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REGULAR ARTICLE

PIT TAG APPLICATION IN NATIVE FRESHWATER MUSSELS: CASE STUDIES ACROSS SMALL, MEDIUM, AND LARGE RIVERS

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ABSTRACT

Since their first use in the mid-1980s, external passive integrated transponder (PIT) tags have facilitated innovative investigations into multiple biological traits of animals. For native freshwater mussels, PIT tags are frequently used in capture-mark-recapture applications because they allow repeated, noninvasive sampling, are easy to apply, have high retention rates, and have negligible short-term effects on growth and survival. Because of these traits, resource managers and scientists are using PIT-tagged animals to estimate survival and movement of mussels associated with restoration efforts. However, consistency is limited in how PIT tags are affixed, monitored, and reported. Thus, our objectives were to (1) share our collective experiences in PIT tagging mussels across three case studies in small, medium, and large rivers and (2) propose guidelines for tagging and reporting data from PIT tag studies with native freshwater mussels to facilitate comparisons across future studies. The number of studies that have marked mussels with PIT tags has increased over the past 10 years. The ability to detect mussels using PIT tags has substantially advanced research in three areas of mussel ecology: (1) estimating vital rates (e.g., growth and survival), (2) tracking movements and behaviors of captively propagated, wild, and translocated individuals, and (3) improving our understanding of life history traits, such as reproductive timing. Each case study offers insights on tagging methods, tag loss, tag retention, and monitoring frequency across multiple species that range in conservation status from common to rare. We conclude with best-practice guidelines for placing PIT tags on freshwater mussels and a list of variables that could be reported in future studies to facilitate cross-system comparisons.

KEY WORDS: passive integrated transponder tag, native freshwater mussels, tagging methods, tag retention, monitoring, survival, movement

REVIEW OF TAGGING METHODS

Movement is a fundamental trait of animals, and tracking animals under natural conditions has facilitated research on behavior, ecology, and conservation science. Landscape alterations, such as changes in land use and cover, invasive species, and climate change, have accelerated studies to assess the effects of global change on animals and their habitats. The field of biotelemetry, the remote measurement of physiological, behavioral, or energetic status of free-living animals (Cooke et al. 2004), has changed substantially over time. Traditional approaches to animal tracking often relied on visual observations and recordings of a few dozen observations per animal, resulting in general movement patterns. The advent of Global Positioning System (GPS)–based telemetry automated this process, but early GPS configurations were large and costly and had limited accuracy (Bijleveld et al. 2022).

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Exponential improvements in tracking technology have led to smaller tracking devices that can record millions of observations per animal for ever-smaller animals (Kays et al. 2015). Today, tagging of animals with electronic sensors (i.e., archival tags, satellite positioning tags, and passive integrated transponder, or PIT, tags) is a common approach to research and monitor animal movements. Large, spatially explicit datasets resulting from high-resolution movement trajectories facilitate new scientific inquiries on ecology, evolution, physiology, social networks, competition, and predation (Kays et al. 2015). The ability to predict animal movements, and to understand the mechanisms behind those movements, play a key role in conservation and management.

As with terrestrial animals, the movements of aquatic animals and their interactions over time and space facilitate ecological processes (Ogburn et al. 2017). They transport nutrients, biomass, and energy across ecosystems. Historically, efforts to acquire and process information on aquatic animal movements were impeded by the vastness, complexity, and opacity of their environments (Hussey et al. 2015). However, recent advances in acoustic tracking technology have revolutionized the scope and scale of questions that can be asked about the causes and consequences of the movements of aquatic animals (Villegas-Ríos et al. 2020). Telemetry data have defined home ranges, delineated species distribution, identified breeding sites, and characterized habitat use (Citta et al. 2018; Bouyoucos et al. 2020; Novak et al. 2020; Williamson et al. 2021). Acoustic telemetry is a widely used aquatic tracking method, in which the signals transmitted from implanted or externally attached acoustic transmitters are detected and logged by nearby acoustic receivers (Reubens et al. 2021). Rapid advances in acoustic telemetry have allowed scientists to monitor a range of species and animal sizes from 10-cm salmon smolts (*Oncorhynchus tshawytscha*) to 29-m blue whales (*Balaenoptera musculus*) across freshwater, brackish, and marine environments (Bailey et al. 2009; Rechisky et al. 2013).

Tagging is also used in capture-mark-recapture analyses to estimate reproduction and survival rates (e.g., Stodola et al. 2017); however, the accuracy of these estimates depends partly on tag retention (Jung et al. 2020). Lost or unrecognized tags can result in unreliable estimates (McDonald et al. 2003), which may lead to misleading ecological inferences. Thus, the choice of tagging method is critical and should be driven by research objectives. In choosing a tagging method, scientists should consider tag longevity, tag requirements, and the need to identify batches of animals or individuals. If research objectives do not require data at the individual level, many methods are available for marking animals. Initial tagging studies used rudimentary technology such as fin clips, oxytetracycline, and coded wire tags to mark batches of animals (Neely et al. 2021). For example, in fish-stocking efforts, visible implant elastomer tags are frequently used because they are inexpensive, relatively easy to apply, and a viable tool for short-term tagging experiments (e.g., Simon and Dorner 2011). Henry and Jarne (2007) assessed marking techniques for the gastropod *Physella acuta* and recommended glued plastic marks for long-term studies and paint marks for mass marking. Fluorescence marking of juvenile mussels by immersion in a calcein solution offers a quick and reliable method to batch mark animals (Eads and Layzer 2002).

Most of the early tagging studies used large-bodied organisms because they were easy to handle, withstood the stress of tagging and recapture, and had high retention rates and because their behaviors were less affected by tag size (Sandford et al. 2019). Small, individually identifiable tags to study smaller animals and earlier life stages have facilitated long-term studies that examine population-level changes in abundance or survival and the mechanisms responsible for these changes (Roberts et al. 2021). Desirable traits of individual-based tags include high tag retention, minimal handling time, and minimal effects on survival or behavior (Roberts et al. 2021). If research objectives necessitate data at the individual level, there are multiple marking methods. Coded wire tags are commonly used in stock enhancement programs and have a high retention rate for long-term use (Simon and Dorner 2011; Zhu et al. 2016). Visible implant alphanumeric tags have been used successfully in salamanders (Moon et al. 2022). High-resolution Vemco positioning system tags and receivers can provide representative estimates of fine-scale movements of larger aquatic species such as the European perch *Perca fluviatilis* (Guzzo et al. 2018).

TAGGING NATIVE FRESHWATER MUSSELS

Native freshwater mussels (hereafter mussels) are long-lived endobenthic organisms that provide critical ecological services in aquatic systems (Vaughn 2018). North America is the global center of mussel diversity, and ~70% of the ~300 species in North America are considered endangered, threatened, or of special concern (Lopes-Lima et al. 2018); thus, resource managers are aptly concerned about their conservation and management. To be effective, tags must be retained throughout the study duration, not cause undue stress on the animal, and not adversely affect survival or behavior. These criteria can be challenging because mussels are long lived (e.g., >30 yr; Haag 2012) and reside in abrasive habitats (e.g., some species burrow into sand and gravel substrates, others reside associated with large boulders). Multiple methods to tag juvenile and adult mussels have been assessed (e.g., Lemarié et al. 2000). Marks made by etching adult shells with a knife, file, or Dremel tool can remain visible for decades (Patterson et al. 2018), although few studies have evaluated the long-term effects of this tagging method. Coded wire tags inserted into the hinge ligament of adult *Reginaia ebenus* were successfully retained for 2 yr (Layzer and Heinricher 2004). Individually numbered polyethylene shellfish tags have been successfully used to track mussels over time (e.g., Lymbery et al. 2021). Because they are inexpensive and easy to apply, this method is frequently used with mussels.

Table 1. Advantages and limitations of using passive integrated transponder (PIT) tags in studies to conserve and restore native freshwater mussels.

Advantages	Limitations
Noninvasive and efficient	Acoustic technology is changing rapidly, so tags and readers may not communicate
Tags can be read indefinitely	Tag interference could result in unreliable data if marked animals are in close proximity
Small (8–32 mm) and lightweight (100–600 mg)	Smaller tags have shorter read ranges
Can be used across life-history stages from subadults to adults	Mussels <20 mm in shell length may not be suitable for PIT tagging
Relatively inexpensive (tags ~$3–12) Sold in bulk so pricing varies depending upon quantity	Readers have a start-up cost ($500–10,000), which is unique to the intended use (i.e., data logging versus shallow or deepwater recovery)
Tag loss can be minimal	If applied incorrectly, tags can cause mortality or fall off
Few short-term effects on behavior	Long-term effects on behavior are unknown
Easily affixed to shells	Internal marking should be avoided as it could cause shell deformity or tissue damage and may cause undue stress to the mussel
High recapture rates	Individuals must be recaptured to confirm they are alive

Recently, laser engraving of subadult mussels (typically <2 yr old) has increased the efficiency of tagging individuals, and one person can tag several hundred mussels per hour (Patterson et al. 2018). PIT tags have been used frequently in studies with mussels because they allow repeated, noninvasive sampling, are relatively inexpensive, are easy to apply, have high retention rates, and have negligible short-term effects on growth and survival (Kurth et al. 2007; Tiemann et al. 2016; Newton et al. 2020).

ADVANTAGES AND LIMITATIONS OF USING PIT TAGS WITH NATIVE FRESHWATER MUSSELS

Since their first use in the mid-1980s, externally affixed PIT tags have facilitated innovative investigations into multiple biological traits of animals. PIT tags are alphanumeric, battery-free radio frequency identification tags that are activated by a low-frequency radio signal emitted by a scanning device to generate a close-range electromagnetic field (Patterson et al. 2018). Reliable as a fingerprint, they can last throughout the lifespan of the organism studied (Gibbons and Andrews 2004). PIT tags allow researchers to recapture an individual without repeated handling and associated stress on the animal (e.g., Young and Isely 2008). For imperiled species, PIT tags allow an individual to be located and identified without removing it from the substrate (Stodola et al. 2017). Scanners are available as handheld, portable, battery-powered, and automated stationary models (Smyth and Nebel 2013). Their small size (8–32 mm) reduces potential adverse behavioral and physiological effects on the animal, improving animal welfare and scientific results (Table 1; Kays et al. 2015). For these reasons, PIT tags have become a common choice for marking animals, especially mussels. However, their use with mussels has limitations, and it is important to be aware of these prior to initiating a study (Table 1).

APPLICATION OF PIT TAGS IN MUSSEL CONSERVATION

The ability to detect mussels using PIT tags has advanced ecological research, notably by (1) improving estimates of vital rates, such as growth and survival, (2) tracking movements and behaviors of captively propagated, wild and translocated individuals, and (3) improving our understanding of life-history traits, such as reproductive timing. A goal of many conservation programs is to estimate vital demographic rates to assess the vulnerability of mussels to threats from disease, invasive species, habitat loss, and climate change (Roberts et al. 2021). PIT tags have been used to assess growth, survival, movement, behavior, and reproductive timing (e.g., Gough et al. 2012; Tiemann et al. 2016; Sotola et al. 2021; Nakamura et al. 2022). Estimates of background rates of growth and survival have informed management decisions by providing information on how vital rates govern mussel populations and how they vary across physical and biological factors (Newton et al. 2020). Information on how population vital rates vary among species and over time gives managers a tool to understand how mussels might respond to management actions, such as habitat restoration projects or translocations.

Survival and reproductive success are benchmarks to evaluate the effectiveness of translocation efforts; PIT tags can improve recapture rates to more effectively estimate these parameters. Translocations are used to restore mussel populations by moving individuals from one location to another, often in response to in-river activities (i.e., bridge replacement or channel dredging). In one of the first studies with mussels, Kurth et al. (2007) PIT tagged 238 *Lampsilis cariosa* and reported a mean recapture rate of 78% after 21 mo. The effectiveness of translocation also depends on translocated individuals surviving until they reproduce and replace themselves. Tiemann et al. (2016) measured 3-yr survival rates of 71% and 93% for PIT-tagged *Lampsilis cardium* and

Ortmanniana ligamentina, respectively, after translocation. Survival rates vary across species, and some species are inherently more difficult to translocate. For example, survival of *Pleurobema clava* was five-fold greater than *Epioblasma rangiana* 4 yr after translocation (Stodola et al. 2017). High recapture rates of PIT-tagged mussels can improve the accuracy of survival estimates and provide robust data to assess the success of translocation as a restoration tool to conserve imperiled mussels.

Mussels' high imperilment rate, coupled with the important ecological services they provide, prompted the creation of large-scale propagation programs to culture juveniles in captivity and release them in the wild. Initial propagation efforts often stocked newly released juveniles that were typically too small to be tagged individually, and thus the success of these programs could not be accurately assessed. Today, most propagation programs stock older juveniles (~2 yr old), which have higher survival rates and can be individually tagged (Southwick and Loftus 2017). The long-term success of propagation efforts is not well understood, but some results are encouraging (Inoue et al. 2023). However, post-release monitoring of propagated mussels is inconsistent (Rytwinski et al. 2021). The ability to PIT tag juveniles before they are released into the wild allows scientists to monitor survival. For example, Hua et al. (2015) PIT tagged 5- to 10-mm hatchery-propagated *Epioblasma brevidens* and estimated detection probabilities and survival rates of released individuals that averaged 98 and 99%, respectively, over a 2-yr period. Release and monitoring of tagged juveniles are critical steps in the propagation process (Patterson et al. 2018). Although this field is relatively new, the available data indicate that noninvasive tracking of mussels using PIT tags could advance our ability to conserve and restore imperiled species.

Although many studies have documented the efficacy of PIT tags in facilitating recapture of mussels, notably fewer studies have assessed the effects of tagging on behavior. Wilson et al. (2011) cautioned that marking individual mussels with PIT tags significantly decreased burrowing rate. However, the results were likely influenced by methodological details, such as holding mussels out of water for 40 min to allow the epoxy resin adhesive to dry. A 40-min processing time is 10–20× longer than recent studies (Newton et al. 2015; Ashton et al. 2017). Longer-duration studies would be helpful to assess the long-term effects of PIT tagging on the physiology and behavior of mussels.

Since Kurth et al. (2007), the use of PIT tags in mussels in the peer-reviewed literature has increased, with 19 of 28 studies published since 2016 based on a search of "freshwater mussels" and "PIT tags" in Web of Science and Google Scholar (Fig. 1). This increase occurred despite limited guidelines on the appropriate use, size, and placement of PIT tags or on the efforts required to recapture mussels. Because PIT tags increasingly are being applied to mussels, our objectives were to (1) share our collective experiences in PIT tagging mussels across three case studies in small, medium, and large rivers and (2) propose guidelines for tagging and reporting data from PIT tag studies with native freshwater mussels to facilitate comparisons across future studies. Development of consistent guidelines for PIT tagging could reduce handling and other stressors that might adversely affect individuals (e.g., Henry and Jarne 2007). Below are three case studies that offer insights on tagging methods, tag loss, tag retention, and monitoring frequency (Table 2). Nomenclature for species names follows the Integrated Taxonomic Information System (ITIS 2024).

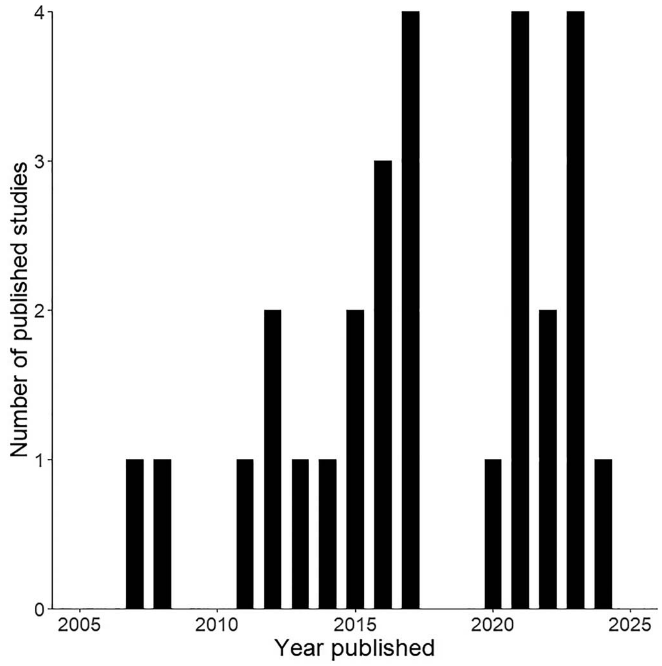

Figure 1. Cumulative number of studies published in the peer-review literature based on a search of the terms "freshwater mussels" and "PIT tags" in Web of Science and Google Scholar searches. Twenty-eight papers were published between 2007 and 2024 (as of May 8, 2024).

CASE STUDY 1 (SMALL RIVER): NANJEMOY CREEK AND BROWNS BRANCH, MARYLAND

The Maryland Department of Natural Resources began PIT tagging *Prolasmidonta heterodon* (also known as *Alasmidonta heterodon*) in Nanjemoy Creek (4,160 ha) and Browns Branch (694 ha), Maryland, in 2020 and 2021, respectively. Both streams are in the Atlantic Coastal Plain, are relatively small (wetted width <5 m), are dominated by sand and gravel substrates, and flow directly into Chesapeake Bay. In prior surveys (2001–2006), shellfish tags were used to mark 165 *P. heterodon* and 13% were recaptured (MDNR 2022). The low recapture rates created uncertainty about their population status. There was no a priori information on known stressors outside of natural factors (e.g., predation, drought, floods). Mussels were PIT tagged to facilitate capture-mark-recapture sampling (e.g., Stodola et al. 2017) following visual surveys to estimate apparent survival and detection probability within

Table 2. Comparison of methods used to place passive integrated transponder (PIT) tags on adult native freshwater mussels across three river systems in North America. Adhesives include marine epoxy and cyanoacrylate gel. All case studies used cyanoacrylate accelerator except for 2b. Mean % recaptures are mussels found with the PIT tag reader at least once.

Case Study	River Size	Species Tagged	Tag Size, mm	Adhesive	No. Tagged	Mean % Tag Loss	Mean % Recaptures	Monitoring Frequency
1	Small	*Prolasmidonta heterodon*	10	Cyanoacrylate gel	163	16.5	5	Annually, biweekly during fall, spring, and summer
2a	Medium	*Alasmidonta marginata*	9, 12.5	Marine epoxy and cyanoacrylate gel	515	4.0	35	Annually for 8–10 yr
		Lampsilis cardium					81	
		Lasmigona compressa					100	
		Lasmigona costata					45	
		Ligumia recta					45	
		Ortmanniana ligamentina					93	
		Venustaconcha ellipsiformis					56	
2b	Medium	*Epioblasma rangiana*	12.5	Marine epoxy	4,314	0	69	Annually for 13 yr
		Pleurobema clava					78	
3	Large	*Amblema plicata*	20, 23	Cyanoacrylate gel	573	0.5	44	Annually for 4 yr
		Pustulosa pustulosa					47	
		Obliquaria reflexa					51	
		Pleurobema sintoxia					61	

and across years. Tagging also provided a supplemental means of recapturing mussels to assist visual surveys where annual relative abundance and growth are measured. Monitoring with PIT tags also was initiated because of a need for demographic data to develop and potentially implement management actions in the watershed. Available data on vital rates from the nearest population of *P. heterodon* were from a watershed with substantially different characteristics (Galbraith et al. 2016). The population in Nanjemoy Creek could be one of the largest in the Chesapeake Bay because the watershed is mostly forested and has good water quality. Conversely, Browns Branch is in an agricultural watershed where the water quality is degraded, and the population appears to be in decline (Pinkney et al. 2020).

Mussels were PIT tagged in the field after conducting visual surveys and promptly returned to their source location, as indicated by a weighted marker. Due to their small size (22–52 mm in length), a 10-mm, 134.2-kHz FDX-B PIT tag was used. Tags were externally affixed to the shell—anterior to the posterior ridge and below the umbo—with a fine tip gel cyanoacrylate glue (Loctite, Henkel Corporation, Rocky Hill, Connecticut). The adhesive and the tag were cured to the shell with a drop of cyanoacrylate accelerant (Palm Labs Adhesives, DeBary, Florida). The tag was surrounded by a thin layer of cyanoacrylate and another drop of accelerant. This process took ~2 min/mussel and was typically done with the mussel at least partially submerged in water. Monitoring with a handheld PIT tag reader and submersible wand antenna (Biomark BP Lite Antennae and HPR Plus Reader, Boise, Idaho) was done at four sites in each river on four to five dates about every 2 wk during August to October (following initial visual surveys), March to April (Nanjemoy Creek only), and again the following May to July (prior to another visual survey). The number of PIT-tagged *P. heterodon* at each site ranged from 5 to 55 individuals, and sites ranged in size from 24 to 112 m^2. Each site was searched by one person for 15 to 45 min (depending on size) by systematically walking upstream with the submersible reader and making a second pass walking downstream. An additional 5 m downstream from each site was searched also.

In Nanjemoy Creek, the percentage of PIT tags detected among the four sites ranged from 57% to 78% in the fall and from 26% to 44% the following spring. Tag detection ranged from 48% to 54% in early summer. A visual survey in July 2021 recaptured 15 of 88 (17%) tagged mussels, which took 14 person-hours of effort. Five of the recaptured mussels (33%) lost their PIT tag. An additional 37 untagged *P. heterodon* were obtained and tagged. Monitoring across four additional events through the summer and fall of 2021 detected 50% to 59% of the first cohort and 73% to 89% of the second cohort. In the second annual survey, nine mussels from the first cohort (10%) and seven from the second cohort (19%) were recaptured during 26 person-hours of effort. Three of the recaptured *P. heterodon* from the first cohort (33%) and zero from the second cohort had lost their tags. This rate of tag loss could result from the small number of recaptures, insufficient cure time for the glue, low pH water, and changes in tagging personnel.

In Browns Branch, 38 *P. heterodon* were obtained at four sites in August 2021 and PIT tagged using methods like those used in Nanjemoy Creek. Monitoring started ~14 days after tagging and was conducted four times through the fall. Tag detection averaged 74% to 95% across monitoring events following the initial tagging of *P. heterodon* from visual surveys in 2021. The higher detection rate in Browns Branch, relative to Nanjemoy Creek, may be due to the smaller area of sites in Browns Branch and the clustered nature of *P. heterodon*. For example, 13 *P. heterodon* were found in a single 10-m^2 pool at one site associated with large woody debris. Detection of PIT tags across four events the following spring and summer was similar (45–68%) to rates observed in Nanjemoy Creek. A visual survey across all four sites in July 2022 recaptured 10 of 38 (26%) tagged mussels and took 7 person-hours of effort. No tag loss was observed in recaptured mussels.

Rates of tag detection indicate that a relatively high number of *P. heterodon* were undetected in visual surveys even after considerable effort. This pattern of low abundance and cryptic behavior affecting detection is well documented across a range of mussel species and habitats (e.g., Wisniewski 2013; Sanchez and Schwalb 2021). Even in relatively small rivers with low density ($<$0.5 mussels/m^2) and well-defined monitoring plots, variation in detection appeared to correspond with seasonal changes in discharge and water temperature. These covariates are known to affect detection probability, presumably due to the physiological demands on mussels to maintain their position in the substrate (Meador et al. 2011; Wisniewski 2013). Clustering of *P. heterodon* alongside large woody debris and under masses of roots of aquatic vegetation can exacerbate tag interference. This issue persisted, even though upstream and downstream sampling passes were made. The modest effort (1–3 person-hours per event) expended to detect most tagged mussels with a handheld reader and submersible wand antenna provided high rates of detection that consistently exceeded the rates in more labor-intensive visual surveys. Thus, PIT tags provided an efficient way to document site fidelity of the tagged population and to estimate apparent survival; four PIT tag monitoring events could be conducted in the time required for one visual survey. Although relatively high rates of tag loss were observed in *P. heterodon* in Nanjemoy Creek, the presence of ghost tags (PIT tags found in the environment from loss or mortality) does not account for such a sustained rate of tag reads given the number of untagged mussels found in visual surveys. An alternate hypothesis is that the population may have multiple endobenthic individuals at any given time. Combining PIT tag monitoring with traditional visual surveys allowed us to understand if nondetections in visual surveys represent true loss from the population (i.e., mortality or emigration) or if they are attributable to other factors (i.e., temperature). Future studies could place a shellfish tag on one valve and a PIT tag on the other valve to assess the degree to which ghost tags influence tag loss.

CASE STUDY 2 (MEDIUM RIVER): VERMILION RIVER AND KISHWAUKEE RIVER, ILLINOIS

The Illinois Natural History Survey began a PIT tag study in 2010 to monitor translocated mussels from the Allegheny River, Pennsylvania, into the Vermilion River, Illinois (Stodola et al. 2017). Since then, PIT tags have been used to monitor translocations of mussels from bridge construction sites (e.g., Kishwaukee River, Illinois; Tiemann et al. 2016). The methods developed to tag and monitor mussels across the state are based on studies in the Vermilion and Kishwaukee rivers in Illinois.

The Vermilion River Basin in east-central Illinois has a rich and diverse aquatic fauna, and the lower portions of the Middle Fork and Salt Fork Vermilion rivers are medium-sized rivers dominated by sand, gravel, and cobble (Page et al. 1992; Stodola et al. 2017). Between 2010 and 2016, 2,006 federally endangered *P. clava* and 2,308 federally endangered *E. rangiana* were obtained from the Allegheny River, Pennsylvania, PIT tagged, and translocated to the Middle Fork (109,447 ha) or Salt Fork Vermilion (131,571 ha) rivers. These individuals, ranging in shell length between 15 and 89 mm, were tagged with a Biomark PIT tag using Devcon marine-grade epoxy (Danvers, Massachusetts) on one valve and a shellfish tag using cyanoacrylate glue (e.g., Loctite or Gorilla Glue, Sharonville, Ohio) on the other valve. The 2010 translocated animals had 12.5-mm, 125-kHz PIT tags, while those translocated during 2012 to 2016 had 12.5-mm, 134-kHz PIT tags. The mussels have been monitored at least annually since placement.

The Kishwaukee River (163,350 ha) in northern Illinois is rated a Biologically Significant River because of high mussel and fish diversity (Bertrand et al. 1996; ILDNR 2000). A study was initiated because bridge construction on the Jane Addams Memorial Highway (Interstate 90) required mussels to be translocated, providing an opportunity to assess the effects of short-distance ($<$0.2 km) translocation (Tiemann et al. 2016). Sand and gravel substrates dominate this portion of the river, which is $\sim$50 m wide and has a mean depth $<$1 m during base flow. In 2013, 100 mussels of two common species, *L. cardium* and *O. ligamentina*, were obtained, affixed with 12.5-mm, 134-kHz PIT tags in Devcon marine-grade epoxy, and released about 200 m upstream from the bridge (refer to Tiemann et al. 2016 for further details). Mussels were monitored monthly from May to October during 2013 to 2015.

In 2015 a capture-mark-recapture study was initiated to evaluate population dynamics and movement of the mussel community present in the Kishwaukee River (Tiemann et al. 2016). Five species were affixed with a 9- or 12.5-mm, 134-kHz PIT tag on one valve and a single shellfish tag on the opposite valve. A drop of cyanoacrylate glue (Loctite or Gorilla Glue) was applied on one valve, the tag was placed on the drop of glue, and the area was sprayed with a cyanoacrylate accelerant (Palm Labs Adhesives). Once the glue dried, another layer of cyanoacrylate glue was placed on top of the tag and sprayed again with the accelerant. Tags were placed near the hinge line or below the umbo. Animals were returned to the point of capture. Since 2015, 415 animals of five species were tagged (182 *Ligumia recta*, 146 *Alasmidonta marginata*, 77 *Lasmigona costata*, nine *Venustaconcha ellipsiformis*, and one *Lasmigona compressa*). Seven species have been PIT tagged, including two common (*L. cardium* and *O. ligamentina)* and five Species of Greatest Conservation Need in Illinois (*A. marginata*, *L. compressa*, *L. costata*, *L. recta*, *V. ellipsiformis*). Across all species, shell length of tagged individuals ranged from 39 to 169 mm.

Tagged mussels in the Kishwaukee and Vermilion rivers were largely monitored with Biomark BP Lite or Portable Antennae and HPR Plus readers. The experimental design allowed comparisons of detection rates across seasons (Stodola et al. 2017). In the Vermilion River, the greatest detection rates were observed in autumn, likely due to low water levels. The Kishwaukee River has been monitored from late spring to early fall (Tiemann et al. 2016). In 2019, a single-cable inflatable (floating) antenna was incorporated into monitoring efforts to cover more area. Rather than walking the river in a systematic manner with a handheld antenna (detectability range of ~0.3 m), Biomark's floating antenna can cover ~1 m up to depths of >2 m. The floating antenna is typically used once a year (often during the summer) in the Vermilion and Kishwaukee rivers. The antenna is battery powered and can be pulled behind a kayak or canoe (Fig. 2).

Figure 2. Example of a single-cable inflatable (floating) antenna used to cover large areas (~1 m) to improve detection of passive integrated transponder tags placed on native freshwater mussels in the Vermilion and Kishwaukee rivers, Illinois. Photo by Alison Stodola.

CASE STUDY 3 (LARGE RIVER): UPPER MISSISSIPPI RIVER, MINNESOTA AND WISCONSIN

Scientists from the U.S. Geological Survey, Upper Midwest Environmental Sciences Center, and the Minnesota Department of Natural Resources were interested in estimating population vital rates of mussels as a measure of relative health in the Upper Mississippi River (defined here as upstream from the mouth of the Ohio River, excluding the Missouri River). The Upper Mississippi River contains varied habitats for mussels including the main navigation channel, side channels, backwater lakes, and impounded areas. PIT tags were affixed on 578 mussels of four species (*Amblema plicata*, *Pustulosa pustulosa* (formerly *Cyclonaias pustulosa*), *Obliquaria reflexa*, and *Pleurobema sintoxia*) in a well-studied mussel assemblage in a side channel of the Mississippi River (15,247,620 ha). Mean (± 1 standard deviation) shell lengths of tagged mussels were 73.6 ± 14.4, 63.8 ± 11.3, 50.1 ± 8.1, and 61.4 ± 10.2 mm for *A. plicata*, *P. pustulosa*, *O. reflexa*, and *P. sintoxia*, respectively. Growth and survival of tagged mussels were assessed annually for 4 yr across core (high density, ~11.1 mussels/m^2) and peripheral (low density, ~0.5 mussel/m^2) areas of the assemblage. Details about the study design and research results can be found in Newton et al. (2020).

To begin the tagging process, the shells of each mussel were scrubbed to remove existing Dreissenid mussels. Next, a thick elliptical bead of cyanoacrylate glue (Gorilla Glue) was applied in the crevice adjacent to the hinge line to the extent possible. A 20- or 23-mm PIT tag (Biomark) was placed in the bead of cyanoacrylate and another thick bead of cyanoacrylate was placed over the tag. Last, a 1-mL syringe was used to apply ~0.5 mL of a cyanoacrylate accelerant (Palm Labs Adhesives) to the PIT tag area to facilitate drying. To reduce stress on the mussel from the PIT tag, tags that were <1% of the mussel's body mass and below the maximum suggested threshold of 4% were used (Theuerkauf et al. 2007). Rapid application of PIT tags can reduce handling stress. The process from scrubbing the shell to placement of a PIT-tagged mussel into an experimental grid took <4 min/mussel. Handling stress was further reduced by gluing a standard length of buoyant fly-fishing line (included in the 4-min processing time) to the shell, which facilitated recapture rates and allowed us to estimate burial depth without handling each mussel. The PIT-tagging method worked well in this large dynamic river, and only two broken tags were encountered during the recapture of 294 individuals over the course of the study.

Prior to initiating this study, a preliminary experiment was conducted to identify how near a PIT tag the receiver must be to locate a mussel (Newton et al. 2015). A 20-mm PIT tag and an 18-cm loop antenna allowed mussels to be recaptured within <30 cm using the PIT tag reader alone and to a depth of at least 20 cm. Positional accuracy was assessed by estimating position errors due to field measurements based on trilateration error surfaces. About 80% to 86% of the locations had error polygons of ≤300 cm^2 (i.e., equivalent to ~10-cm radius circle) and 96% to 98% had error polygons ≤600 cm^2 (i.e., equivalent to ~20-cm radius circle).

This study had a relatively large sample size (578 tagged mussels) and modest sampling frequency (annually for 4 yr), and it yielded >500 observations of tagged mussels. The resulting data allowed us to estimate growth and survival of mussels. Of the 578 tagged mussels, 294 (51%) were recaptured at least once, 100 were recaptured in multiple years, and 44 were recaptured in all 4 yr (Newton et al. 2020). Results indicate considerable variability in rates of survival and growth in natural mussel assemblages. This variation warrants being

Table 3. Best practice guidelines for affixing passive integrated transponder (PIT) tags to native freshwater mussels.

Category	Guideline	Rationale
Tag size	Use the smallest tag size needed to meet study objectives	Larger tags can create a body burden due to their mass
Tag placement	If possible, affix the PIT tag in the crevice adjacent to the hinge line	Tag placement (i.e., posterior, anterior) can influence read range and potential tag loss
Adhesive type	Select adhesive based on study duration	Tag loss can affect the quality and quantity of data obtained; some adhesives require an extended period out of water for curing that can stress mussels
Accelerant	If using a cyanoacrylate glue, use a cyanoacrylate accelerant	The accelerant can substantially reduce the amount of time mussels are out of the water for tagging, which can reduce tagging-associated mortality
Tagging time	Reduce the amount of time mussels are out of the water to the extent possible	Mussels should remain submerged during processing to reduce handling mortality
Demarcation of study site	Release PIT-tagged mussels in well-marked areas	Improves sampling efficiency and potentially improves recapture rates; can facilitate estimating temporary and permanent immigration rates
Tag density	Avoid clustering PIT-tagged mussels	Tags in proximity can interfere with one another or can affect the probability of detection, depending upon reader type

accounted for when assessing the response of mussels to habitat restoration projects.

GUIDELINES FOR PIT TAGGING MUSSELS

These case studies represent varied applications of PIT tag use for research and monitoring of mussel assemblages in different-sized rivers. Based on our collective experiences in small, medium, and large rivers, we offer the following as considerations for future PIT tag studies with mussels (Table 3).

Tag Size

Tag sizes typically used on mussels include 9-, 10-, 12.5-, or 23-mm tags. Size affects the read range of the antenna; larger tags have a greater detection range (Table S1), but smaller tags reduce the weight burden on small-bodied mussels. Thus, the choice of tag size is a compromise between the desired proximity to detect a tagged mussel and the potential adverse effects due to the mass of the tag.

Tag Placement

A key consideration for PIT tag longevity is placement of the tag on the mussel valve (Fig. 3). Protecting the tag from abrasion and shear forces is critical. In the Kishwaukee River, erosion of the glue surrounding the PIT tag rendered the tag obsolete. If using a species with sculptured shells, affix the tag in the crevice near the hinge line or parallel to a ridge. If using a species with nonsculptured shells, affix the tag near the hinge line. In the Kishwaukee River, smooth-shelled species (e.g., *L. recta*) lost PIT tags more frequently than other species (Douglass et al. 2022). Tag orientation also can affect the read range. Tags orientated perpendicular to the antenna have a larger read range than those oriented parallel to the antenna (https://www.biomark.com/pit-tags/). For mussels that bury into river sediments, tags placed adjacent to the hinge line are typically in the optimal orientation.

Adhesive Type

Most case studies used cyanoacrylate glue in gel form to affix PIT tags to mussels (Table 2). Typically, a bead of cyanoacrylate glue was applied to the periostracum, a PIT tag was placed in the bead and a second coat of cyanoacrylate glue was applied to completely enclose the tag. Initially, studies in the Kishwaukee (Tiemann et al. 2016) and Vermilion (Stodola et al. 2017) rivers used marine-grade epoxy (Table 2). Epoxy can last longer and may not erode as often; however, initial cure time can be lengthy (>45 min; mussels can be held in water during this time but should not be allowed to burrow).

Accelerant Use

All case studies used an accelerant to speed cyanoacrylate drying time. The accelerant is easily photodegraded, so store it in an amber bottle. Apply the accelerant by spraying (60-mL bottle) or by using a 1.0-mL syringe. Using an accelerant reduces drying time of the cyanoacrylate from ~4 to ~1 min at 25°C (https://palmlabsadhesives.com/).

Tagging Time

Recent studies indicate that an individual mussel can be tagged in <4 min (Newton et al. 2020). Prolonged handling can impose physiological stress that can lead to indirect mortality or dislodgement from river substrates (Zigler et al. 2008). Repeated handling also can influence growth rates. Growth of *P. pustulosa* that were excavated, measured, and tagged twice in 2 yr was lower than that of individuals

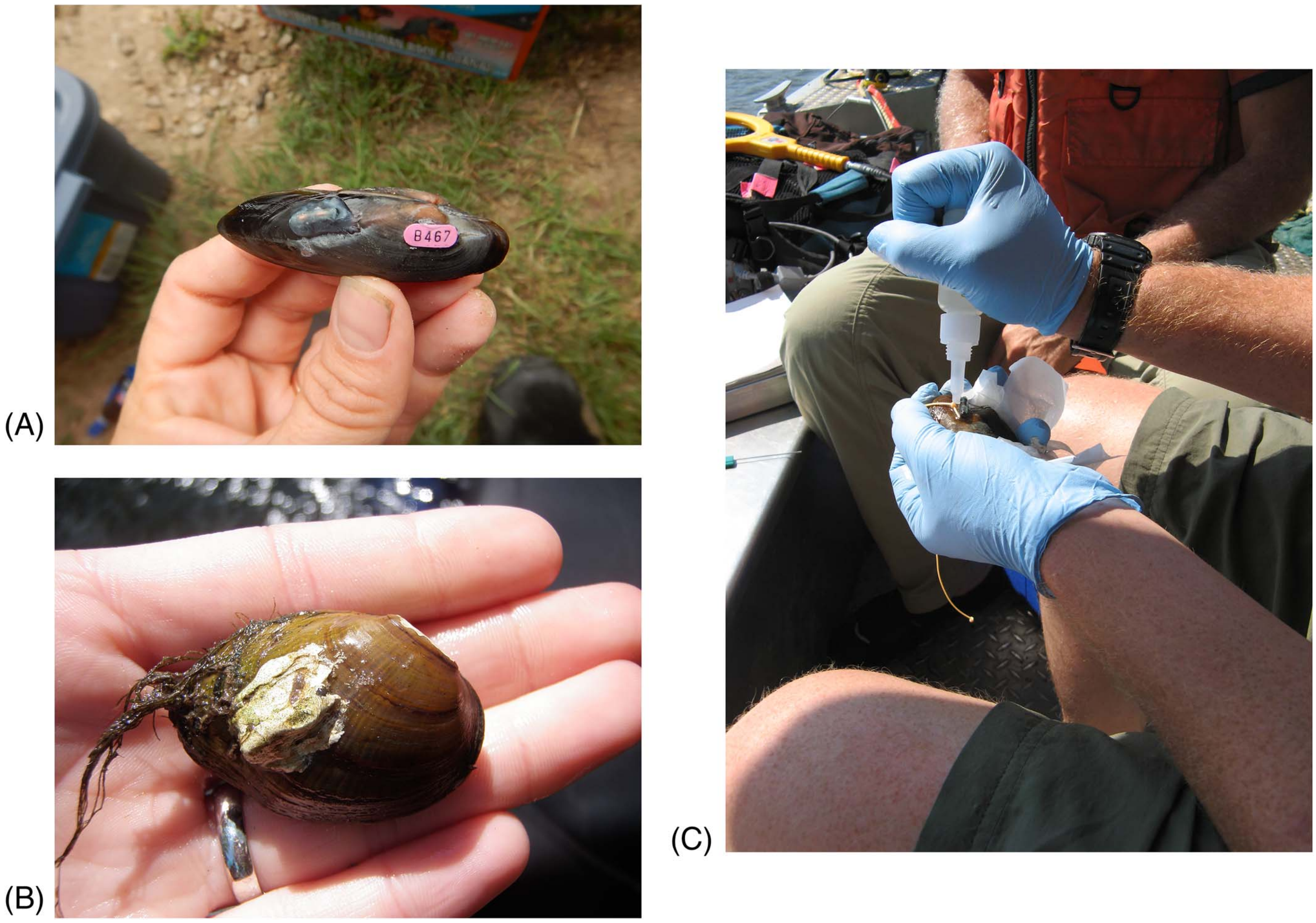

Figure 3. Examples of methods to affix passive integrated transponder (PIT) tags on native freshwater mussels used across three case studies in small, medium, and large rivers: (A) a 9-mm PIT tag affixed to *Ligumia recta* in the Kishwaukee River, (B) a 12.5-mm PIT tag in epoxy affixed to *Epioblasma rangiana*, and (C) affixing a 20-mm PIT tag to *Amblema plicata* in the Upper Mississippi River. Photos by Sarah Douglass (A), Alison Stodola (B) and Teresa Newton (C).

disturbed only once in 2 yr (Haag and Commens-Carson 2008). Training staff to apply PIT tags prior to the day of tagging could reduce tagging time, benefiting mussels.

Demarcation of Study Site

The longer it takes to relocate the site, the less time there is to recapture mussels. In the Upper Mississippi River, Newton et al. (2020) marked sites with GPS coordinates, polyvinyl chloride stakes, and concrete blocks with lead lines and after ~6 h of diving were only able to relocate 14 or 16 (dependent on year) of the 20 sites. Similarly, in the Vermilion River, sites were marked with GPS coordinates, whereas in the Kishwaukee River, Tiemann et al. (2016) marked the site with a steel fence post, and the subsequent study was marked with GPS coordinates (Douglass et al. 2022). These results highlight the benefits of marking sites with multiple methods to facilitate relocating sites.

Tag Density

Experiences in the Illinois studies indicate that tags in proximity ($\geq$10 tags/m^2) substantially reduced the detection of mussels (Fig. 4). Because detection range and interference can vary with antenna type and tag density (Fischer et al. 2012), an experiment was conducted to assess detection rate as a function of tag density and antenna type. Tagged mussels were simulated by placing one PIT tag in a 2-mL plastic tube; tubes were positioned in a 1-m $\times$ 1-m cell on the ground, in an area free of metal or inductive material, at densities of 1, 3, 5, 10, 20, 30, and 90 tags/m^2 to mimic natural densities (Schwalb and Pusch 2007). Cells were arranged as a single cell or as three joined cells (3 m $\times$ 1 m), and tags were read by passing each antenna within 10 cm of the tag for three replicate trials. The proportion detected for each density, cell width, and antenna type was averaged for each replicate. We fit linear models using arcsine of the proportion of tags detected as the response variable and density (log-transformed), cell width, and antenna type as predictors. We predicted tag detection rate and 95% confidence intervals for each scenario using R Statistical Software (v4.0.3; R Core Team 2020). Regardless of antenna type, detection rates declined as tag density increased. The handheld antenna had greater detection rates than the floating antenna ($P < 0.001$; Fig. 4).

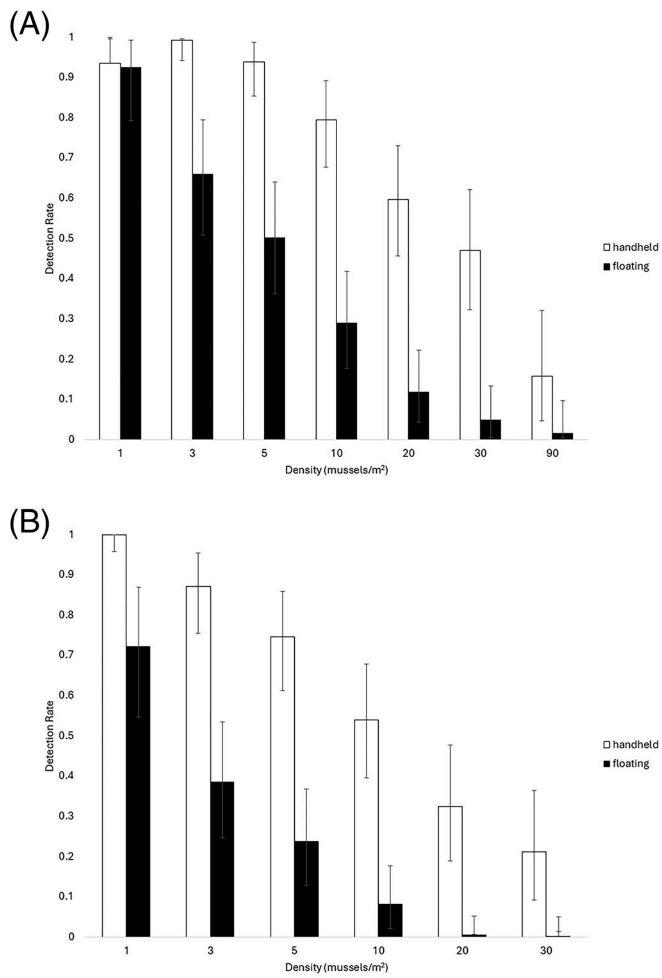

Figure 4. Linear model predictions of detection rate of tags and 95% confidence intervals using handheld (white) and floating (black) antennas as a function of tag density in a (A) 1-m × 1-m cell and (B) 3-m × 1-m cell ($P < 0.001$).

Guidelines for Reporting Results of Studies with PIT-Tagged Mussels

A research priority for mussel conservation is standardizing monitoring methods (Ferreira-Rodríguez et al. 2019). To facilitate future comparisons across PIT-tagging studies with mussels, we suggest six guidelines for reporting data. First, report the species-specific number of mussels tagged and the size frequency of individuals to document the frequency in which tags are used on mussels and to identify species that could be less (or more) amenable to tagging. Second, report the size of the search area and the time required to search a given area to help future studies maximize search efficiency. Third, report tag size and tag placement to facilitate metanalyses of the effects of these covariables on behavior and subsequent rates of tag loss. Fourth, estimate the time a mussel is out of water, so that future studies can develop empirical relationships between time-out-of-water and subsequent rates of mortality. Fifth, record rates of tag loss, including tags that do not read or evidence (epoxy or glue on the shell) of a mussel having been tagged; this information could help future studies interpret recapture data and evaluate conditions that may have contributed to tag loss. Placing a shellfish tag on one valve and a PIT tag on the other valve could identify if an individual had a PIT tag or was untagged during a given study. Sixth, document environmental variables such as water temperature and substrate type to help future studies explore the associations among water temperature, substrate type, burrowing rates, tag loss, and tag detection, especially considering that substrate types likely affect tag retention and mussel mobility.

CONCLUSIONS

Multiple factors warrant consideration when using PIT tags to recapture mussels in studies that advance conservation and restoration of native freshwater mussels. PIT tags provide a noninvasive method for tracking mussels and offer advantages such as ease of application and long-term durability. However, PIT tags are not without limitations, and assessing these relative to stated objectives for each study would be beneficial. Because PIT tag use in studies with native freshwater mussels are increasing, guidelines for PIT tagging mussels and development of a consistent reporting of PIT tag-associated variables can facilitate comparisons across future studies. Our synthesis of collective experiences across small, medium, and large rivers can provide researchers and managers with best practice guidelines for PIT tagging and monitoring native freshwater mussels.

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