the tag size. Internal insertion is generally avoided for freshwater mussels in favor of external affixation because it can result in premature tag rejection or animal mortality (Kurth et al. 2007). Although mussels have been tagged internally (e.g., Layzer and Heinricher 2004), external placement of shellfish tags is the predominant method used to mark mussels in capture–recapture studies (Lemarie et al. 2000; Villela et al. 2004), especially when using PIT tags (Kurth et al. 2007; Peck et al. 2007) and sensors (Hauser 2015; Hartman et al. 2016a, 2016b). Cyanoacrylate and epoxy resin adhesives have been primarily used to externally affix PIT tags to mussel shells, and they have variable curing times, costs, and chemical compositions, in addition to bond strength and longevity. These types of adhesives have shown low rates of mortality and high rates of PIT tag retention in laboratory and in situ settings (Young and Isley 2008). A third, less commonly used adhesive (dental cement) has shown similar performance (Kurth et al. 2007; Hua et al. 2015).

Despite their rapidly increasing use in mussel research and conservation, there has been just a few studies on the effects of external adhesion on mussel behavior, movement, growth, and survival (e.g., Wilson et al. 2011; Peck et al. 2014; Hartmann et al. 2016a; Hua et al. 2016). Furthermore, there has been no evaluation of the trade-offs in material cost and effort (i.e., application and curing time) between the three most widely implemented adhesive types. These could be important factors to consider when developing a conservation plan or ecological study that incorporates PIT tags if the effects of handling or transportation may already be large or if resources are limited. Our objective was to model and evaluate how these factors function over a range of tagging sample sizes for epoxy resin, cyanoacrylate, and dental cement adhesives.

## METHODS

We used data from three case studies that represent field applications of externally affixed PIT tags by using three adhesive types with four freshwater mussel species that have been monitored for $\geq$2 yr.

### Illinois Case Study

Natural resource agencies in Illinois PIT tagged 1,766 Clubshell (*Pleurobema clava*) and 1,983 Northern Riffleshell (*Epioblasma rangiana*) translocated from the Allegheny River beneath the existing U.S. Highway 62 Bridge, Forest County, Pennsylvania, between 2012 and 2014. Clubshell ranged in length from 23 to 62 mm ($\mu = 45.2$ mm), whereas Northern Riffleshell varied from 26 to 78 mm ($\mu = 53.1$ mm). Mussels were shipped in coolers from Pennsylvania to Illinois ($\sim$10 h out of water) and then placed in quarantine holding tanks at the Illinois Natural History Survey Aquatic Research Facility in Champaign-Urbana, Illinois. Each tank provided continuous ground water (temperature ranged from 20 to 22°C), lacked substrate, and was aerated using air pumps. The 2012 cohort was held in quarantine for 14 d, whereas the 2013 and 2014 classes were quarantined for 4–5 d before being released.

While in quarantine, individual mussels were externally affixed with 12.5-mm, 134.2-kHz PIT tags (BioMark, Inc., Boise, ID) by using Devcon 11800 marine grade epoxy resin (Devcon, Danvers, MA). Batches of up to 50 individuals were scrubbed to removed debris (e.g., algae and caddisfly cases), towel dried, and affixed with a PIT tag on the right valve and a uniquely numbered, vinyl shellfish tag (Hallprint, Hindmarsh Valley, South Australia) on the left valve. To affix both PIT and shellfish tags, technicians placed a small bead of cyanoacrylate to hold a tag in place; the brand of cyanoacrylate varied and no accelerant was applied to the glue (Fig. 1a). Once dried, PIT tags were completely encased in epoxy, whereas shellfish tags were encased in cyanoacrylate (Fig. 1b). Individuals were then databased (i.e., recorded species, sex, length, tag numbers, and other information) before being returned to the holding tanks. Out-of-water time averaged 30 min mussel$^{-1}$. Animals were held at least 24 h for the epoxy to fully cure before being hand planted at eight sites in the Vermilion River basin (Wabash River drainage).

Figure 1. Marking of Northern Riffleshell (*Epioblasma rangiana*) and Clubshell (*Pleurobema clava*) by (a) attaching passive integrated transponder (PIT) tags to shells with cyanoacrylate and (b) encapsulating PIT tags in epoxy resin; Eastern Elliptio (*Elliptio complanata*) by using cyanoacrylate by (c) attaching PIT tags to shell and (d) encapsulating the PIT tag in cyanoacrylate; and Cumberland Combshell (*Epioblasma brevidens*) by (e) attaching a PIT tag to the shell with cyanoacrylate and (f) encapsulating the PIT tag in dental cement.

Animals have since been monitored to estimate the survival and gauge the success of the project (Stodola et al. in review). Of the 3,749 animals tagged and relocated, 3,371 (90%) have been encountered at least once during subsequent recapture monitoring by using a portable submersible PIT tag antennae.

## Maryland Case Study

Maryland Department of Natural Resource biologists relocated 2,345 Eastern Elliptio (*Elliptio complanata*) in 2014 from the direct and indirect impact zones of a stream bank stabilization project along Route 24 in Deer Creek, Harford County, Maryland. Particular attention was paid to the effort required to remove, process, and relocate mussels because this was the first large relocation in the state. As a result, an additional 541 mussels were collected in preremoval surveys to assess the potential effects of relocation via capture–recapture monitoring (Ashton et al. 2016). In total, 427 of the 2,866 mussels collected in the removal and preremoval surveys were externally PIT tagged. These mussels have been monitored at five relocation sites and three control sites that received no relocated mussels annually since 2014. This has resulted in an additional 149 (2015) and 112 (2016) naive (i.e., unmarked) mussels being PIT tagged. The Eastern Elliptio PIT tagged ranged in length from 19 to 86 mm ($\mu = 57.3$ mm).

Mussels collected in preremoval, removal, and monitoring surveys were held on site in flowthrough containers or aerated coolers that received frequent changes of river water before processing. After being cleaned of debris, the shell length (millimeters) of each mussel was measured, and each valve was marked with a Hallprint tag adhered using a surface-insensitive, cyanoacrylate gel. Eastern Elliptio <50 mm in shell length and every fifth naive mussel were externally affixed with a 12.5-mm, 134.2-kHz PIT tag. PIT tags were held in place on the shell in a small bead of cyanoacrylate gel (Fig. 1c). Using a separate tube of cyanoacrylate without an application tip, PIT tags were then encapsulated on all sides with additional adhesive (Fig. 1d). In 2014, PIT tags were affixed and encapsulated with LOCTITE gel control (Henkel Corp., Rocky Hill, CT). In 2015 and 2016, Turbo Fuse gel (Palm Labs Adhesives, DeBary, FL) was used to attach tags. Total time to measure and tag was maintained at 2 min mussel$^{-1}$ to minimize aerial exposure by using one or two sprays of a cyanoacrylate curing accelerant (Turbo Set I, Palm Labs Adhesives) in all years. After processing was complete, mussels were kept in flowthrough or aerated holding containers of river water before being hand planted into the substrate. Of the 576 animals PIT tagged in 2014 and 2015, approximately 25% have been relocated through visual survey methods at least once in subsequent monitoring (M.J. Ashton et al., unpublished data).

## Virginia Case Study

Ninety-nine Cumberland Combshell (*Epioblasma brevidens*) were propagated at the Freshwater Mollusk Conservation

Table 1. Comparison of adhesives to attach and encapsulate passive integrated transponder tags to freshwater mussels.

| Study | Adhesive | Adhesive Type | Approximate Time to Apply (min) | Cure Time (min) | Cost (US\$ $g^{-1}$) | Adhesive (g·mussel$^{-1}$) |
|---|---|---|---|---|---|---|
| Illinois | Devcon 11800 | Epoxy resin | 5 | 1,440[a] | 0.14 | 0.72 |
| Maryland | Palm Labs 440 Turbo Fuse Gel | Cyanoacrylate | 1 | 1 | 0.35 | 0.54 |
| Virginia | Fuji Glass Ionomer Luting Cement | Dental cement | 1 | 1 | 2.54 | 0.94 |

[a] We estimated that 2% of the total cure time (30 min) involved costs associated with effort (e.g., transfer of mussels to holding tanks, arrangement within tank, collection for transport).

Center, Department of Fish and Wildlife Conservation, Virginia Tech in Blacksburg, Virginia. Over a 2-yr period, mussels were released from hatchery or in situ culture systems after they reached a minimum length of 20 mm into the Powell River, Claiborne County, Tennessee. Tagged Cumberland Combshell ranged in length from 17.8 to 22.9 mm ($\mu = 19.3$ mm).

While in culture, subadult Cumberland Combshell were marked with a bee tag (The Bee Works, Ontario, Canada) or vinyl shellfish tag by using cyanoacrylate. A three-step process was used to externally affix PIT tags in the field. After being cleaned and dried, PIT tags were held with LOCTITE gel control cyanoacrylate (Fig. 1e). Tags were then completely encapsulated in Fuji Glass Ionomer Luting Cement (Fig. 1f; GC Fuji Luting, Tokyo, Japan). A hypodermic needle was used to mix the dental cement powder and liquid on a manufacturer's supplied application pad and apply the mixed cement onto the PIT tag via syringe. To reduce negative effects of exposure, the PIT tagging process was conducted in the field under shade and took 2 min mussel$^{-1}$. Mussels were hand planted into the substrate at the monitoring site after tagging was complete. The released mussels were monitored using a portable submersible PIT tag antennae to assess individual heterogeneity of demographic rates (Hua et al. 2015). Of the 99 animals tagged and released, 97 (98%) have been encountered at least once during subsequent recapture monitoring (Hua et al. 2015).

## Evaluation

We evaluated the total cost to externally affix PIT tags to freshwater mussels by parameterizing the cost (US\$ $g^{-1}$) of each primary adhesive ($A$), quantity of adhesive (q$A$) used in each case study (g mussel$^{-1}$), time (min mussel$^{-1}$) needed to apply the adhesive and PIT tag (t$A$), and time (min mussel$^{-1}$) actively engaged with tagged mussels during the adhesive curing process (c$A$) (Table 1). Costs of adhesives per unit were calculated from purchase records kept in each case study. We did not include the cost of PIT tags and adhesive used to attach the tag as they were similar among studies. We also did not include adhesive use and tag application data from the 2014 portion of the Maryland case study because it was discovered that a relatively large amount of adhesive remained inside the applicator even after it appeared exhausted.

The quantity of adhesive used per mussel was determined by dividing the number of mussels tagged in each study by the quantity of adhesive consumed. We used the average hourly salary rate published by the General Services Administration's Contract-Awarded Labor Category for project scientists in the environmental services schedule with a Bachelor's or higher education level to determine a constant cost in staff time (US\$96.00 $h^{-1}$) to affix PIT tags (GSA 2016). Cost in time spent to cure adhesive type was calculated in the same manor, but for epoxy the time was estimated at 30 min for batches of 50 mussels instead of for an individual mussel. The parameters of cost were then totaled and extrapolated on a per mussel tagged basis (cost-per-tag-effort; CPTE in \$US) for cyanoacrylate and dental cement as follows:

$$\begin{aligned}\mathrm{CPTE} = {} & [(A \times \mathrm{q}A) \times \mathrm{N}_{\mathrm{mussels}}] \\ & + \left[\left(\$96.00{\cdot}\mathrm{h}^{-1} \times (\mathrm{t}A \times \mathrm{N}_{\mathrm{mussels}})\right]/60\,\mathrm{min} \\ & + \left(\$96.00{\cdot}\mathrm{h}^{-1} \times (\mathrm{c}A \times \mathrm{N}_{\mathrm{mussels}})\right]/60\,\mathrm{min}. \qquad (1)\end{aligned}$$

For epoxy, CPTE was calculated as follows:

$$\begin{aligned}\mathrm{CPTE} = {} & [(A \times \mathrm{q}A \times \mathrm{N}_{\mathrm{mussels}})] \\ & + \left[(\$96.00{\cdot}\mathrm{h}^{-1} \times (\mathrm{t}A \times \mathrm{N}_{\mathrm{mussels}}\right]/60\,\mathrm{min} \\ & + \left(\$96.00{\cdot}\mathrm{h}^{-1} \times (\mathrm{c}A \times \mathrm{N}_{\mathrm{mussels}}/50)\right]/60\,\mathrm{min}. \qquad (2)\end{aligned}$$

To generate a predictive equation for the relationship between CPTE and number of mussels tagged, we constructed ordinary least squares regression models for each adhesive type by using the lmList function in R package nlme (Pinheiro et al. 2016). A linear method was chosen as opposed to fitting the extrapolated parameter values against other distributions because parameters of CPTE increase at a constant rate mussel per mussel (equation 1) or batch per batch (equation 2). We used the lm method of the geom_smooth function in R package ggplot 2 (Wickham 2009) to visualize these relationships.

## RESULTS

The PIT tagging of 3,749 Clubshell and Northern Riffleshell consumed approximately six 454-g epoxy adhesives over the 3-yr period. Tagging of 149 Eastern Elliptio in 2015 and 112 individuals in 2016 consumed four and three 20-g cyanoacrylate adhesives, respectively. Three 35-g dental cement adhesives were used to tag 99 Cumberlandian Combshell in 2009 and 2010. The quantity of adhesive used

Table 2. Costs of materials and effort incurred during the adhesion and curing of passive integrated transponder (PIT) tags to freshwater mussels per mussel and extrapolated per 100 individuals by adhesive type.[a]

| No. Mussels Tagged | Dental cement (US$) | | | | Cyanoacrylate (US$) | | | | Epoxy (US$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Adhesive (q*A*) | Application (t*A*) | Cure (c*A*) | Cost (CPTE) | Adhesive (q*A*) | Application (t*A*) | Cure (c*A*) | Cost (CPTE) | Adhesive (q*A*) | Application (t*A*) | Cure (c*A*) | Cost (CPTE) |
| 1 | 2.40 | 1.60 | 1.60 | 5.60 | 0.22 | 1.60 | 1.60 | 3.42 | 0.10 | 8.00 | 48.00 | 56.10 |
| 100 | 239.76 | 160.00 | 160.00 | 559.76 | 22.46 | 160.00 | 160.00 | 342.46 | 10.30 | 800.00 | 96.00 | 906.30 |
| 200 | 479.51 | 320.00 | 320.00 | 1,119.51 | 44.92 | 320.00 | 320.00 | 684.92 | 20.60 | 1,600.00 | 192.00 | 1,812.60 |
| 300 | 719.27 | 480.00 | 480.00 | 1,679.27 | 67.38 | 480.00 | 480.00 | 1,027.38 | 30.90 | 2,400.00 | 288.00 | 2,718.90 |
| 400 | 959.02 | 640.00 | 640.00 | 2,239.02 | 89.84 | 640.00 | 640.00 | 1,369.94 | 41.19 | 3,200.00 | 384.00 | 3,625.19 |
| 500 | 1,198.78 | 800.00 | 800.00 | 2,798.78 | 112.31 | 800.00 | 800.00 | 1,712.31 | 51.49 | 4,000.00 | 480.00 | 4,531.49 |
| 600 | 1,438.53 | 960.00 | 960.00 | 3,358.53 | 134.77 | 960.00 | 960.00 | 2,054.77 | 61.79 | 4,800.00 | 576.00 | 5,437.79 |
| 700 | 1,678.29 | 1,120.00 | 1,120.00 | 3,918.29 | 157.23 | 1,120.00 | 1,120.00 | 2,397.23 | 72.09 | 5,600.00 | 672.00 | 6,344.09 |
| 800 | 1,918.04 | 1,280.00 | 1,280.00 | 4,478.04 | 179.69 | 1,280.00 | 1,280.00 | 2,739.69 | 82.39 | 6,400.00 | 768.00 | 7,250.39 |
| 900 | 2,157.80 | 1,440.00 | 1,440.00 | 5,037.80 | 202.15 | 1,440.00 | 1,440.00 | 3,082.15 | 92.69 | 7,200.00 | 864.00 | 8,156.69 |
| 1,000 | 2,397.55 | 1,600.00 | 1,600.00 | 5,597.55 | 224.61 | 1,600.00 | 1,600.00 | 3,424.61 | 102.99 | 8,000.00 | 960.00 | 9,062.99 |

[a] q*A*, quantity of adhesive used in each case study (g mussel$^{-1}$); t*A*, time (min mussel$^{-1}$) needed to apply the adhesive and PIT tag; c*A*, time (min mussel$^{-1}$) actively engaged with tagged mussels during the adhesive curing process; CPTE, cost-per-tag-effort.

to PIT tag these mussels was similar across years by adhesive type.

Parameters of adhesive consumption, application, and curing effort varied by adhesive type (Table 1). Cyanoacrylate required 24% less adhesive to affix a PIT tag to an individual mussel than the epoxy and 43% less than dental cement. In contrast, epoxy was 2.5 times less costly per gram than cyanoacrylate and 18 times less costly than dental cement. Epoxy required 5 times more effort to apply and encapsulate a PIT tag than both dental cement and cyanoacrylate. Total cure time for epoxy was considerably greater than other adhesives, yet little of this time was spent handling mussels. Consequently, less effort associated with the process of adhesive curing accumulated as more mussels were tagged with epoxy than with cyanoacrylate and dental cement by handling mussels in batches of 50 (e.g., 100 mussels cured in 60 min vs. 60 mussels in 60 min).

Linear models of total cost (US$) per PIT-tagged mussel based on our cost and consumption parameters illustrated that cyanoacrylate ($\text{CPTE} = \$3.42 \times N_{\text{mussels}} - 1.23^{-10}$) was less costly than dental cement ($\text{CPTE} = \$5.60 \times N_{\text{mussels}} - 2.52^{-13}$) or epoxy ($\text{CPTE} = \$9.04 \times N_{\text{mussels}} + \$14.96$) (Table 2 and Fig. 2a). Costs associated with adhesive consumption increased at a greater rate for dental cement and cyanoacrylate than epoxy (Fig. 2b). The rate at which CPTE increased as the number of mussels tagged increased was higher for epoxy than cyanoacrylate and dental cement due to higher costs associated with adhesive application effort (Fig. 2c). An initial investment of effort to cure the first batch of 50 mussels led to higher upfront costs (i.e., larger y-intercept) for epoxy, but ultimately resulted in lower costs in comparison with cyanoacrylate and dental cement as the number of mussels tagged increased (Fig. 2d).

## DISCUSSION

External attachment of PIT tags is a marking technique that can increase detection rates of freshwater mussels (Kurth et al. 2007) and improve the accuracy of survival and demographic rates (Hua et al. 2015; Tiemann et al. 2016). For this reason, PIT tags seem especially suited for use in mussel relocation and conservation monitoring due to historically low recapture rates (Cope et al. 1995, 2003). A primary goal in studies that employ recapture sampling is reduced stress from handling, especially out of water time (Dunn et al. 2000). Aerial exposure to apply and adhere tags to freshwater mussels by using cyanoacrylate was generally <15 min mussel$^{-1}$ (Lemarie et al. 2000; Villella et al. 2004), yet this can be reduced to 2 min mussel$^{-1}$ by using a curing accelerant. Dental cement has a similar curing time. Using underwater epoxy to affix PIT tags can negate the reduced handling time goal as it requires more handling and total curing time than cyanoacrylate (Table 1 and Fig. 2c).

In this evaluation of the materials and staff time needed to affix and encapsulate PIT tags to freshwater mussels from three studies, cyanoacrylate was overall less costly than dental cement and epoxy on a per mussel basis. Absolute differences in total cost compared to cyanoacrylate are relatively small when the number of mussels tagged is low, but they increased by more than $2 mussel$^{-1}$ for dental cement and almost $6 mussel$^{-1}$ for epoxy. We suggest that dental cement and waterproof epoxy resin may be an appropriate choice of adhesive for transmitters when the number of study animals is low. In this scenario, differences in costs among adhesive types will be negligible, and dental cement or epoxy may be better suited to protect PIT tags from damage should even minimal tag loss affect the statistical power to detect a change in population size or condition. A quicker, more controlled method of applying epoxy warrants investigation as the effort

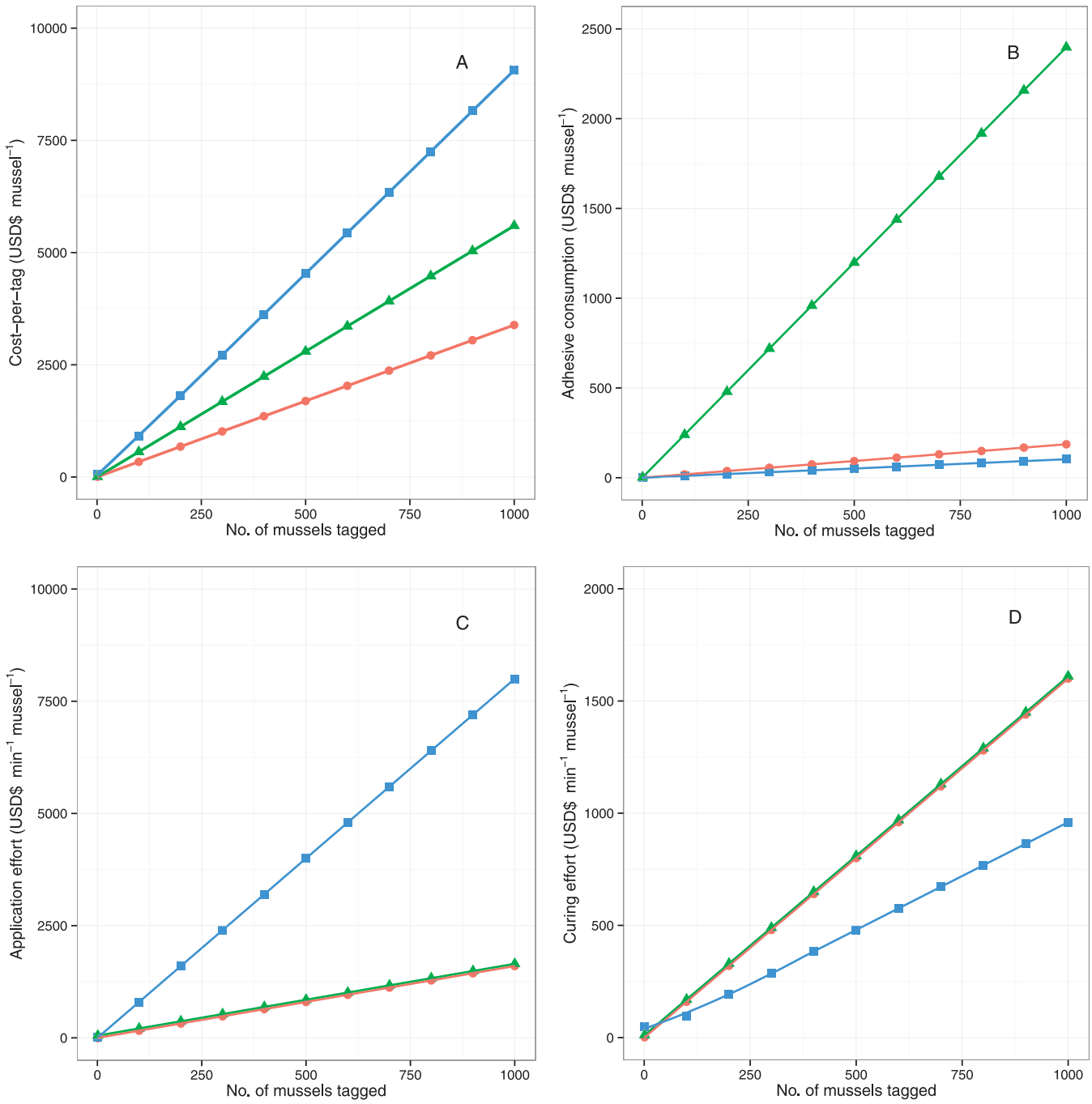


Figure 2. Linear models for epoxy resin (blue squares), cyanoacrylate (red circles), and dental cement (green triangles). Relationships between (a) cost-per-tagged mussel versus number of mussels with externally affixed PIT tags and individual cost-per-tag-effort (CPTE) parameters of (b) adhesive consumption, (c) application time, and (d) curing time versus number of mussels tagged.

associated with its application evaluated in this study was 5 times more than that of cyanoacrylate or dental cement. This difference in effort drove CPTE higher for epoxy (Fig. 2a, c), even though the cost of adhesive consumption per tag was less and curing in batches may reduce and even reverse any cost advantage achieved from using a faster curing adhesive (Fig. 2b, d). A more controlled applicator could also reduce the quantity of epoxy consumed per tag, thus realizing additional savings in materials. Because application and curing times were similar for cyanoacrylate and dental cement, differences in CPTE could be mitigated by more conservative cement application or a less costly formula.

Prices of adhesives can vary widely, especially when considering the advent of online shopping, buying in bulk, or discounts some groups receive (e.g., governmental agencies). The difference in adhesive cost per unit may in part be because

the epoxy evaluated in this study is sold in a greater quantity per standard package than both dental cement and cyanoacrylate. On average, 600 individuals could be affixed with PIT tags by using a 454-g package of epoxy. In contrast, about 30 individuals could be tagged using a 35-g package of dental cement. Other factors to consider are the ability to rapidly procure adhesive, surcharges when not ordering in bulk, or unintended curing of unused product. For example, acquiring dental cement can be challenging because its intended use is in a regulated industry. Also, unexpected demand for additional adhesive (e.g., tagging more mussels than expected or more liberal adhesive application) requires the need for impromptu purchasing. We have observed prices varying by 10–30% among major retailers for the same cyanoacrylate adhesive. Cyanoacrylate adhesives and accelerants are often sold in cases of 10 or 12 and have a suggested shelf life of a year. There are often surcharges to purchase units less than a case, which would increase cost per unit parameters if a relatively small number of mussels are to be tagged. With adequate planning time, comparison shopping should help keep actual costs comparable to our studies; however, we noted a 30% increase in the price of epoxy since the last purchase from the same vendor.

Although we focused our effort on resources required to affix PIT tags, the cost of tags can also vary depending on the quantity, size, and manufacturer. For the data evaluated in our models, tag cost would have been constant because large quantities were procured from the same vendor at or about the same time. However, over the course of these studies tag price has fluctuated year to year and vendor to vendor by (+) 150 to (–) 250% (e.g., prices have ranged from $2 to $5 per tag). Other costs we did not measure and account for in our evaluation should also be considered when choosing an adhesive type for PIT tagging of freshwater mussels. For example, the curing time associated with underwater epoxies could reduce the number of mussels that can be tagged and returned to a stream in a day or require travel between study sites and laboratory facilities thus extending the number of field days. Specialized facilities and equipment may also be necessary to hold mussels in captivity during the curing time, whereas mussels can be immediately returned to the stream after cyanoacrylate and dental cures. Tiemann et al. (2016) speculated that prolonged handling and exposure may have contributed to the initial mortality observed following relocation. Factors other than cost may also warrant consideration, including the presence of potentially harmful compounds, adhesive durability, and ability to reapply in the field. For example, Hartmann et al. (2016a) chose not to adhere sensors to Duck Mussel (*Anodonta anatina*) with epoxy resin due to its complex application and presence of bisphenol-A. Environmental factors (e.g., air temperature and relative humidity) can also affect adhesive viscosity and curing time.

We propose that PIT tag retention is generally not an important factor in choosing an adhesive as previous studies have shown that retention rates do not seem to vary substantially by adhesive type (e.g., Young and Isley 2008). However, PIT tag attachment may fail regardless of adhesive type if debris causes the bond between shell and adhesive or adhesive and tag to break. Insufficient PIT tag encapsulation could cause them to be damaged if mussels become dislodged or struck with coarse particles during high flow events. Still, externally affixed PIT tag loss appears to be low over 1–2-yr periods and comparable to retention rates of vinyl shellfish tags (e.g., Lemarie et al. 2000). For example, Ashton et al. (2016) observed the loss or failure of eight (2%) cyanoacrylate-affixed PIT tags 12 mo after relocation on Eastern Elliptio that were recovered 650 to 1,500 m downstream of the point of their relocation in a coarse substrate stream. Similar levels of tag damage due to cyanoacrylate erosion were observed after 18 mo by Young and Isely (2008), but they observed no tag damage due to adhesive loss for underwater epoxy. Tiemann et al. (2016) reported one (1%) tag failure during their assessment of short-distance mussel relocation with epoxy encapsulated PIT tags. Hua et al. (2016) observed no failure of tags embedded in dental cement. We are unaware of any published studies that have evaluated PIT tag retention beyond 3 yr so we cannot speculate whether a particular type is more suited for long-term (>10-yr) study.

The findings of our evaluation are likely limited in their scope to the adhesives we evaluated (gel cyanoacrylate, dental cement, and 24-h curing waterproof epoxy resin); however, the assumptions used to parameterize our model are flexible to other costs and adhesive properties. Accordingly, the costs incurred from applying and handling with the epoxy used in this study would have been likely similar if a quicker curing formula was used based on observations of others (e.g., Young and Isley 2008). For this reason, we expect that epoxy resin would sustain higher total costs per mussel tagged without reductions in application time while also maintaining a minimal level of effort during the curing process. Further limitations in our findings may arise from a lack of quantified variation within each case study and by adhesive type. Variation when applying model parameters could arise from fluctuations in adhesive costs, level of adhesive applicator experience, and staffing. For example, actual staff costs incurred in the Illinois and Maryland case studies may have been lower than our model because some tag applicators were volunteers. However, a relocation or reintroduction involving a federally listed, cryptic species may necessitate primary investigators with specialized experience, which could lead to higher salary rates. Added variation could result from adhesive brand and environmental factors, including air temperature and relative humidity. We believe a more thorough comparison of commercially available adhesives used to externally PIT tag mussels is warranted.

## ACKNOWLEDGMENTS

Funding for the Illinois project was provided in part by the U.S. Fish and Wildlife Service (USFWS) through the Illinois Department of Natural Resources' (IDNR) Office of Resource Conservation to the Illinois Natural History Survey (INHS)

(grants R70470002 and RC09-13FWUIUC); the USWFS's Ohio River Basin Fish Habitat Partnership (award F14AC00538); the IDNR's Natural Resource Damage Assessment settlement from the Heeler Zinc - Lyondell Basell Companies (reference documents OREP1402 and OREP1504); the Illinois Wildlife Preservation Fund (grant RC07L25W); and the Illinois Department of Transportation. Funding for the Maryland project was provided to the Maryland Department of Natural Resources by the State Highway Administration of the Maryland Department of Transportation. The grant and facility support for the Virginia project was provided by the USFWS, U.S. Geological Survey (USGS), and Department of Fish and Wildlife Conservation at Virginia Polytechnic Institute and State University. Permits for Illinois were provided by the USFWS (TE73584A-1); Pennsylvania Fish and Boat Commission (PFBC) (e.g., 2014-02-0837, 2013-756); IDNR (e.g., S-16-047, #S-10-30); the Illinois Nature Preserves Commission; and the University of Illinois (U of I). Staff from the USFWS (Rock Island, Ohio and Pennsylvania field offices), PFBC, IDNR, INHS, U of I, and EnviroScience, Inc. (ESI), assisted with the tagging and relocation in Illinois; specifically, Alison Stodola (INHS), Rachel Vinsel (INHS), Sarah Douglass (INHS), Kevin Cummings (INHS) assisted with relocations, monitoring, database management, and have offered thoughtful insight to this project and subsequent manuscripts. James McCann (Maryland Department of Natural Resource [MDNR]), Dave Brinker (MDNR), staff of the MDNR's Monitoring and Non-Tidal Assessment Division and USFWS Maryland Fisheries Resource Office conducted the relocation in Maryland. Special thanks are given to Martha Stauss (MDNR) for coordinating logistics with Maryland State Highway Administration. We thank the staff and students at Freshwater Mollusk Conservation Center, Virginia Tech for their assistance in propagation, culture, release, and recapture of juvenile mussels. Heidi Dunn (ESI), Emily Robins (ESI), Teresa Newton (USGS), and Andrew Peck (The Nature Conservancy) shared their experiences with PIT-tagging mussels.

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Notes of the *Northeastern Naturalist,* Issue 24/4, 2017

# Some Phoretic Associations of Macroinvertebrates on Transplanted Federally Endangered Freshwater Mussels

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**Abstract** - Benthic macroinvertebrates were washed from nearly 1000 federally endangered freshwater mussels that had been collected from Pennsylvania during a reintroduction project to 2 eastern Illinois streams. Most benthic macroinvertebrates collected were larvae of the *Neophylax fuscus*, but other caddisflies and segmented worms were also observed. No unoccupied caddisfly cases were observed on live mussels, leaving open the question as to the seasonal fate of these microhabitats after caddisflies pupate and emerge in the Autumn of each year. Unionid mussel shells might modify local-scale species diversity by influencing physical and hydraulic properties of microhabitats.

*Life-history knowledge gaps.* Basic attributes of macroinvertebrate life histories are often poorly known and under-reported in the scientific literature. Gaps in the information on the ecology of individual organisms are one of a number of knowledge gaps that systematically limit the effective management and conservation of species, as well as our understanding of the factors that constrain species diversity and the evolution of new traits and taxa (Cardoso et al. 2011, Hortal et al. 2015). The prospect of improving the management of species under special conservation protections provides an additional impetus for reporting basic life-history and ecological attributes of these species, and the other members of the ecological communities in which they persist.

*Relocation project.* Beginning in 2005, biologists from the Illinois Natural History Survey (INHS) partnered with personnel from the US Fish and Wildlife Service and from several state resource management agencies in Ohio and Pennsylvania to rescue individuals of 2 federally endangered mussel species, *Epioblasma rangiana* (Lea) (Northern Riffleshell) and *Pleurobema clava* (Lamarck) (Clubshell). The mussels were collected from the footprint of a proposed bridge construction on the Allegheny River in Forest County, PA, in areas of swiftly flowing water with clean and stable sand, gravel and cobble substrates (Stodola et al. 2017, Tiemann 2014). Mussels were relocated to the Vermilion River basin in Champaign and Vermilion counties, IL, with the goal of re-establishing viable populations of these 2 species into areas where they were considered extirpated (Cummings and Mayer 1997, Tiemann 2014). This paper concerns macroinvertebrates collected from live mussels transplanted during 26–27 August 2013.

Individual mussels (249 Northern Riffleshell and 758 Clubshell) were quarantined in a holding facility at the University of Illinois at Urbana-Champaign (UIUC), tagged with passive integrated transponder (PIT) tags, and then resituated at 8 different sites in the Middle Fork (5) and Salt Fork (3) of the Vermilion River (Stodola et al. 2017, Tiemann 2014). During tagging, the external shell of individual mussels was scrubbed and temporarily dried to facilitate the attachment of tags. This process rinsed and removed attached sediment and epibionts, including caddisfly cases and other aquatic macroinvertebrates. Most of this material was retained in 95% ethanol for later microscopic inspection and identification.

We identified 152 individual macroinvertebrates, representing 4 species (Table 1). The macroinvertebrates we report herein are a nonrandom and limited subset of the complete

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Manuscript Editor: Trip Krenz

phoretic fauna associated with these 2 mussel species, because many organisms were certainly detached or disassociated during the process of removal and quarantine.

*Life history and local ecology.* Both of the mussel species considered here are typically found in medium to large rivers in clean, stable sand, gravel, and cobble riffles, where they may live several inches beneath the streambed surface (Cummings and Mayer 1992, Watters et al. 2009). These 2 species vertically migrate to the streambed surface during their reproductive period; Northern Riffleshell are bradytictic (brooding from September to the following June), whereas Clubshells are tachytictic (brooding from early May to July). The macroinvertebrate assemblage associated with the shells of these 2 mussel species could be different during these reproductive periods compared to the rest of the year. Associations of macroinvertebrates and unionids within this interstitial microhabitat are likely to experience temporal progression within each year, as a function of mussel vertical migration and/or macroinvertebrate life history (e.g., adult emergence of *Neophylax* sp. in autumn).

Table 1. List of macroinvertebrates dislodged from 249 *Epioblasma rangiana* (Northern Riffleshell) and 758 *Pleurobema clava* (Clubshell) from the Allegheny River (Route 62 Bridge, 4.5 km SW Tionesta, Forest County, PA, 41.472348°N, 79.499838°W), collectors J.S. Tiemann, K.S. Cummings, S.A. Douglass, A.L. Price, et al.

| Phylum | Class | Order | Family | Species | Count |
|---|---|---|---|---|---|
| Arthropoda | Insecta | Trichoptera | Thremmatidae | *Neophylax fuscus* | 113 |
| Arthropoda | Insecta | Trichoptera | Leptoceridae | *Oecetis inconspicua* | 2 |
| Annelida | Clitellata (Hirudinea) | Rhynchobdellida | Glossiphoniidae | *Helobdella papillata* | 35 |
| Annelida | Clitellata | Tubificida | Naididae | *Nais bretscheri* | 1 |
| Annelida | Clitellata | Tubificida | Naididae | Unidentified Naidinae | 1 |

Figure 1. Ventral view of *Neophylax fuscus* case, with larva enclosed. Remnants of mussel shell are visible at anterior and posterior attachment sites.

*Neophylax* cases are tube-like, typically with large stones flanking the tube along the axis of the body of the organism (Fig. 1). The insect prepares for pupation by enclosing the tube and firmly attaching the larval case to some firm substrate (including mussel shells) with silk produced from glands located in the mouth of the larva (Sehnal and Akai 1990). Cases may persist, in some habitats, for several years after the emergence of adults. Larvae occupied all cases removed from mussels in this study, and no empty cases from previous seasons were observed. This finding is curious because *Neophylax fuscus* Banks cases were so firmly attached to mussels that removing the cases during our prepping procedure also removed small pieces of periostracum (the non-living outer layer of the shell) at the attachment sites (Fig. 2). Lawfield et al. (2014) suggested that Trichoptera case attachment might not harm or damage the shell of mussels because this attachment is confined to the periostracum.

The aquatic annelids rinsed from external mussel shells included the leech *Helobdella papillata* (Moore) (5 brooding adults, 5 non-brooding adults, and 25+ young of the year that had detached from parents), 1 aquatic oligochaete (*Nais bretscheri* Michaelsen) and 1 other unidentified naidid oligochaete (Table 1). Several leech species in the family Glossiphoniidae (including *H. papillata*) are known associates of freshwater mollusks, feeding primarily if not exclusively on mollusks (Sawyer 1986). Several species in the oligochaete genus *Chaetogaster* (most commonly, *Chaetogaster limnaei* von Baer) are often collected from pulmonate snails (externally, from within the mantle cavities, around the apertures, and as parasites in the kidneys; Klemm 1985), from unionid bivalves (externally and from within the mantle cavities; Anderson and Holm 1987, Beckett et al. 1996, Kelly 1988), and occasionally from freshwater sponges, bryozoans, and crayfishes (Sawyer 1986; Stephenson 1930; Wetzel et al. 2009; M.J. Wetzel, pers. observ.).

Surprisingly, no *Chaetogaster* specimens were present in the material washed from the mussels from our study, but as noted above, many organisms were certainly detached or

Figure 2. Close up view of remnants of freshwater mussel shell remaining at the posterior attachment site on a *Neophylax fuscus* case.

disassociated during the process of removal and quarantine. Neither *Nais bretscheri* nor other congeners are known to have commensal or parasitic relationships with mollusks. It is possible that aquatic oligochaetes washed from the mussels were living within silt and sand on the shell of the mussels, and also possible that these individuals were only incidentally using areas around caddisfly cases as habitat or refugia. We note a litany of personal field and lab experiences on oligochaetes and other macroinvertebrate fauna in the contents of caddisfly cases or puparia, which frequently contain more macroinvertebrate species when examined in detail (Beckett et al. 1996; Bodis et al. 2014; Lawfield et al. 2014; J.L. Robinson and M.J. Wetzel, pers. observ.; pers. comm. from caddisfly taxonomists D. Denson [Reedy Creek Improvement District, Lake Buena Vista, FL], C. Parker [ret. USGS, Gatlinburg, TN], D. Etnier [ret. UT-Knoxville, Knoxville, TN], and D. Ruiter [ret. USEPA, Centennial, CO]) and sometimes provide a substrate for *Podostemum ceratophyllum* Michx. (Hornleaf Riverweed) (Vaughn et al. 2002).

Among our observations, all cases we report were occupied by living caddis, and older cases from which caddis had previously emerged were completely absent. Little is known about the behaviors or ecological significance of burrowing mussels (Newton et al. 2015). Although mussels are known to vertically migrate to escape predation (Burlakova et al. 2000) and control zebra mussel infestation (Nichols and Wilcox 1997), we hesitate to speculate that vertical migration can remove spent cases. Regardless, caddisfly cases may help to create and maintain fine-scale structural and hydraulic and ecological diversity widely reported from freshwater mussel habitats (Commito and Rusignuolo 2000, Gutierrez et al. 2003, Lawfield et al. 2014, Taniguchi and Tokeshi 2004, Vaughn and Spooner 2006).

We believe that this report is the first literature discussion of a phoretic association between living unionid mussels and any of the confirmed eastern North American species of *Neophylax* (Trichoptera: Thremmatidae) in the ecological literature. However, phoretic associations of Trichoptera with unionids have been reported from fossils dating to the Paleocene of North Dakota, where psychomyiid caddisfly retreats and net were preserved on a unionid (Erickson 1983). Trichoptera associations must be known or familiar to malacologists who observe organisms in the field, and associations with case-building Trichoptera have previously been suggested from photographs of organisms attached to dead shells (Lawfield et al. 2014), but not identified to genus or species. Images posted on the USFWS website for this specific project clearly depicted *Neophylax* cases on specimens in situ, and images published in Lawfield et al. (2014) suggest hydropsychids, hydroptilid, and glossosomatid caddisflies may successfully colonize the surface of unionids. Interestingly, Anderson and Vinikour (1984) reported the use of unionid mussels and viviparid snails as pupation sites for the leptocerid caddisfly *Oecetis inconspicua* (Walker), but no associations with other extant Trichoptera species have yet been reported. Interspersed among *Neophylax* cases were 2 very early instar larvae of some species of *Oecetis,* in the *O. inconspicua* group (Floyd 1995)—instars that could not be confidently associated with any of the morphologically distinguishable forms within this group.

Although interesting as ecological trivia, this observation raises issues about quarantine and unionid reintroduction efforts. Our results are at best an underestimate of the fauna attached to or living on the mussels in situ but a great example of how organisms can unintentionally be transported great distances. Predicting which species might be most likely to be introduced might prove difficult, because numerous observations of epibiotic relations suggest that many different taxa can form these associations without specificity (Wahl and Mark 1999). Caddis cases, or macrophytes, might be obvious to

most observers and frequently removed during basic quarantine. Oligochaetes or other invertebrates inhabiting the mantle cavities or internal organs of mollusks, as well as those capable of hiding in the crevices of the shells, may be more difficult or impossible to positively remove. The chance for accidental introduction of non-native macroinvertebrates obviously increases when hundreds, if not thousands, of mussels are translocated into new river basins across multiple years. To avoid potential contamination and unwanted introduction of macroinvertebrates, careful, stringent quarantine procedures should be considered when transporting freshwater mussels.

*Acknowledgments.* This relocation project is being funded, in part, by a natural resource damage assessment settlement (Hegeler Zinc–Lyondell Basell Companies) to the US Fish and Wildlife Service (USFWS) and to the State of Illinois. USFWS, Pennsylvania Fish and Boat Commission (PFBC), and Illinois Department of Natural Resources (IDNR) issued collecting permits. Staff and volunteers from EnviroScience (Ohio), IDNR, Illinois Natural History Survey, Ohio State University, Columbus Zoo and Aquarium (Ohio), PFBC, Prairie Rivers Network, USFWS, and West Virginia Department of Natural Resources assisted with collecting and scrubbing mussels. Dead mussel specimens, trichopterans and annelid specimens identified during this study are deposited, respectively, in the INHS Mussel, Insect, and Annelid Collections (*Epioblasma rangiana*- INHS 46825, *Pleurobema clava*- INHS 46829, *Neophylax fuscus*- INHS 797483, *Oecetis inconspicua group*- INHS 797484, *Helobdella papillata* INHS_ANNELID_10269_431, *Nais bretscheri* INHS_ANNELID_10270_164, unidentified Naidinae INHS_ANNELID_10271_102).

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*Freshwater Mollusk Biology and Conservation* 20:89–102, 2017
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REGULAR ARTICLE

# SURVIVAL OF TRANSLOCATED CLUBSHELL AND NORTHERN RIFFLESHELL IN ILLINOIS

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***ABSTRACT***

**Translocation of freshwater mussels is a conservation tool used to reintroduce extirpated populations or augment small populations. Few studies have evaluated the effectiveness of translocations, mainly because estimating survival is challenging and time-consuming. We used a mark-recapture approach to estimate survival of nearly 4,000 individually marked Clubshell (*Pleurobema clava*) and Northern Riffleshell (*Epioblasma rangiana*) translocated to eight sites over a five-year period into the Salt Fork and Middle Fork Vermilion rivers in central Illinois. Survival differed among sites and between species; Clubshell were approximately five times more likely to survive than Northern Riffleshell. Survival also increased in the fourth year following a release and decreased following high-flow events. Translocating numerous individuals into multiple sites over a period of years could spread the risk of catastrophic high-flow events and maximize the likelihood for establishing self-sustaining populations.**

***KEY WORDS:*** reintroduction, freshwater mussel, high flow, PIT tag, unionids

## INTRODUCTION

North American freshwater mussels have undergone drastic population declines during the past century and are one of the most imperiled groups of animals in the world (Williams et al. 1993; Lydeard et al. 2004; Strayer et al. 2004). Translocation has been used for decades to augment populations or reintroduce mussels into regions where species have declined or are extirpated (Coker 1916; Ahlstedt 1979; Sheehan et al. 1989). Much time and effort is placed on collecting, marking, and transporting mussels for translocation, but few studies have evaluated the effectiveness of mussel reintroductions. More than a quarter of all translocation projects conducted prior to 1995 failed to report on the efficacy of those efforts (Cope and Waller 1995).

Obtaining precise and unbiased estimates of mussel survival is challenging, even for translocated individuals. Mussels often burrow beneath the substrate surface when not actively feeding or reproducing, making them difficult to detect (Amyot and Downing 1998; Watters et al. 2001; Strayer and Smith 2003). Furthermore, an unequal proportion of the population is often sampled, such as larger individuals, those found in easy-to-sample areas, or those at or near the surface (Strayer and Smith 2003; Meador et al. 2011). Reliable estimates of survival can be obtained using capture-mark-recapture techniques (Hart et al. 2001; Meador et al. 2011). Capture-mark-recapture methods are often time-intensive due to the effort needed to capture and mark a large number of individuals, but marking individuals already captured for translocation can be easily incorporated.

The federally endangered Clubshell (*Pleurobema clava*) and Northern Riffleshell (*Epioblasma rangiana*) were formerly widespread in the Ohio River and Great Lakes basins but have experienced significant range reductions during the last century. The recovery plan for the Clubshell and Northern Riffleshell set objectives of reestablishing viable populations in 10 separate river drainages across the species' historical range via augmentation and reintroduction (USFWS 1994). Bridge construction on the Allegheny River, Pennsylvania, which supports large populations of both species, prompted a salvage operation to remove thousands of individuals from the impacted area. In an attempt to meet recovery plan objectives, these individuals were translocated to multiple streams within seven states where the species had declined or had been extirpated.

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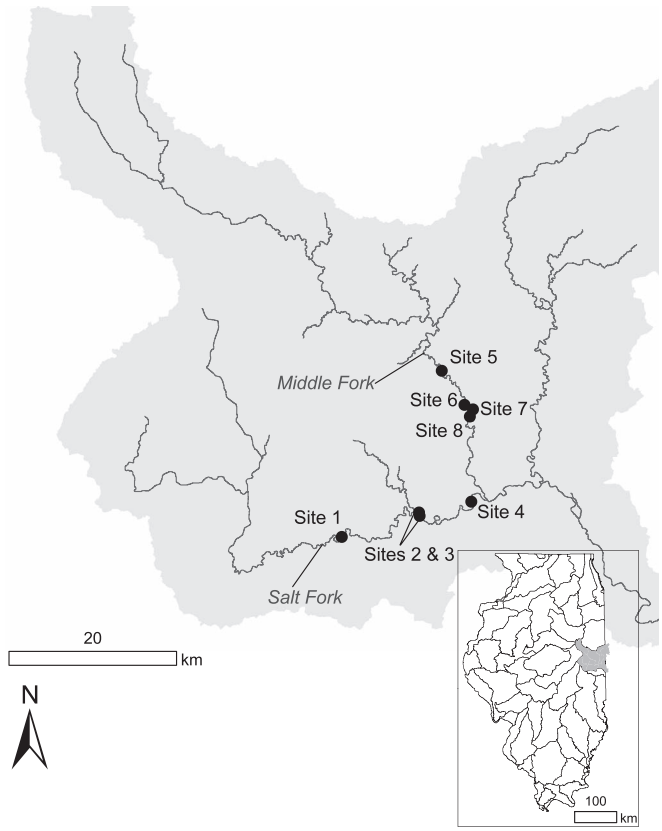


Figure 1. The Clubshell and Northern Riffleshell release sites in the Vermilion River basin (Wabash River drainage), Illinois.

Beginning in 2006, the Illinois Department of Natural Resources and the Illinois Natural History Survey partnered with the U.S. Fish and Wildlife Service and state agencies in Ohio, Pennsylvania, and West Virginia to translocate Clubshell and Northern Riffleshell from the Allegheny River to the Vermilion River system (Wabash River basin) in Illinois, where both species occurred historically (Cummings and Mayer 1997; Tiemann et al. 2007). Pilot translocations ($n <$ 75 individuals) first occurred in 2010 at one site each in the Salt Fork and Middle Fork Vermilion rivers, and more widespread translocations occurred at eight sites in 2012, 2013, and 2014. We conducted a five-year capture-mark-recapture study focusing on those individuals released in 2012, 2013, and 2014 to estimate survival of translocated mussels. Specifically, our goals were to evaluate (1) how survival differed according to species, sex, and mussel size, (2) how survival varied spatially (among sites and between rivers), and (3) how survival varied temporally after release.

## METHODS

### Mussel Collection and Transportation

Mussels were collected from the Allegheny River at the U.S. Highway 62 Bridge, Forest County, Pennsylvania. The Allegheny River at this site is approximately 200 m wide and drains an area of approximately 10,000 km$^2$. Mean daily discharge is approximately 56 m$^3$/s at the end of August and nearly 425 m$^3$/s at the beginning of April (average of 71 yr; USGS gage 03016000). We collected 197, 758, and 807 Clubshell and 957, 249, and 777 Northern Riffleshell in 2012, 2013, and 2014, respectively. We measured total length of each individual as the greatest distance from the anterior to posterior shell margin (nearest 1 mm), and affixed a 12.5 mm, 134.2 kHz PIT tag (BioMark, Inc., Boise, Idaho) to the right valve and a uniquely numbered HallPrint Shellfish tag (HallPrint, Hindmarsh Valley, South Australia) to the left valve. Northern Riffleshell averaged 45.6 mm long (range 15–70 mm) and Clubshell averaged 52.2 mm long (range 18–84 mm). We also determined the sex of each Northern Riffleshell based on shell morphology, although a few smaller individuals were classified as "unknown" (male:female ratio = 1.34:1); Clubshell sexes cannot be differentiated by external shell morphology and were all classified as "unknown." Clubshell and Northern Riffleshell were placed in coolers between damp towels and transported in climate-controlled vehicles to Illinois.

### Mussel Translocation and Release

We selected release sites based on the presence of presumably suitable habitat for Northern Riffleshell and Clubshell, which consisted of clean, stable sand, gravel, and cobble riffles (Watters et al. 2009), abundant and diverse mussel populations (INHS 2017), and presence of suitable host fishes (i.e., darters and minnows) for both mussel species (Cummings and Mayer 1992; Tiemann 2008a, 2008b; Watters et al. 2009). Based on these criteria, we selected four sites each in the Salt Fork and Middle Fork Vermilion rivers in east-central Illinois (Fig. 1). These streams are an order of magnitude smaller than the Allegheny River, each 30–40 m wide and draining approximately 1,100 km$^2$. Mean daily discharge in the Salt Fork is 0.4 m$^3$/s at the end of August and 4.3 m$^3$/s at the beginning of April (average of 45 yr; USGS gage 03336900); mean daily discharge in the Middle Fork is 0.9 m$^3$/s at the end of August and 8.5 m$^3$/s at the beginning of April (average of 38 yr; USGS gage 03336645).

We released 3,745 mussels (both species combined) among all eight sites from 2012 to 2014 (Table 1). Mussels were released in the late summer, following a quarantine and acclimatization period (14 d for 2012 mussels and 4–5 d for 2013–2014 mussels, differences between years due to logistics). We hand-placed mussels into the substrate at each site within an area demarcated by site-specific landmarks (such as trees, boulders, water willow beds, or other discernible feature) to facilitate recapture surveys. The size of marked release areas varied with site and were between 3–10 m wide and 20–100 m long. Sites with greater suitable area received more mussels, but all sites were stocked at less than 50% of the density observed at the collection site on the Allegheny River, which is 5.5/m$^2$ for Northern Riffleshell and 7.5/m$^2$ for

Table 1. Number of Clubshell and Northern Riffleshell released into the Salt Fork and Middle Fork Vermilion rivers in 2012, 2013, and 2014.

| | 2012 | | 2013 | | 2014 | |
|---|---|---|---|---|---|---|
| Site | Clubshell | Riffleshell | Clubshell | Riffleshell | Clubshell | Riffleshell |
| Salt Fork | | | | | | |
| 1 | - | 291 | - | - | - | - |
| 2 | 106 | 196 | 258 | - | - | - |
| 3 | 91 | 470 | 250 | - | - | - |
| 4 | - | - | 50 | 50 | 277 | 290 |
| Middle Fork | | | | | | |
| 5 | - | - | 50 | 50 | - | - |
| 6 | - | - | 50 | 50 | 175 | 180 |
| 7 | - | - | 50 | 50 | 181 | 174 |
| 8 | - | - | 50 | 49 | 174 | 133 |
| Totals | 197 | 957 | 758 | 249 | 807 | 777 |

Clubshell (Enviroscience, Inc., personal communication); these densities are similar to those seen for these species at other locations (Crabtree and Smith 2009). We stocked Clubshell at greater densities than Northern Riffleshell due to presumed historical presence based on historical shell collection records (INHS 2017). Logistical constraints (e.g. land access, previous stocking, mussel availability) largely dictated which sites received mussels in multiple years.

## Field Surveys

We surveyed for PIT-tagged Clubshell and Northern Riffleshell during 12 sampling periods from 2012 to 2016 (Appendix 1). We used a robust design sampling protocol that included primary and secondary samples (Fig. 2; Kendall and Nichols 1995; Kendall et al. 1997). We attempted to conduct primary samples every 3–4 mo to represent each season (spring, summer, autumn, winter), but environmental conditions prevented us from collecting all samples during every year. We used two to three observers during each primary sample. Each observer was considered an independent sample and represented a secondary sample in the robust design framework. We detected PIT-tagged mussels using BioMark FS2001F-ISO or BioMark HPR Plus receivers with portable BP antennas (BioMark). Each observer independently traversed the stream in a systematic manner from a unique starting point while slowly sweeping the streambed with an antenna. Surveys continued until the release site was covered completely and extended 5–10 m downstream after detections ceased. Each sample typically required 2–3 h/site.

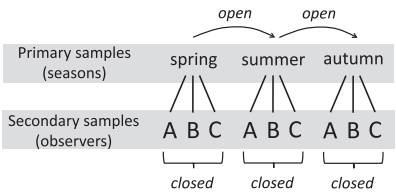


Figure 2. Robust design as employed in this study, with primary samples (seasons) and secondary samples (observers).

## Statistical Analyses

We used the Huggins Robust Design model (Huggins 1989, 1991) to estimate apparent survival while accounting for imperfect detection and to estimate of the numbers of individuals remaining after each sampling period. Population estimates from the Huggins Robust Design model (Huggins 1989, 1991) are derived using the actual number of individuals observed during a primary sample and detection probability. We were interested in the influence of individual traits (sex, length, and species), environmental factors (site within river and whether or not flood events had occurred between primary sampling periods), and number of years following release on survival. We fit a single model that included all covariates instead of fitting a suite of models and comparing model fit (Burnham and Anderson 2002). Consequently, we attained estimates for each species released at each site during each year by estimating a species effect, site effect, and an effect of years following release, along with the individual covariates of sex and length and the environmental covariate of the presence of a flood. We did not include group (site or species) by sampling period interactions because we had no reason to believe that survival would vary along that spatio-temporal scale (Anderson and Burnham 2002). We constrained our model so there was no immigration or emigration between primary samples, which we believed was biologically reasonable given the limited vagility of freshwater mussels (Amyot and Downing 1998; Schwalb and Pusch 2007). We fit detection as a function of sampling period and site to encompass differences in sampling efficiency due to variation in flow, temperature, and depth among dates and variation in habitat conditions among sites. We did not account for species-specific differences in detection because we used PIT tags and hand-held readers for both species and did not believe detection would differ by species when using this method.

Table 2. Parameter estimates (β coefficients), standard errors (SE), log-odds ($e^{\beta}$), and log-odds lower and upper 95% confidence limits (CL) of monthly survival of translocated Clubshell and Northern Riffleshell relative to site, years following release, species, sex, mussel length, and presence of flood between primary samples. Parameter estimates should be interpreted in relation to the baseline, which was Northern Riffleshell of average length and unknown sex at Site 1, four years postrelease, and during a period with no flooding, as indicated.

| Parameter | Estimate | SE | Log-odds | Lower CL log-odds | Upper CL log-odds |
|---|---|---|---|---|---|
| Intercept | 4.760 | 0.891 | | | |
| Individual traits | | | | | |
| Clubshell versus Riffleshell | 1.670 | 0.623 | 5.312 | 1.567 | 18.011 |
| Male versus unknown | 0.207 | 0.620 | 1.230 | 0.365 | 4.150 |
| Female versus unknown | −0.117 | 0.621 | 0.890 | 0.263 | 3.004 |
| Length | 0.009 | 0.004 | 1.009 | 1.003 | 1.016 |
| Environmental factors | | | | | |
| Site 2 versus Site 1 | −0.853 | 0.085 | 0.426 | 0.361 | 0.504 |
| Site 3 versus Site 1 | −1.402 | 0.079 | 0.246 | 0.211 | 0.287 |
| Site 4 versus Site 1 | −0.007 | 0.165 | 0.993 | 0.718 | 1.374 |
| Site 5 versus Site 1 | −0.999 | 0.130 | 0.368 | 0.286 | 0.475 |
| Site 6 versus Site 1 | −1.063 | 0.132 | 0.345 | 0.267 | 0.448 |
| Site 7 versus Site 1 | −1.757 | 0.128 | 0.173 | 0.134 | 0.222 |
| Site 8 versus Site 1 | −0.958 | 0.142 | 0.384 | 0.290 | 0.507 |
| Flood versus No Flood | −0.530 | 0.077 | 0.589 | 0.506 | 0.685 |
| Years following release | | | | | |
| Year 1 versus Year 4 | −1.260 | 0.658 | 0.284 | 0.078 | 1.030 |
| Year 2 versus Year 4 | −1.666 | 0.661 | 0.189 | 0.052 | 0.691 |
| Year 3 versus Year 4 | −1.228 | 0.660 | 0.293 | 0.080 | 1.066 |

Post hoc analyses indicated that inclusion of species-specific detection had very little influence on survival probabilities (i.e., estimates were within 0.01%). We determined if a flood occurred between primary samples using the Indicators of Hydrologic Alteration software package (IHA; Richter et al. 1996) and discharge data for both streams from the U.S. Geological Survey National Water Information System (https://waterdata.usgs.gov/il/nwis/rt; gages 03336900 and 03336645). We did not differentiate between small floods and large floods as identified by IHA, and anything equivalent to or greater than a 2-yr flood event was considered a flood. We used the Huggins' p and c extension in Program MARK (White and Burnham 1999) with initial capture probability ($p$, probability of detecting an individual at least once during a primary sample) equal to recapture probability ($c$, probability of detecting an individual during a primary sample given it is detected) because secondary samples occurred via the same method on the same day. We interpreted the strength and biological meaning of each model covariate using the beta coefficients (β) and their 95% confidence intervals and log-odds ratios, which approximate how much more likely it is for an event (survival) to occur based on the beta coefficient (log-odds ratio = $e^{\beta}$, Gerard et al. 1998; Hosmer and Lemeshow 2010).

## RESULTS

Detection rate averaged 0.78 across both species (range of averages = 0.66–0.90; Appendix 1). Detection was generally greatest in autumn. Average detection in autumn samples was about 1.25 times greater than for spring and summer samples; we had only one winter sample because of high flows and frozen conditions. However, detection probabilities were highly variable among sites and sampling periods (Appendix 1).

Monthly survival varied among species, sites, and sampling periods. Average monthly survival was 0.981 for Clubshell and 0.905 for Northern Riffleshell; these values translate to an approximate annual survival of 0.79 for Clubshell and 0.30 for Northern Riffleshell, irrespective of site, individual traits, and years following release. The β coefficient and log-odds ratio showed that, overall, Clubshell was approximately 5 times more likely to survive than Northern Riffleshell, but the precision of this estimate was low (95% confidence interval = 1.57–18.00×; Table 2). There was no difference in survival among males, females, and mussels of unknown sex; confidence intervals included zero for all coefficients (Table 2). There was no appreciable effect of size on survival. The log-odds ratio indicated that individuals were 1.009 times more likely to survive (95% confidence interval = 1.003–1.016) for every mm increase in length (Table 2).

Survival was greatest at Sites 1 and 4 on the Salt Fork and lowest at Site 7 on the Middle Fork (Figs. 3–6). Log-odds ratios showed that mussels were nearly 6 times less likely to survive at Site 7 than Site 1, and mussels were 2–4 times less likely to survive at Sites 2, 3, 5, and 6 (Table 2). Survival was reduced following floods. The log-odds ratio showed that

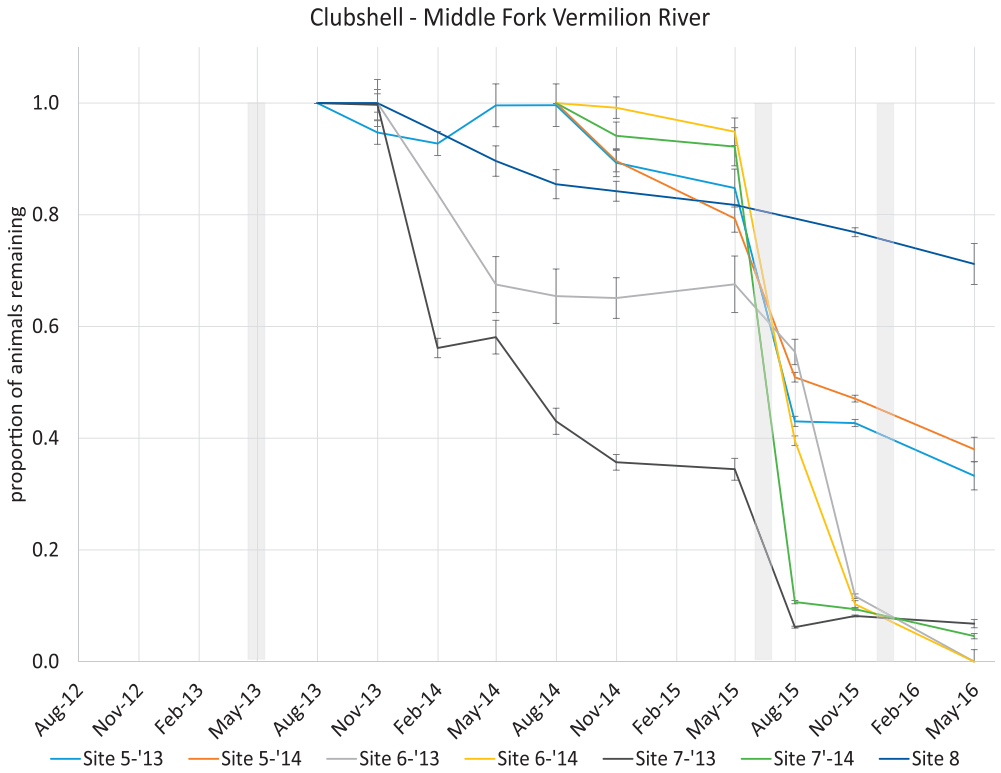


Figure 3. Derived estimates of proportion of Clubshell remaining at each release site in the Middle Fork from 2012 to 2016. Gray boxes indicate when a flood occurred. Numbers of individuals released per year per site can be viewed in Table 1.

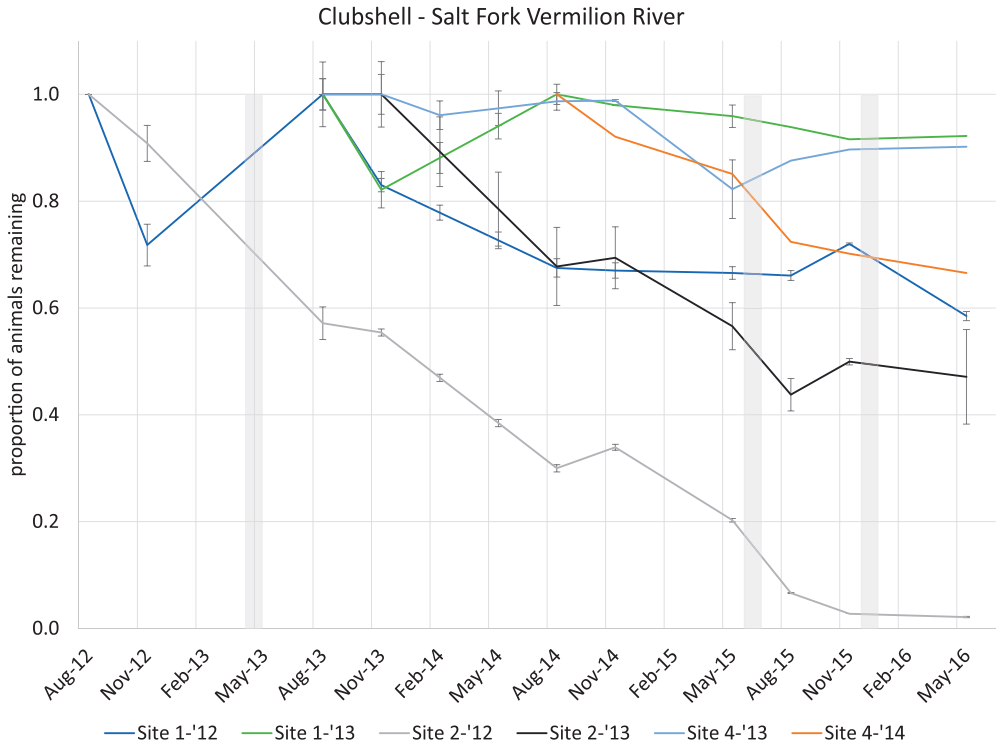


Figure 4. Derived estimates of proportion of Clubshell remaining at each release site in the Salt Fork from 2012 to 2016. Gray boxes indicate when a flood occurred. Numbers of individuals released per year per site can be viewed in Table 1.

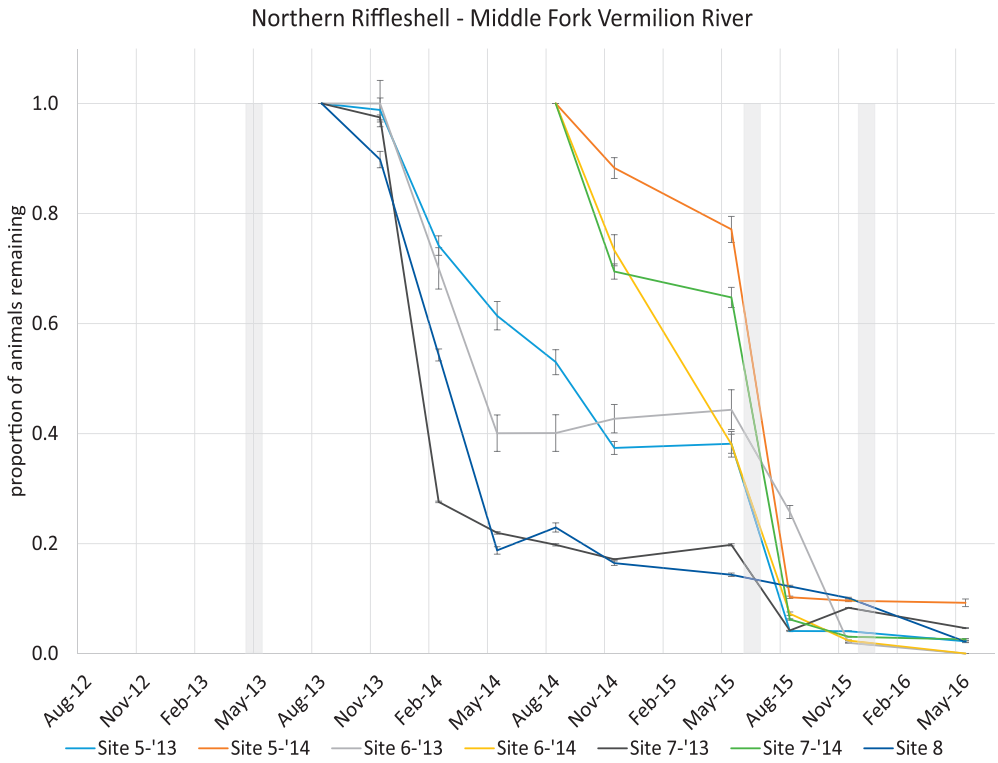


Figure 5. Derived estimates of proportion of Northern Riffleshell remaining at each release site in the Middle Fork from 2012 to 2016. Gray boxes indicate when a flood occurred. Numbers of individuals released per year per site can be viewed in Table 1.

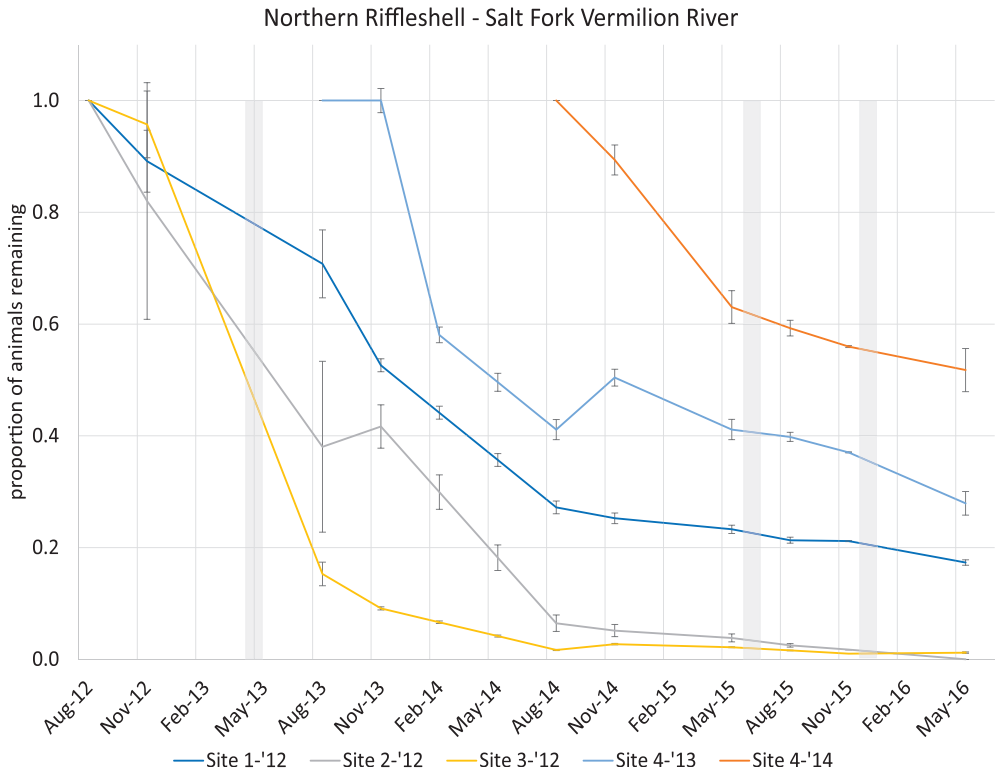


Figure 6. Derived estimates of proportion of Northern Riffleshell remaining at each release site in the Salt Fork from 2012 to 2016. Gray boxes indicate when a flood occurred. Numbers of individuals released per year per site can be viewed in Table 1.

mussels were 1.70 times less likely to survive after floods (95% confidence interval: 1.46–1.98) than after periods with no floods; this is equivalent to a reduction of monthly survival from 0.950 to 0.917 (average of all species and sites). The occurrence of a flood on the Middle Fork during June–July 2015 was associated with a sharp decline in population size for both species (Figs. 3, 5), but the influence of other flood events was not associated with similar declines. We did not model river as a separate factor (see Methods), but survival appeared to be greater in the Salt Fork than in the Middle Fork. An average of 62% of Clubshell and 19% of Northern Riffleshell were alive in the Salt Fork in 2016 compared with only 21% of Clubshell and 4% of Northern Riffleshell in the Middle Fork in 2016 (Figs. 3–6). This difference was apparent despite the fact that most mussels were translocated to the Salt Fork 1–2 yr earlier than in the Middle Fork (Table 1).

Number of years following release was an important determinant of survival. Survival was greatest in the fourth year following a release; individuals were 3.52 times more likely to survive in the fourth year following release (95% confidence interval: 0.97–12.80) compared to the first year following release (Table 2). Survival was lowest in the second year following release; individuals were 1.50 times less likely to survive (95% confidence interval: 1.30–1.70) compared to the first year (Table 2).

## DISCUSSION

The long-term efficacy of a reintroduction program depends on the establishment of a self-sustaining population, which requires translocated individuals to survive until they reproduce and replace themselves. It is too early to tell if the Clubshell and Northern Riffleshell reintroduction program into Illinois has been a success because no recruitment has been documented. Reintroduction of the Clubshell appears to have been more successful initially than reintroduction of Northern Riffleshell. Reintroduced Clubshell survived at a much greater rate and represented the majority of individuals remaining after five years of monitoring. Annual survival for Clubshell (0.79) is within the estimated range for other mussel species in the wild, (0.50–0.99, Hart et al. 2001; Villella et al. 2004) and near the estimates of the closely related Southern Clubshell (*Pleurobema decisum*) (0.91, Haag 2012). However, annual survival for Northern Riffleshell (0.30) was well below those values, those reported from French Creek, Pennsylvania, which averaged 0.60 (Crabtree and Smith 2009), and those of the closely related Oystermussel (*Epioblasma capsaeformis*) (0.73, Jones and Neves 2011; Haag 2012).

Some species may be inherently more difficult to translocate. There is high variability in the success of translocation projects, ranging from nearly all individuals remaining after a few years to very few if any (e.g., Ahlstedt 1979; Sheehan et al. 1989; Cope et al. 2003). Some of this variation may be explained by inherent life history differences among species, and Clubshell probably lives longer than Northern Riffleshell. For instance, the Southern Clubshell, a congener of Clubshell, can reach 45 yr of age (Haag and Rypel 2011), while Northern Riffleshell is a relatively short-lived species with a maximum age reported in French Creek, Pennsylvania, of 11 yr (Crabtree and Smith 2009). Based on these differences, Northern Riffleshell is expected to have lower survival than Clubshell even in wild populations, and our data show that translocated populations may have even lower survival. Consequently, translocation of short-lived species such as Northern Riffleshell may require larger numbers of individuals and repeated translocations to overcome high mortality and ensure that translocated individuals experience conditions favorable for recruitment.

Differences in hydrology, either between rivers or even within the same river, may play an important role in determining the suitability of sites for freshwater mussel reintroduction (Cope et al. 2003; Carey et al. 2015). The hydrology, land use, and watershed size of the Vermilion River basin differ from the source location of the Allegheny River (Larimore and Smith 1963; Smith 1968; Larimore and Bayley 1996; White et al. 2005), thus some discrepancy in survival between the source and recipient basins may be expected. However, the Salt Fork Vermilion and Middle Fork Vermilion rivers are comparable in size and have similar land use and hydrology, yet we found that survival varied even among sites within a river. Local-scale differences among sites, such as substrate or gradient, can lead to biologically significant differences that influence survival (McRae et al. 2004). We selected release sites based on the best available habitat and species assemblage data, yet unmeasured habitat differences and stochastic events appeared to have a large effect on survival. Similar results have been observed in other translocations, such as siltation due to bank failure following flow diversion (Bolden and Brown 2002), possible washout due to earthen causeway removal (Tiemann et al. 2016), or diminished recovery of relocated individuals in sites with high current velocity in the two years following relocation (Dunn et al. 2000).

High-discharge events present an ongoing threat to the reintroduction of Clubshell, Northern Riffleshell, and similar translocation projects. High-flow events have been problematic in other translocation projects (e.g., Sheehan et al. 1989; Carey et al. 2015) and were clearly detrimental for translocated Clubshell and Northern Riffleshell. Following the flood in June–July 2015, we examined the nearest downstream gravel bar at a few sites and found numerous stranded and dead individuals. Existing native mussel communities in the Salt and Middle Fork Vermilion rivers have persisted throughout similar high-flow events, but translocated mussels may be at a disadvantage. PIT tags can decrease the burrowing rate of individuals (Wilson et al. 2011), and translocated mussels may have lower energetic status (Patterson et al. 1997), which could reduce their ability to anchor themselves in the substrate or rebury after a flood event (Killeen and Moorkens 2016). Additionally, the native mussel community represents individuals that have found optimal locations to withstand scouring and dislodging. The

Clubshell and Northern Riffleshell we translocated may not have had enough time to find optimal locations, which may have made them more vulnerable to dislodgement and may partly explain why individuals survived at a greater rate 4 yr following release.

We provide the following recommendations for conducting and monitoring reintroduction efforts. The best time to monitor Clubshell and Northern Riffleshell was during autumn, when stream flows were low and we observed the greatest probability of detection. Sampling was difficult or impossible during the spring because of high stream flows, which resulted in reduced detectability using handheld readers; sampling also was difficult in winter because of high flows and occasional ice cover. Spreading reintroduction efforts over several geographically separate river systems could lessen risk of failure due to stochastic events such as floods, chemical spills, and biological invasion (e.g., Griffith et al. 1989; Trdan and Hoeh 1993). Translocating individuals over a period of several years might also reduce the overall risk of failure due to isolated events occurring in a particular year. For instance, many Clubshell and Northern Riffleshell, especially in the Middle Fork, were lost during a late spring/early summer high-flow event in 2015. Finally, stocking greater numbers of individuals in multiple translocations for species with naturally low annual survival, such as Northern Riffleshell, may be necessary to maximize chances for natural recruitment.

## ACKNOWLEDGMENTS

This project is a collaborative effort among the U.S. Fish and Wildlife Service (USFWS); Pennsylvania Fish and Boat Commission (PFBC); Pennsylvania Department of Transportation; Illinois Department of Natural Resources (IDNR), including the Illinois Nature Preserves Commission and the Illinois Endangered Species Protection Board; Illinois Natural History Survey; University of Illinois, Urbana-Champaign; Champaign County Forest Preserve District; the Ohio State University; Columbus Zoo and Aquarium; West Virginia Department of Natural Resources; Indiana Department of Natural Resources; Kentucky Department of Fish and Wildlife; and EnviroScience, Inc. Permits were provided by the USFWS (no. TE73584A-1); PFBC (e.g., no. 2014-02-0837, no. 2013-756); IDNR (e.g., no. SS16-047, no. S-10-30); the Illinois Nature Preserves Commission; and the University of Illinois. Funding was provided in part by the USFWS (through the IDNR's Office of Resource Conservation to the Illinois Natural History Survey, Grant no. R70470002 and no. RC09-13FWUIUC); the USWFS's Ohio River Basin Fish Habitat Partnership (Award no. F14AC00538); the IDNR (through the Natural Resource Damage Assessment settlement: Heeler Zinc–Lyondell Basell Companies, Reference Document no. OREP1402 and no. OREP1504); the Illinois Wildlife Preservation Fund (Grant no. RC07L25W); and the Illinois Department of Transportation.

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Appendix 1. Estimates of detection for each site and during each period; 95% confidence intervals are provided in parentheses.

| | Middle Fork | | | | Salt Fork | | | |
|---|---|---|---|---|---|---|---|---|
| Sample Period | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 | Site 8 |
| Summer 2012 | - | - | - | - | - | - | - | - |
| Autumn 2012 | 0.71 (0.68–0.74) | 0.67 (0.64–0.71) | 0.68 (0.64–0.72) | - | - | - | - | - |
| Summer 2013 | 0.72 (0.68–0.75) | 0.68 (0.63–0.73) | 0.69 (0.63–0.74) | - | - | - | - | - |
| Autumn 2013 | 0.79 (0.77–0.81) | 0.76 (0.74–0.70) | 0.76 (0.72–0.80) | 0.87 (0.85–0.89) | 0.83 (0.80–0.85) | 0.77 (0.73–0.80) | 0.81 (0.77–0.85) | 0.85 (0.82–0.88) |
| Winter 2014 | - | - | - | 0.80 (0.76–0.84) | 0.84 (0.80–0.88) | - | 0.83 (0.78–0.87) | - |
| Spring 2014 | - | - | - | - | 0.76 (0.72–0.80) | 0.69 (0.63–0.74) | 0.71 (0.66–0.76) | 0.79 (0.75–0.84) |
| Summer 2014 | 0.70 (0.67–0.72) | 0.66 (0.63–0.69) | 0.67 (0.64–0.71) | 0.81 (0.77–0.84) | 0.75 (0.71–0.78) | 0.67 (0.63–0.72) | 0.73 (0.68–0.78) | 0.78 (0.74–0.82) |
| Autumn 2014 | - | 0.75 (0.72–0.78) | - | 0.85 (0.81–0.87) | 0.80 (0.76–0.83) | 0.73 (0.68–0.77) | 0.78 (0.73–0.82) | 0.82 (0.78–0.86) |
| Spring 2015 | - | - | - | 0.72 (0.67–0.77) | 0.77 (0.73–0.82) | 0.70 (0.64–0.75) | 0.75 (0.69–0.81) | - |
| Summer 2015 | 0.80 (0.78–0.82) | 0.78 (0.75–0.80) | 0.78 (0.74–0.82) | 0.88 (0.86–0.90) | 0.84 (0.81–0.87) | 0.78 (0.74–0.82) | 0.83 (0.78–0.86) | - |
| Autumn 2015 | 0.86 (0.84–0.87) | 0.83 (0.81–0.85) | 0.84 (0.80–0.87) | 0.92 (0.90–0.93) | 0.88 (0.86–0.91) | 0.84 (0.80–0.87) | 0.87 (0.84–0.90) | 0.90 (0.88–0.92) |
| Spring 2016 | 0.78 (0.74–0.81) | 0.75 (0.71–0.79) | - | 0.87 (0.83–0.89) | 0.82 (0.78–0.86) | - | 0.81 (0.75–0.85) | 0.85 (0.81–0.88) |

Appendix 2. Monthly apparent survival estimates for Clubshell. Years (2012–2014) represent the year animals were released. Numbers in parentheses beside primary sample indicate the number of months since the preceding sample; 95% confidence intervals are provided in parentheses beside survival estimates. Bold rows indicate a flood occurred during that period (e.g., between Su 2013 and Au 2013). Sp = spring, Su = summer, Au = autumn, Wi = winter.

| | Salt Fork Vermilion River | | | | | | |
|---|---|---|---|---|---|---|---|
| | Site 1 | | Site 2 | | Site 3 | Site 4 | |
| Primary Samples (mo) | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2014 |
| Su 2012–Au 2012 (2) | 0.994 (0.993–0.995) | - | 0.977 (0.974–0.981) | - | 0.987 (0.984–0.989) | - | - |
| Au 2012–Su 2013 (9) | 0.990 (0.989–0.992) | - | 0.962 (0.956–0.967) | - | 0.978 (0.973–0.982) | - | - |
| **Su 2013–Au 2013 (2)** | **0.992 (0.990–0.993)** | **0.994 (0.993–0.995)** | **0.966 (0.962–0.971)** | **0.977 (0.974–0.981)** | **0.980 (0.976–0.984)** | **0.994 (0.992–0.996)** | **-** |
| Au 2013–Wi 2014 (4) | 0.992 (0.990–0.993) | 0.994 (0.993–0.995) | 0.966 (0.962–0.971) | 0.977 (0.974–0.981) | 0.980 (0.976–0.984) | 0.994 (0.992–0.996) | - |
| Wi 2014–Sp 2014 (2) | 0.992 (0.990–0.993) | 0.994 (0.993–0.995) | 0.966 (0.962–0.971) | 0.977 (0.974–0.981) | 0.980 (0.976–0.984) | 0.994 (0.992–0.996) | - |
| Sp 2014–Su 2014 (2) | 0.992 (0.990–0.993) | 0.994 (0.993–0.995) | 0.966 (0.962–0.971) | 0.977 (0.974–0.981) | 0.980 (0.976–0.984) | 0.994 (0.992–0.996) | - |
| Su 2014–Au 2014 (4) | 0.995 (0.993–0.996) | 0.992 (0.990–0.993) | 0.978 (0.973–0.982) | 0.966 (0.962–0.971) | 0.987 (0.983–0.990) | 0.991 (0.988–0.994) | - |
| Au 2014–Sp 2015 (5) | 0.995 (0.993–0.996) | 0.992 (0.990–0.993) | 0.978 (0.973–0.982) | 0.966 (0.962–0.971) | 0.987 (0.983–0.990) | 0.991 (0.988–0.994) | 0.994 (0.992–0.996) |
| **Sp 2015–Su 2015 (3)** | **0.991 (0.988–0.993)** | **0.986 (0.983–0.988)** | **0.963 (0.955–0.97)** | **0.944 (0.934–0.953)** | **0.979 (0.972–0.983)** | **0.986 (0.980–0.990)** | **0.990 (0.986–0.993)** |
| Su 2015–Au 2015 (3) | 0.995 (0.993–0.996) | 0.992 (0.990–0.993) | 0.978 (0.973–0.982) | 0.966 (0.962–0.971) | 0.987 (0.983–0.990) | 0.991 (0.988–0.994) | 0.994 (0.992–0.996) |
| **Au 2015–Sp 2016 (6)** | **0.997 (0.990–0.999)** | **0.991 (0.988–0.993)** | **0.989 (0.961–0.997)** | **0.963 (0.955–0.970)** | **0.994 (0.977–0.998)** | **0.991 (0.986–0.994)** | **0.986 (0.98–0.990)** |

Appendix 2, extended.

| Middle Fork Vermilion River | | | | | | |
|---|---|---|---|---|---|---|
| Site 5 | | Site 6 | | Site 7 | | Site 8 |
| 2013 | 2014 | 2013 | 2014 | 2013 | 2014 | 2013 |
| - | - | - | - | - | - | - |
| - | - | - | - | - | - | - |
| **0.985 (0.980–0.988)** | **-** | **0.984 (0.979–0.988)** | **-** | **0.968 (0.959–0.975)** | **-** | **0.985 (0.981–0.989)** |
| 0.985 (0.980–0.988) | - | 0.984 (0.979–0.988) | - | 0.968 (0.959–0.975) | - | 0.985 (0.981–0.989) |
| 0.985 (0.980–0.988) | - | 0.984 (0.979–0.988) | - | 0.968 (0.959–0.975) | - | 0.985 (0.981–0.989) |
| 0.985 (0.980–0.988) | - | 0.984 (0.979–0.988) | - | 0.968 (0.959–0.975) | - | 0.985 (0.981–0.989) |
| 0.977 (0.971–0.982) | - | 0.976 (0.969–0.981) | - | 0.953 (0.940–0.963) | - | 0.978 (0.972–0.983) |
| 0.977 (0.971–0.982) | 0.985 (0.980–0.988) | 0.976 (0.969–0.981) | 0.984 (0.979–0.988) | 0.953 (0.940–0.963) | 0.968 (0.959–0.975) | 0.978 (0.972–0.983) |
| **0.962 (0.950–0.971)** | **0.974 (0.966–0.981)** | **0.960 (0.946–0.97)** | **0.973 (0.964–0.980)** | **0.922 (0.898–0.941)** | **0.947 (0.931–0.959)** | **0.964 (0.951–0.973)** |
| 0.977 (0.971–0.982) | 0.985 (0.980–0.988) | 0.976 (0.969–0.981) | 0.984 (0.979–0.988) | 0.953 (0.940–0.963) | 0.968 (0.959–0.975) | 0.978 (0.972–0.983) |
| **0.975 (0.966–0.982)** | **0.962 (0.950–0.971)** | **0.974 (0.963–0.981)** | **0.960 (0.946–0.97)** | **0.953 (0.940–0.963)** | **0.922 (0.898–0.941)** | **0.976 (0.967–0.983)** |

Appendix 3. Monthly apparent survival estimates for Northern Riffleshell. Years (2012–2014) represent the year animals were released. Numbers in parentheses beside primary sample indicate the number of months since the preceding sample; 95% confidence intervals are provided in parentheses beside survival estimates. Bold rows indicate a flood occurred during that period (e.g., between Su 2013 and Au 2013). Sp = spring, Su = summer, Au = autumn, Wi = winter.

| | Salt Fork | | | | | | |
|---|---|---|---|---|---|---|---|
| | Site 1 | | Site 2 | | Site 3 | Site 4 | |
| Primary Samples (months) | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2014 |
| Su 2012–Au 2012 (2) | 0.971 (0.907–0.991) | - | 0.891 (0.706–0.965) | - | 0.934 (0.806–0.98) | - | - |
| **Au 2012–Su 2013 (9)** | **0.951 (0.852–0.985)** | **-** | **0.828 (0.586–0.942)** | **-** | **0.893 (0.711–0.966)** | **-** | **-** |
| Su 2013–Au 2013 (2) | 0.957 (0.867–0.987) | 0.971 (0.907–0.991) | 0.844 (0.614–0.949) | 0.891 (0.706–0.965) | 0.904 (0.735–0.97) | 0.970 (0.904–0.991) | - |
| Au 2013–Wi 2014 (4) | 0.957 (0.867–0.987) | 0.971 (0.907–0.991) | 0.844 (0.614–0.949) | 0.891 (0.706–0.965) | 0.904 (0.735–0.97) | 0.970 (0.904–0.991) | - |
| Wi 2014–Sp 2014 (2) | 0.957 (0.867–0.987) | 0.971 (0.907–0.991) | 0.844 (0.614–0.949) | 0.891 (0.706–0.965) | 0.904 (0.735–0.97) | 0.970 (0.904–0.991) | - |
| Sp 2014–Su 2014 (2) | 0.957 (0.867–0.987) | 0.971 (0.907–0.991) | 0.844 (0.614–0.949) | 0.891 (0.706–0.965) | 0.904 (0.735–0.97) | 0.970 (0.904–0.991) | - |
| Su 2014–Au 2014 (4) | 0.972 (0.909–0.991) | 0.957 (0.867–0.987) | 0.894 (0.71–0.967) | 0.844 (0.614–0.949) | 0.936 (0.809–0.98) | 0.956 (0.862–0.987) | - |
| Au 2014–Sp 2015 (5) | 0.972 (0.909–0.991) | 0.957 (0.867–0.987) | 0.894 (0.71–0.967) | 0.844 (0.614–0.949) | 0.936 (0.809–0.98) | 0.956 (0.862–0.987) | 0.970 (0.904–0.991) |
| **Sp 2015–Su 2015 (3)** | **0.953 (0.855–0.986)** | **0.928 (0.793–0.978)** | **0.832 (0.59–0.944)** | **0.762 (0.483–0.916)** | **0.896 (0.715–0.967)** | **0.928 (0.785–0.979)** | **0.951 (0.846–0.986)** |
| Su 2015–Au 2015 (3) | 0.972 (0.909–0.991) | 0.957 (0.867–0.987) | 0.894 (0.71–0.967) | 0.844 (0.614–0.949) | 0.936 (0.809–0.98) | 0.956 (0.862–0.987) | 0.97 (0.904–0.991) |
| **Au 2015–Sp 2016 (6)** | **0.986 (0.923–0.997)** | **0.953 (0.855–0.986)** | **0.944 (0.746–0.99)** | **0.832 (0.59–0.944)** | **0.967 (0.836–0.994)** | **0.952 (0.849–0.986)** | **0.928 (0.785–0.979)** |

Appendix 3, extended.

| Middle Fork | | | | | | |
|---|---|---|---|---|---|---|
| Site 5 | | Site 6 | | Site 7 | | Site 8 |
| 2013 | 2014 | 2013 | 2014 | 2013 | 2014 | 2013 |
| - | - | - | - | - | - | - |
| **-** | **-** | **-** | **-** | **-** | **-** | **-** |
| 0.924 (0.78–0.977) | - | 0.920 (0.768–0.975) | - | 0.851 (0.624–0.952) | - | 0.927 (0.785–0.978) |
| 0.924 (0.78–0.977) | - | 0.920 (0.768–0.975) | - | 0.851 (0.624–0.952) | - | 0.927 (0.785–0.978) |
| 0.924 (0.78–0.977) | - | 0.920 (0.768–0.975) | - | 0.851 (0.624–0.952) | - | 0.927 (0.785–0.978) |
| 0.924 (0.78–0.977) | - | 0.920 (0.768–0.975) | - | 0.851 (0.624–0.952) | - | 0.927 (0.785–0.978) |
| 0.890 (0.702–0.966) | - | 0.884 (0.688–0.963) | - | 0.792 (0.525–0.929) | - | 0.894 (0.709–0.967) |
| 0.890 (0.702–0.966) | 0.924 (0.78–0.977) | 0.884 (0.688–0.963) | 0.920 (0.768–0.975) | 0.792 (0.525–0.929) | 0.851 (0.624–0.952) | 0.894 (0.709–0.967) |
| **0.827 (0.578–0.943)** | **0.878 (0.675–0.961)** | **0.818 (0.563–0.94)** | **0.871 (0.66–0.959)** | **0.691 (0.391–0.887)** | **0.771 (0.493–0.921)** | **0.833 (0.587–0.946)** |
| 0.890 (0.702–0.966) | 0.924 (0.78–0.977) | 0.884 (0.688–0.963) | 0.920 (0.768–0.975) | 0.792 (0.525–0.929) | 0.851 (0.624–0.952) | 0.894 (0.709–0.967) |
| **0.881 (0.679–0.963)** | **0.827 (0.578–0.943)** | **0.874 (0.665–0.961)** | **0.818 (0.563–0.940)** | **0.776 (0.498–0.924)** | **0.691 (0.391–0.887)** | **0.885 (0.687–0.964)** |