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Survey of Native Freshwater Mussel Diversity and Survivorship After Reintroduction in Cub Creek, Homestead National Historical Park

2020–2024

Freshwater mussel species found during diversity surveys in Cub Creek.
NPS / ROBERT HARTWIG

Survey of Native Freshwater Mussel Diversity and Survivorship After Reintroduction in Cub Creek, Homestead National Historical Park: 2020–2024

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Abstract

Freshwater mussels are one of the most endangered taxonomic groups in North America, with native mussels having undergone severe losses in the Midwest due to land conversion, loss of host fish, threats from invasive mussels, and hydrological alterations. Nebraska was historically home to 30 species of freshwater mussels, but only about a third of those are currently considered stable in the state. Cub Creek is a tributary of the Big Blue River and runs through Homestead National Historical Park (HOME) in southeastern Nebraska. The Nebraska Game and Parks Commission has been pursuing the hatchery propagation of two native mussel species for reintroduction. Cub Creek was identified as a location to reintroduce the extirpated species. National Park Service staff and volunteers conducted surveys to characterize freshwater mussel populations in Cub Creek from 2020 to 2024. This resulted in the collection of 2,330 individuals representing ten species. Mapleleaf consistently had the highest relative abundances. Additionally, plain pocketbook and fatmucket mussels were reintroduced in 2020 and restocked in the subsequent three years. A subset of mussels each year were fitted with Passive Integrated Transponder (PIT) tags to allow for recapture of individuals and therefore, evaluation of their growth and survival within reintroduction habitats. Surviving mussels demonstrated significant growth rates, with both species doubling shell length within four years. Fatmucket mussels had higher survival rates than plain pocketbook mussels. This project highlights the potential for successful mussel restoration in Nebraska streams and underscores the importance of continued long-term monitoring.

List of Terms or Acronyms

FM: fatmucket

HOME: Homestead National Historical Park

LDA: linear discriminant analysis

NGPC: Nebraska Game and Parks Commission

NPS: National Park Service

PIT: passive integrated transponder

PP: plain pocketbook

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Introduction

The Unionidae are freshwater filter feeding bivalves that have a nearly worldwide distribution. North America is a significant hotspot for freshwater mussel diversity (Bogan 1993), with approximately 300 recognized species. Freshwater mussels play a number of crucial economic and ecological roles. Native Americans used them as a food source and for construction of tools, pottery, and jewelry. In the early 1900s, European settlers began using them for pearl buttons, which developed into a multimillion-dollar industry (Mueller 1993). Today, they are used as nuclei for commercially farmed saltwater mussel pearls.

As sedentary suspension feeders, mussels have profound influences on aquatic ecosystems by providing critical ecosystem functions (Vaughn 2018). Their burrowing behavior releases nutrients into the water column and oxygenates the substrate (Vaughn and Hakenkamp 2001). They clarify water by filtering sediment, organic matter, bacteria, and phytoplankton from the water and into the benthos, thus providing a link between water column and benthic habitats (Howard and Cuffey 2006). Additionally, they make energy and nutrients available to aquatic invertebrates which are an important food source for fish (Haag 2012). Mussels are prey for other species including racoons, muskrats, otters, turtles, fish, and birds. Density of mussels in streambeds affects the variety of other macroinvertebrates present, stabilizes streambeds, offers microrefugia for benthic life, and provides a substrate on which other organisms such as periphyton can grow (Vaughn and Hakenkamp 2001; Vaughn and Spooner 2006; Zimmerman and Szalay 2007).

Long-lived freshwater mussels are sensitive to a wide variety of environmental factors such as changes in water quality and can serve as a sentinel species, acting as biological indicators of water quality and ecosystem health. When populations begin to decline, it often signals that other species are at risk as well. Because of this, the global population declines and high species extinction rates of unionid mussels are a significant concern (Strayer et al. 2004).

Freshwater mussels are one of the most endangered groups in North America with nearly three quarters of them considered to be endangered, threatened, or of special concern (Williams et al. 1993; Strayer et al. 2004). They are the single largest group of federally listed endangered or threatened invertebrates (The Nature Conservancy 1996; USFWS 2024), with at least 37 species now presumed extinct (Master et al. 2000).

The decline is attributed to pollution, water quality degradation, habitat destruction, damming, exploitation and harvesting, recruitment failure, lack of host fish, watershed and riparian alterations, and predation (Strayer et al. 2004). Native mussels have undergone particularly severe losses in the Midwest due to land conversion, agricultural runoff, hydrologic alterations, loss of host fish, and threats from invasive mussels (e.g., zebra mussels) that outcompete native mussels for food and habitat (Hoke 2011). Hoke (2011) documented 30 unionid species in Nebraska. Twenty-three percent of those were only documented by relic shells or early museum vouchers. These have probably been extirpated from the state, reflecting the poor current condition of Nebraska mussel fauna. Even species that were once relatively widely distributed have undergone significant range contractions

(Hoke 2011). Less than a third of species historically found in Nebraska are currently considered stable in the state.

Without improved habitat quality and connectivity, including environmental protections, all North American mussel fauna may become extinct (Neves 1993; Williams et al. 1993). As the United States' longest standing conservation system, the protected habitats that the National Park Service (NPS) units provide are vital to the preservation of native biodiversity. Many NPS units will become important refuges for this significantly endangered group (Jennings 1998). Preliminary surveys showed that at least 70 species occur in NPS units, including those of global significance (Jennings 1997). Given the preservation goals of the NPS and the importance of mussels to aquatic habitats, the protection of mussels should become integrated in resource management and public education (Jennings 1998).

Lack of understanding of the ecology of unionid populations limits our ability to address the cause of declines. Because of this, research, monitoring, and conservation of freshwater mussel populations have begun to receive increasing attention at the regional and national level. Conservation efforts have increased over the past few decades, including the creation of a national strategy for native mussel conservation (National Native Mussel Conservation Committee 1998; FMCS 2016; Bouska et al. 2018), horizon scans (Aldridge et al. 2023), and a USGS Science Vision report on native mussel research in the United States (Newton et al. 2023). Despite our advancements, we still lack significant data on abundance, distribution, and health of mussels.

Restoration of native freshwater mussels to rivers and streams is of growing interest as a conservation tool to combat their decline and improve water quality and aquatic ecosystem health. Historically, efforts to restore populations of unionid mussels through stocking or relocation have faced challenges, with varied success depending on factors such as habitat quality and stocking density (Cope et al. 2003). Additionally, there is often little monitoring to track progress on conservation action plans to determine effectiveness of different strategies (Strayer et al. 2019). Given the high temporal variability in mussel populations and their long generation time, monitoring should extend for many years. Long-term monitoring and reporting of both successful and unsuccessful reintroductions are vital to advance the field (Strayer et al. 2019).

Haag and Williams (2014) proposed that while mussel conservation efforts should primarily focus on habitat restoration to reduce fragmentation, propagation and translocation should be used as supplementary strategies in appropriate cases such as defaunated systems. Advancements in captive propagation techniques have resulted in the ability to propagate large numbers of freshwater mussels. As a result, propagation and reintroduction are commonly recommended in species recovery plans. The Nebraska Natural Legacy Project serves as a blueprint for conserving Nebraska's flora, fauna, and natural habitats, with goals of the Project including reversing the decline of at-risk species, recovering listed species and allowing for their de-listing, keeping common species common, and conserving natural communities (Schneider et al. 2011, 2018). One of the requirements of a State Wildlife Action plan is that it identifies Species of Greatest Conservation Need within the state. Many of the mussel species listed in the Nebraska Natural Legacy Project have been found in Cub

Creek within the boundaries of Homestead National Historical Park (HOME) (Table 1). Recommended research and inventory of these mussel species listed in the Project include conducting surveys to assess distribution and abundance of mussels, evaluating potential for reintroduction, and determining specific habitat requirements.

Table 1. Mussel species listed in the 2018 Nebraska Legacy Project State Wildlife Action Plan. Tier I species are globally or nationally most at-risk of extinction and occur in Nebraska. Tier II species are typically not at risk from a global perspective but are rare or imperiled within Nebraska (Schneider et al. 2018). G1 = critically imperiled globally, G2 = imperiled globally, G3 = very rare or found locally in a restricted range globally, G4 = widespread, abundant, and apparently secure globally but it may be rare in parts of its range and is of long-term concern, G5 = demonstrably widespread, abundant, and secure globally but may be rare in part of its range, S1 = critically imperiled in state, especially vulnerable to extirpation, S2 = imperiled in the state, SNR = state conservation status not yet assessed.

Common Name	Scientific Name	Tier	Global Rank	State Rank
Flat floater	*Anodonta suborbiculata*	I	G5	S1
Pimpleback [A]	*Quadrula pustulosa*	I	G5	S2
Pistolgrip	*Tritogonia verrucosa*	I	G4G5	S1
Plain pocketbook [A]	*Lampsilis cardium*	I	G5	S2
Scaleshell	*Leptodea leptodon*	I	G1G2	S1
Fatmucket [A]	*Lampsilis siliquoidea*	II	G5	SNR
Pondmussel	*Ligumia subrostrata*	II	G5	S1
Threeridge	*Amblema plicata*	II	G5	SNR
Yellow sandshell [A]	*Lampsilis teres*	II	G5	SNR

[A] Denotes species that have been found in HOME.

The Nebraska Game and Parks Commission (NGPC) works to conserve Nebraska's threatened and endangered species and has developed a native mussel restoration plan. Two native mussels were identified as targets for restoration, including plain pocketbook (*Lampsilis cardium*) and fatmucket (*Lampsilis siliquoidea*). Historically, both species were widely distributed across the state before being either extirpated or confined to a single waterbody (Schainost 2016). Prior to reintroduction efforts, surveys revealed that the plain pocketbook was restricted to the upper Elkhorn River (Smith 2009), while the fatmucket was represented by a single live individual found in the Big Blue River (Hoke 2011).

In 2016, NGPC biologists initiated efforts to spawn and rear juvenile plain pocketbook and fatmucket for reintroduction into watersheds within their historical range. By 2017, a sizable stock of juveniles had been reared and a portion of these had been stocked into Nebraska watersheds (Schainost 2017). The Big Blue Basin, including Cub Creek in HOME, is a target for these native mussel restoration efforts.

According to the Nebraska Freshwater Mussel Restoration Plan, “The loss of freshwater mussel populations is our fault and restoration in Nebraska must occur if we are to ensure their presence for future generations” (Ohlman et al. 2019). In alignment with this goal of preserving mussel biodiversity, the NGPC developed a structured restoration framework including identifying mussel species in need of conservation, outlining restoration strategies, and selecting reintroduction sites.

Reintroduction site selection was guided by several criteria, including historical species presence, habitat suitability, the presence of appropriate host fish species, and logistical constraints. Cub Creek, located within the boundaries of HOME, was selected as a reintroduction site because it falls within the historical range of both the plain pocketbook and fatmucket mussels. Additionally, research conducted by park staff and the Heartland Inventory and Monitoring Network demonstrated that the creek meets the full life history requirements of both species, including suitable water quality parameters (Bowles et al. 2013) and the presence of host fish (Dodd and Cribbs 2012). Importantly, Cub Creek’s location on public land within HOME ensures long-term protection of the site and the presence of an on-site staff biologist further enhances the site’s value by providing increased opportunity for monitoring, research, and management following stocking efforts.

Objectives

The overall objective of this report is to document and synthesize the results of mussel monitoring conducted at HOME between 2020 and 2024. While the core goals of the monitoring program remained consistent, specific objectives evolved slightly over the course of the study period.

The primary objective of the first year of the project (2020) was to establish a baseline of mussel diversity and abundance in Cub Creek within the boundaries of HOME, prior to the planned reintroduction of fatmucket and plain pocketbook mussels. The survey aimed to address the following questions: Do the environmental conditions in Cub Creek support freshwater mussels? If so, which mussel species are present within HOME boundaries? Which species are most abundant? Are fatmucket and plain pocketbook mussels currently found within the park? The results of the baseline survey were subsequently used to guide decisions regarding the suitability of reintroducing the target species into Cub Creek, including the identification of optimal reintroduction sites.

The second objective was to reintroduce the plain pocketbook and fatmucket mussels back into Cub Creek and evaluate growth and survival. We aimed to determine: Was the initial stocking successful and are further stockings warranted? What are the growth rates of plain pocketbook and fatmucket mussels? What are the survival rates? What is an ideal study design moving forward for collecting long-term monitoring data? Tactile surveys were continued to assess mussel diversity and to determine whether juvenile plain pocketbook and fatmucket mussels without hatchery markings were present, indicating natural reproduction rather than results of recent stocking efforts.

Methods

Study Site

HOME preserves 211 acres in Gage County, Nebraska. Natural vegetation in the park is managed to approximate the ecosystem prior to the arrival of homesteaders. Restored tallgrass prairie accounts for around 120 acres, riparian woodland accounts for about 60 acres and developed areas account for the remainder (Leis and Morrison 2024).

Cub Creek is a 56-km-long perennial spring fed creek that flows through HOME before joining the Big Blue River approximately 3 km downstream. The Cub Creek watershed encompasses 374 km^2 and land use within the watershed is predominately agricultural. Surrounding land use is primarily row crop agriculture, which may disrupt ecosystem functioning and affect water quality in Cub Creek (NPS 1999).

The stream is moderately sinuous with steep banks. The substrate consists primarily of sand, but patches of silt, gravel, clay, and organic material are also present. Stream width and depth are mostly consistent throughout HOME, with an average width of 5.7 m and an average depth of 25 cm. There is a narrow riparian zone lining the creek, with vegetation extending halfway down creek banks, except for on the outside of meanders where active erosion is occurring.

The NGPC identified Cub Creek as a site for plain pocketbook and fatmucket reintroduction (see above for details). Most species of freshwater mussels use fish as hosts to complete their unique life cycle. Immature freshwater mussels called glochidia parasitize host fish (Lefevre and Curtis 1910). The glochidia encyst in the host fish gills and mature into juveniles before dropping off and burrowing into the stream bottom. Fish hosts for both species have been documented in the stream, including largemouth bass, bluegill, and common shiner (Dodd and Cribbs 2012; Williams and Dodd 2016). Thus, host fish availability is not likely to pose a barrier to population restoration efforts.

An initial survey of Cub Creek conducted in 2020 confirmed the absence of fatmucket and plain pocketbook mussels and the presence of other species of mussels with ecological niches similar to those of fatmucket and plain pocketbook. This suggested that the environmental conditions in Cub Creek may be broadly suitable habitat. However, the absence of fatmucket and plain pocketbook mussels raises the possibility that their persistence in the creek may be limited by species-specific habitat requirements or other unidentified ecological stressors. The reintroductions of fatmucket and plain pocketbook mussels provided an opportunity to directly evaluate whether the current habitat can support these species.

Field Methods

Mussel surveys of Cub Creek were initiated in July 2020, with subsequent seasonal sampling in July 2021, June/July 2022, and July/August 2024. As Cub Creek within HOME is narrow and relatively short, we aimed to conduct a qualitative survey of the entire reach in 2020 and 2021 and subsections in 2022 and 2024. Qualitative searches optimize rare species detection and are generally more efficient for species detection than quadrat-based surveys (Strayer and Smith 2003; Smith 2006).

Sampling was conducted in summer to maximize chances of accurately recording mussel abundance when river water levels and discharge are low, and mussels are more likely to reside at the substrate surface (Watters et al. 2001; Hart et al. 2016). We recorded dissolved oxygen, pH, conductivity, turbidity, surface velocity, and depth of the creek prior to visually and tactically searching for mussels.

For all individuals, we recorded a preliminary species identification, noted mussels as live or dead, and captured at least two pictures of the shell valve and umbo (Cannon EOS Rebel T5i). Mussels were considered live if they responded to touch or the shell was closed. We measured maximum shell length (widest point parallel to hinge), height (widest point perpendicular to hinge), and thickness (widest point across closed right and left valves) to the nearest tenth of a millimeter using a digital caliper (Mitutoyo, Aurora, Illinois). A cloth measuring tape was used to measure mussels larger than 150 mm to the nearest millimeter.

We noted the approximate GPS location of live specimens (Appendix A). To reduce handling stress, captured individuals were kept submerged except when being processed, which required less than two minutes per individual. Once live individuals were measured and photographed, they were returned to the substrate in the approximate location in which they were found. Dead mussels were catalogued and removed from the study area. After surveys, mussel images were sent to Steven Schainost for species confirmation (Schainost 2016). For the 2022 and 2024 diversity surveys, we focused efforts on recording all live and dead mussels of every species except mapleleaf, given their abundance and our limited personnel for surveys. We did not survey in 2023, as mussels had been recently stocked prior to the monitoring window, and we chose to avoid disturbing them to maximize their chances of survival.

In addition to surveys, mussel reintroduction efforts also began in July 2020, with additional stockings in July 2021 and May 2023. These subsequent stockings occurred after annual surveys were completed. Fatmucket and plain pocketbook mussels were propagated with an in-state source of broodstock at the North Platte Fish Hatchery in North Platte, Nebraska. All introduced mussels were marked with a dot of white (plain pocketbook) or black (fatmucket) glue on each valve near the umbo and initial length was measured. This allows for cultured mussels to be identified as hatchery origin if they are recaptured, while fatmucket or plain pocketbook juveniles without marks may provide evidence of a newly recruited mussel.

A subset of mussels was also marked with Passive Integrated Transponder (PIT; BioMark, Boise, Idaho) tags (Table 2). PIT tags allow for the long-term tracking of the survival of individual mussels and are relatively inexpensive. Kurth et al. (2007) found that the use of PIT tags in mussels almost doubled recapture rates over visual observation alone. We stocked mussels along approximately 100-meter stretches at upper (40.2872625 N, 96.8374481 W to 40.287161 N, 96.8359732 W) and lower (40.2909797 N, 96.83467 W to 40.2916357 N, 96.8344894 W) sites within Cub Creek (Figure 1). Introduction sites were chosen based on the 2020 baseline diversity survey, which identified areas within Cub Creek where mussel populations appeared healthy and where site access was favorable.

Table 2. Quantity of stocked mussels and the subset with PIT tags per species per stocking year.

Species	Year	Stocking Category	Quantity
Fatmucket	2020	Total	500
	2020	PIT-tagged subset	100
	2021	Total	450
	2021	PIT-tagged subset	100
	2023	Total	400
	2023	PIT-tagged subset	100
Plain pocketbook	2020	Total	500
	2020	PIT-tagged subset	100
	2021	Total	500
	2021	PIT-tagged subset	100
	2023	Total	296
	2023	PIT-tagged subset	49

Recapture sampling was conducted using a BioMark model HPR Plus Handheld receiver and submersible model BP Plus Antenna (BioMark, Boise, Idaho). A BioMark Fish Sample PIT tag was first used to validate the reader. We scanned the immediate downstream section (approximately 100 meters) of the area where stocking occurred and then worked upstream through the stocked areas. When a PIT tag was detected, the unique ID, time stamp, and GPS location were recorded. We then searched the substrate until the mussel was recaptured. If we could not relocate the mussel or PIT tag, the detection was not considered a recapture and was recorded as "Not recovered." All recovered mussels were identified by PIT tag, recorded as live or dead, and measured for shell length, width, and thickness. Individuals were returned to approximate location of recapture.

Detections were classified as "Not recovered" when a PIT tag was scanned, but neither the mussel nor the tag could be relocated despite thorough search efforts. These instances were most common in deep water and areas with fine sediment, where excavation led to sediment collapse and the substrate grain size closely matched the size of the PIT tag, making recovery more difficult. Detections where only the PIT tag was found with no shell attached were classified as "Tag only." If the tag was found with a piece of shell or dead mussel, it was considered dead. We noted individuals with loose tags that may dislodge prior to the next survey. In rare instances, we were able to locate both a dislodged tag and the nearby corresponding mussel, which allowed us to match the tag to the remaining glue residue outline. In such instances, the individual was categorized as either live or dead, rather than as "tag only." In 2024, we also located three individuals that had PIT tag glue outlines, but no PIT tags. These were noted and recorded as live or dead.

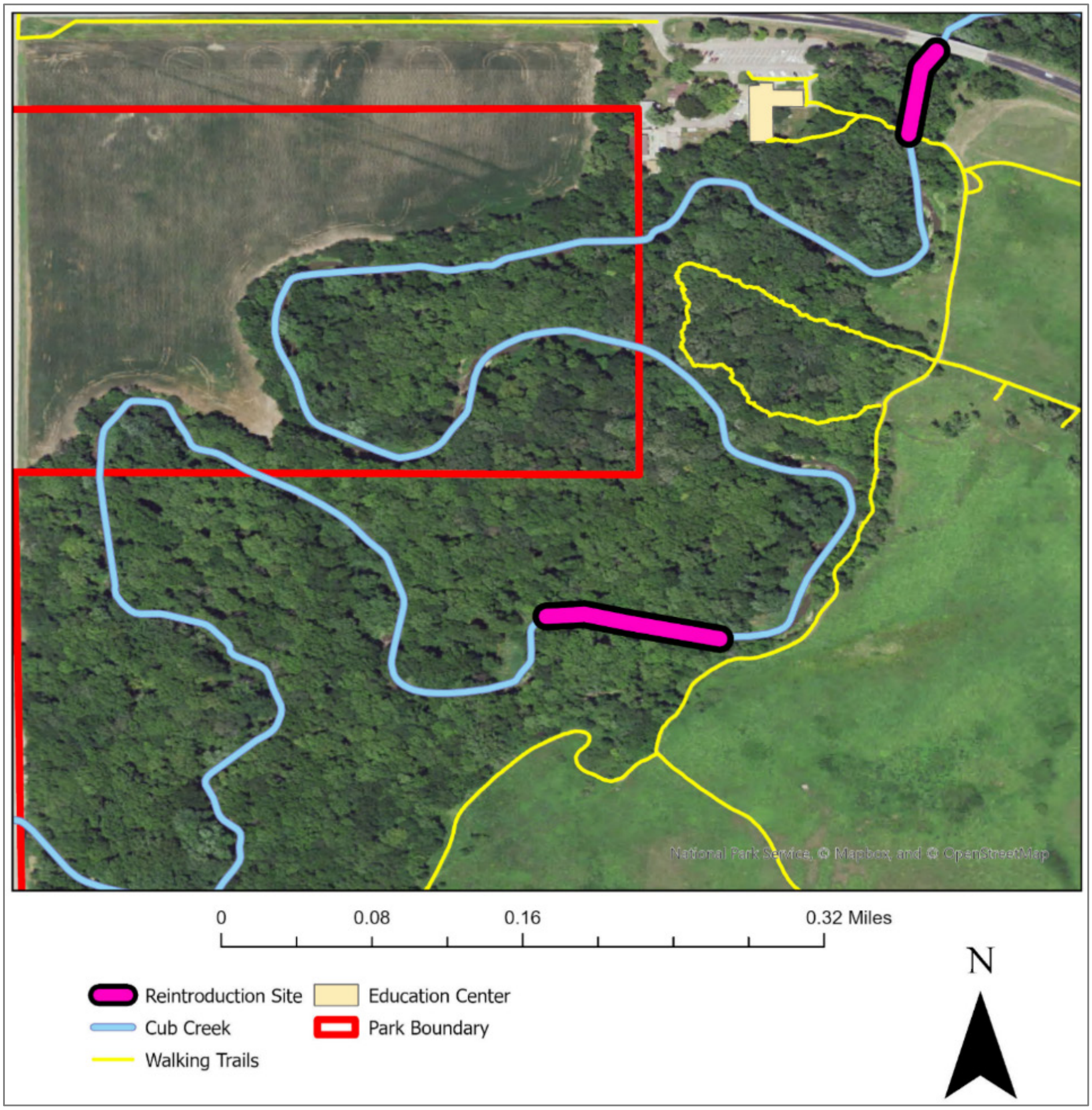

Figure 1. Locations of mussel stocking sites (shown in dark pink with black outlines) along two sections of Cub Creek within the HOME boundaries, where plain pocketbook and fatmucket mussels were reintroduced in 2020, 2021, and 2023. Cub Creek is shown in blue, yellow lines indicate park walking trails, and the HOME park boundary is outlined in red.

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Data Analysis

Prior to analysis, any individuals with unknown identifications due to missing pictures, small shell fractions only, or extremely small unidentifiable juveniles were removed from the dataset. Fingernail clams were also removed. This resulted in the removal of 23 fingernail clams, seven juvenile unionid mussels, and 11 fragments or missing pictures across all years combined. All figures and statistical analyses were performed in R statistical software (v4.1.3, R Core Team 2022) using the following packages: ggplot2 (Wickham 2016), tidyr (Wickham et al. 2024), dplyr (Wickham et al. 2023), and FSA (Ogle et al. 2025).

Environmental Parameters

We evaluated spatial variability in environmental parameters (dissolved oxygen, pH, temperature, conductivity) along the creek to determine whether distinct sections exhibited statistically significant values that could influence habitat quality or mussel distribution. We tested the normality of the data using a Shapiro-Wilk test. Temperature and conductivity data were not normally distributed. Therefore, Kruskal-Wallis tests (Kruskal and Wallis 1952) were used to determine if differences existed among waypoint sections.

Morphological Variation

Freshwater bivalves are often difficult to identify morphologically due to the phenotypic plasticity of their shells, although molecular genetic tools have increased the ability to identify mussels (Inoue et al. 2013, 2014; Smith et al. 2018). During surveys, we observed interspecific variation in shell shape that may be helpful in distinguishing between species, particularly for future surveys of fatmucket and plain pocketbook mussels. We wanted to evaluate whether morphological traits can reliably differentiate between fatmucket and plain pocketbook mussels and thereby serve as an additional tool for species confirmation. This would be especially valuable in cases where PIT tags or glue dots have fallen off the shell, as accurate species identification is critical for population assessments.

We used 144 fatmucket and 76 plain pocketbook mussels for morphometric analysis. As shells may suffer damage from erosion or predation, only measurements from live, undamaged individuals were used in the analysis. Several individuals were recaptured across sampling years, resulting in some PIT-tagged mussels being included in the analysis more than once. A total of 12 tagged fatmucket individuals were recorded across two different years, while an additional individual was detected across three years. Five tagged plain pocketbook individuals were repeated once each across years. However, these individuals exhibited notable growth between sampling events, including subtle changes in shell morphology such as increased thickness. Given these changes, recaptured mussels were retained in the overall analysis to capture the natural variation. We calculated the ratios of height/length, width/length, and width/height to standardize variables for size and then normalized data using an arcsine square root transformation (Gangloff et al. 2006; Inoue et al. 2013, 2014). Normality was verified using a Shapiro–Wilk test (Sokal and Rohlf 1995). We tested homogeneity of variance between groups using Box's M test (Box 1949) and Levene's test (Brown and Forsythe 1974). We then scaled normalized data to a mean of 0 and a standard deviation of 1 prior to visualizing patterns of variation between groups using linear discriminant analysis (LDA). We initially scaled the full dataset, including all sampling years, to assess overall patterns. To account for potential repeated measurements of the same individuals across years, we also scaled data within each year for separate analyses by year in which each individual only appeared once.

Species Diversity

To assess mussel species diversity and abundance, we calculated the total number of individuals per species for each sampling year, with subcounts for live and dead individuals. To enable comparisons across years, we calculated relative species diversity as the proportion of individuals of a given species relative to the total number of individuals observed. Only live mussels were included in these calculations to accurately reflect the community for that sampling year. Further, during the 2022 and 2024 diversity surveys, sampling efforts were focused on recording both live and dead mussels of every species except mapleleaf, in which dead counts were not consistently recorded. Therefore, only live individuals were used in relative diversity estimates. Overall species totals included PIT-tagged individuals recorded as “tag only” or “not recovered.” However, these individuals were excluded from live-only analyses due to their uncertain status.

Fatmucket and Plain Pocketbook Reintroductions

To determine growth and survival across years, we analyzed the fate of PIT-tagged individuals and calculated shell growth rates. For each species, we calculated the mean shell length and standard deviation at time of release and for each subsequent recapture year. To test for differences in shell length between recapture years within a given reintroduction cohort, we used Kruskal-Wallis tests (Kruskal and Wallis 1952). Only live individuals were used. When significant differences were detected, we conducted pairwise comparisons using Dunn’s test with a Bonferroni correction (Dunn 1964). An exception to the Bonferroni correction was made for the 2022 plain pocketbook cohort for the comparison between the release length and second recapture length, as only two individuals were recaptured at that time point.

We also tracked the fates of PIT tags and their associated mussels each year, assigning each individual to one of five categories: live, dead (including a tag recovered with shell remains), not recovered (tag detected but not located), not detected (tag not scanned during survey), and tag only (tag found without any shell). If a tag was found with a piece of mussel shell attached, the mussel was categorized as dead. Tags categorized as dead or tag only were removed from the stream. Thus, for each cohort and sampling year, we noted the initial number of deployed tags, the number removed, and the number potentially remaining in the stream.

Results

Environmental Parameters

There were no statistically significant differences in dissolved oxygen, temperature, pH, or conductivity between waypoint sections ($p > 0.4$). pH ranged from 7.3 to 8.6. Surface temperature ranged from 21.6 to 31.4 degrees Celsius.

Morphological Variation

A total of 220 individuals were measured and assigned to one of two species: fatmucket (N = 144) and plain pocketbook (N = 76). The analysis yielded a single linear discriminant function (LD1, Figure 2) with the following coefficients: height/length −0.3, width/length 3.3, and width/height −1.5. Morphological separation between species was primarily influenced by width/length. The density plot of LD1 scores revealed two distinct, but slightly overlapping, peaks corresponding to the two species, indicating separation based on shell morphology. The LDA model had an overall classification accuracy of 97.3%, with species specific accuracies of 97.9% for fatmucket and 96.1% for plain pocketbook. When each year was examined individually (Appendix A), overall classification accuracy within year ranged from 94.9% to 100%.

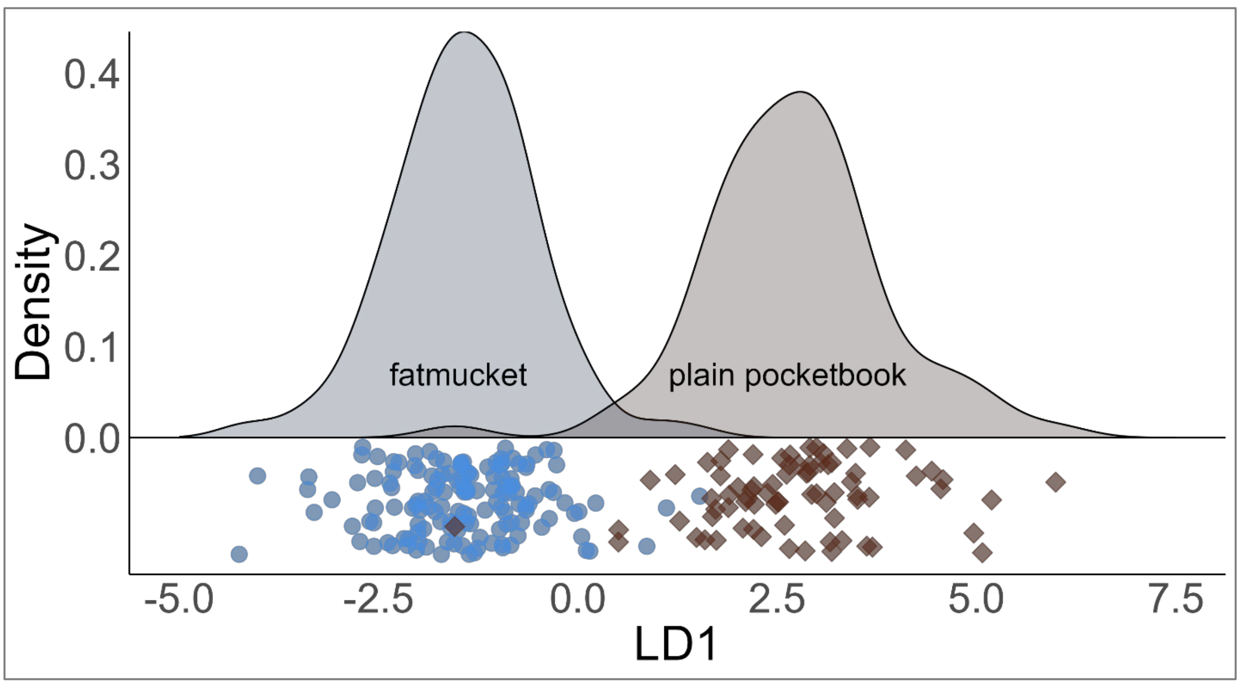

Figure 2. Density plot of LD1 scores from linear discriminant analysis of morphometric ratios of shell height/length, width/length, and width/height for fatmucket (blue, N = 144) and plain pocketbook (brown, N = 76) mussels. Jittered points below density plots represent fatmucket (circle) and plain pocketbook (diamond) individuals. $LD1 = -0.3 \times height/length + 3.3 \times width/length - 1.5 \times width/height$.

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Species Diversity

In 2020, nearly the entire stretch of Cub Creek within HOME boundaries was surveyed (1,402 meters, Appendix A) to determine which species of mussels were already present prior to reintroducing plain pocketbook and fatmucket mussels. A total of 421 mussels were collected, including 311 live individuals (Table 3). Neither fatmucket nor plain pocketbook mussels were detected. The most abundant species was mapleleaf (*Quadrula quadrula*), which accounted for 400 of the 421 mussels collected. Additional species included fragile papershell (*Leptodea fragilis*), giant floater (*Pyganodon grandis*), pimpleback (*Quadrula pustulosa*), pink papershell (*Potamilus ohiensis*), and white heelsplitter (*Lasmigona complanata*; Figure 3).

Approximately the same stretches of Cub Creek were resampled in 2021 (Appendix A). Across 1,310 meters, 636 mussels were found, 489 of which were live. Nine species of mussels were found, including fatmucket, fragile papershell, giant floater, lilliput (*Toxolasma parvum*), mapleleaf, pimpleback, pink papershell, plain pocketbook, and yellow sandshell (*Lampsilis teres*). Lilliput and yellow sandshell mussels were only found as shells. Mapleleaf mussels remained the most abundant species (live, N = 397), followed by pimpleback (N = 38), fatmucket (N = 21) and plain pocketbook (N = 18) mussels (Table 3, Figure 3).

In 2022 and 2024, only subsections of Cub Creek were sampled which included the reintroduction areas and immediate downstream stretches (see maps in Appendix A). In 2022, six species of mussels were found across 323 meters, including fatmucket, giant floater, mapleleaf, pimpleback, pink papershell, and plain pocketbook (Table 3). Similar to 2021, mapleleaf, pimpleback, plain pocketbook, and fatmucket mussels were the most abundant (Figure 3). In 2024, 359 meters of creek were sampled, which resulted in the highest quantity of mussels found across sampling years (N = 909). Mapleleaf remained the most abundant species, followed by fatmucket and plain pocketbook. There was a notable increase in the abundance of giant floaters, including many juveniles. Across all years, lilliput, white heelsplitter, and yellow sandshell were only detected as shells, with no live individuals recorded.

Table 3. Total number of mussels collected at Cub Creek from 2020 to 2024, with a breakdown of the subset of live individuals. Some species were only found as shells. Totals include plain pocketbook and fatmucket tags that were detected but not recovered, as well as instances where only the tag was found.

		2020		2021		2022		2024	
Common name	**Scientific name**	**Total**	**Live**	**Total**	**Live**	**Total**	**Live**	**Total**	**Live**
Fatmucket [A]	*Lampsilis siliquoidea*	0	0	69	21	64	30	218	93
Fragile papershell	*Leptodea fragilis*	2	1	19	8	0	0	7	4
Giant floater	*Pyganodon grandis*	5	0	3	1	9	9	106	72
Lilliput	*Toxolasma parvum*	0	0	1	0	0	0	0	0
Mapleleaf	*Quadrula quadrula*	400	301	408	397	165	165	373	361
Pimpleback	*Quadrula pustulosa*	11	8	40	38	64	64	56	48
Pink papershell	*Potamilus ohiensis*	2	1	7	6	2	2	0	0
Plain pocketbook [A]	*Lampsilis cardium*	0	0	88	18	60	16	149	43
White heelsplitter	*Lasmigona complanata*	1	0	0	0	0	0	0	0
Yellow sandshell	*Lampsilis teres*	0	0	1	0	0	0	0	0
Total	**–**	**421**	**311**	**636**	**489**	**364**	**286**	**909**	**621**

[A] denotes reintroduced species.

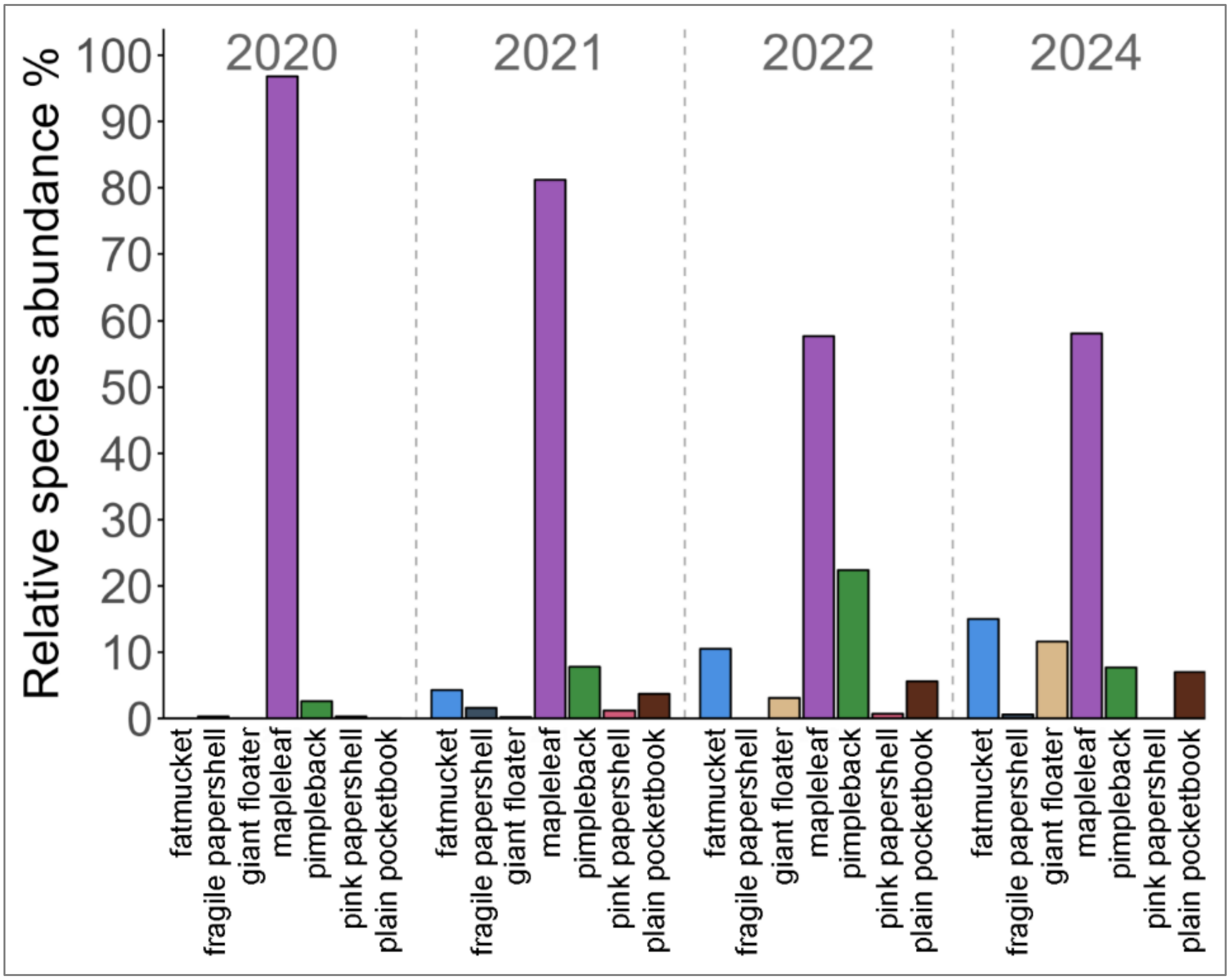

Figure 3. Relative abundances of live mussel species for each sampling year. Lilliput, white heelsplitter, and yellow sandshell were only found as shells and are excluded from this figure. Dashed vertical lines separate sampling years.

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Fatmucket and Plain Pocketbook Reintroductions

Tagged fatmucket mussels exhibited significant growth in shell size over time (Kruskal-Wallis, $p < 0.001$). At the time of release, the average shell length across all cohorts was 53.6 ± 7.1 mm (mean ± standard deviation; Figure 4). By the following summer, the average shell length had increased to 81.3 ± 8.4 mm. By the second recapture, the average shell length reached 96.3 ± 10.8 mm. Finally, the average shell length by the third recapture year had more than doubled the original shell length, averaging 114.4 ± 7.1 mm. The most growth occurred between the release and the first recapture years. For each cohort, mussel size at the first recapture and all subsequent recaptures were significantly larger than at the time of release (Dunn's test, $p < 0.01$).

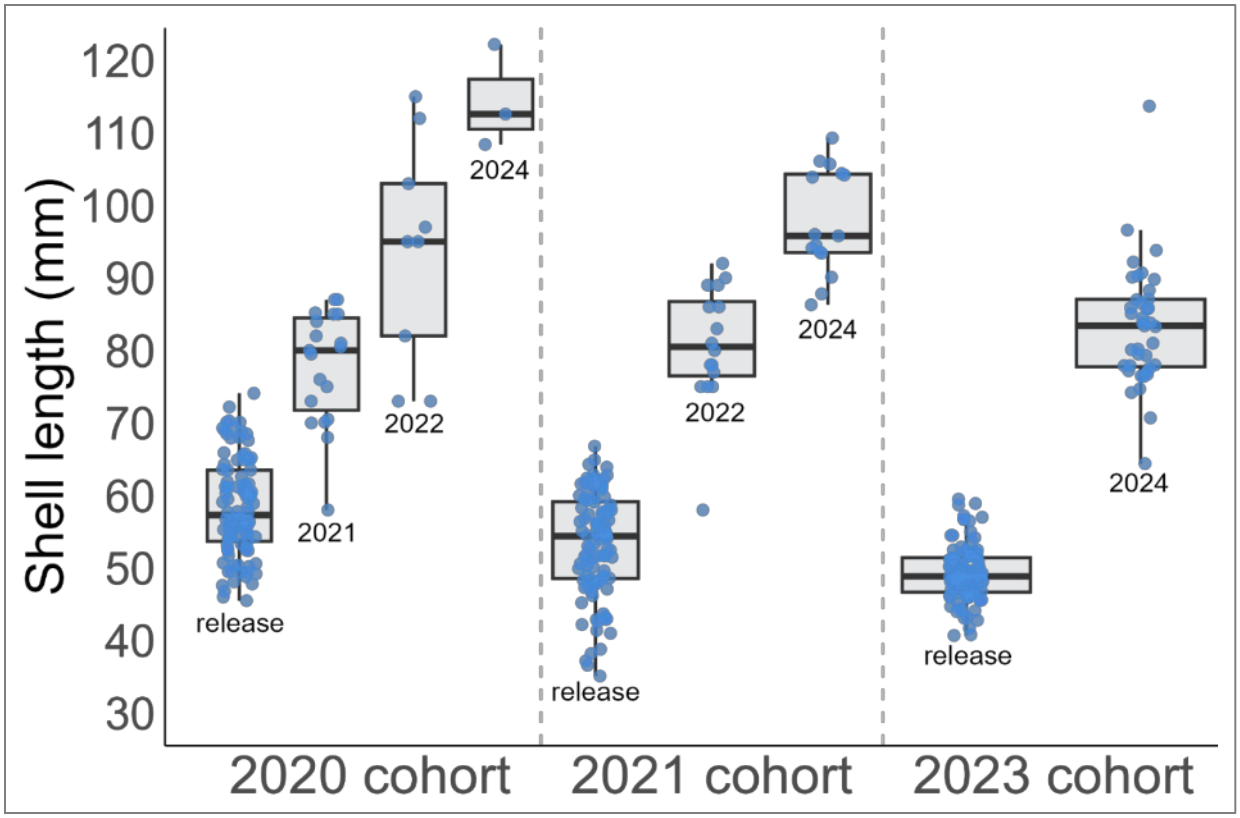

Figure 4. Boxplots illustrate the growth and survival of each fatmucket cohort. Cohorts are plotted along the x-axis by release year, with vertical dashed lines separating cohorts. Boxplots are labeled with initial release, followed by subsequent recapture years. Live individuals are represented by jittered blue circles. Boxes display the interquartile range with the line inside the box indicating the mean; whiskers extend to 1.5× the interquartile range. For all cohorts, mussel size at the first recapture and all subsequent recaptures were significantly larger than at the time of release ($p < 0.01$).

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In addition to the tagged mussels, 300–400 untagged fatmucket mussels were also stocked each year (Table 2). We encountered 69 total fatmucket mussels in 2021 (live N = 21, 30%), 64 in 2022 (live N = 30, 47%), and 218 in 2024 (live N = 93, 43%; Table 3). These numbers likely underestimate the actual number of remaining live fatmucket mussels, as many were found deeply buried in the substrate during sampling. Untagged mussels, in particular, were likely underdetected.

There were also significant increases in shell size between years for plain pocketbook mussels (Kruskal-Wallis, $p < 0.05$), although the average sizes were smaller than those of fatmucket mussels. Across all cohorts, the average shell length at release was 46.8 ± 6.5 mm (mean ± standard deviation; Figure 5). By first recapture, mean shell length had increased to 75.2 ± 7.9 mm. By the second recapture, it reached 89.6 ± 6.4 mm, and by the third recapture it had more than doubled from the original size, reaching an average of 98.6 ± 3.5 mm. The largest year-to-year growth occurred between the release and the first recapture. For each cohort, mussel size at the first recapture and all subsequent recaptures were significantly larger than at the time of release (Dunn's test, $p < 0.05$).

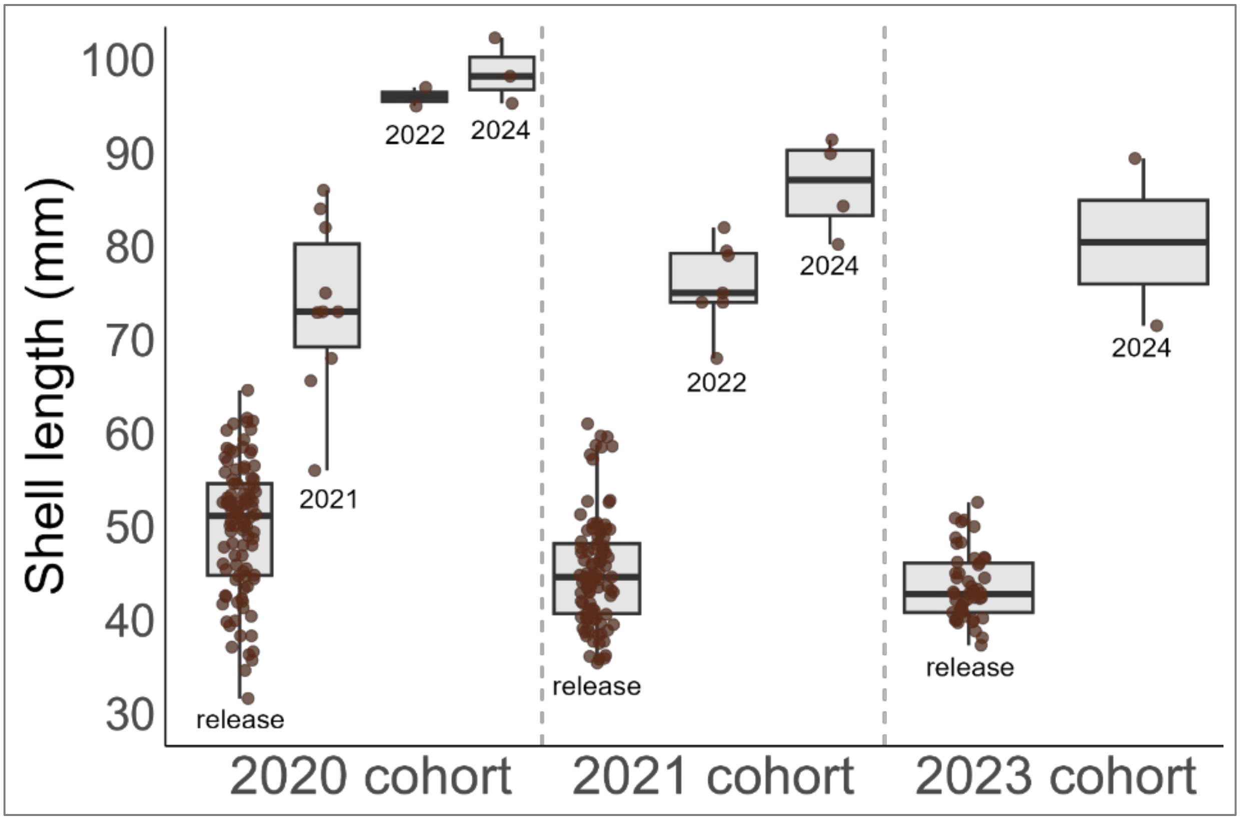

Figure 5. Boxplots illustrate the growth and survival of each plain pocketbook cohort. Cohorts are plotted along the x-axis by release year, with vertical dashed lines separating cohorts. Boxplots are labeled with initial release, followed by subsequent recapture years. Live individuals are represented by jittered brown circles. Boxes display the interquartile range with the line inside the box indicating the mean; whiskers extend to 1.5× the interquartile range. For all cohorts, mussel size at the first recapture and all subsequent recaptures were significantly larger than at the time of release ($p < 0.05$).

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In addition to the tagged individuals, between 247 and 400 individuals were also stocked each year (Table 2). We encountered 88 total plain pocketbook mussels in 2021 (live N = 18, 20%), 60 in 2022 (live N = 16, 27%), and 149 in 2024 (live N = 43, 29%). Again, this is likely an underestimation of the remaining live plain pocketbook mussels, as they were often buried deep within soft substrate and therefore may have been missed during sampling.

One hundred PIT-tagged fatmucket mussels were released each year in 2020, 2021, and 2023. Of the 2020 cohort, 19 individuals were recaptured live in 2021, nine in 2022, and three in 2024 (Table 4, Figure 4). From the 2021 cohort, 17 mussels were confirmed live in 2022 and 15 in 2024. Thirty-nine individuals from the 2023 cohort were detected live during the 2024 sampling. In total, 57 of those were the PIT-tagged individuals that were confirmed live during the most recent 2024 survey. Across all cohorts and sampling years, 88 PIT-tagged individuals (29%) have been confirmed dead. To date, 119 PIT tags or dead mussels (40%) have been recovered and removed from the stream, while 181 of the original 300 tagged mussels remain (60%). However, the proportion of PIT tags that were not detected, relative to the number of tags remaining in the stream, varied widely across years, from 24% to as high as 80% (Table 4, Figure 6).

Table 4. Tag data for the 2020, 2021, and 2023 PIT-tagged fatmucket cohorts. Each cohort includes the release year, followed by subsequent recapture years. Mussels or PIT tags that could not be relocated were recorded as “Not recovered.” Detections where only the PIT tag was found with no shell attached were classified as “Tag only.” “Not detected” indicates the PIT tag was not detected during that year’s survey. The bottom three rows summarize the initial number of tags deployed each year, the number of tags removed from the stream when they were found dead or as tag only, and the final number of tags remaining by cohort and year.

	2020 Fatmucket Cohort				2021 Fatmucket Cohort			2023 Fatmucket Cohort	
Sampling Category	**2020**	**2021**	**2022**	**2024**	**2021**	**2022**	**2024**	**2023**	**2024**
Dead	0	20	5	3	0	17	10	0	33
Live	100	19	9	3	100	17	15	100	39
Not recovered	0	10	1	4	0	1	7	0	3
Not detected	0	47	58	52	0	63	34	0	24
Tag only	0	4	3	6	0	2	15	0	1
Initial tags remaining	100	100	76	68	100	100	81	100	100
Tags removed	0	24	8	9	0	19	25	0	34
Final tags remaining	100	76	68	59	100	81	56	100	66

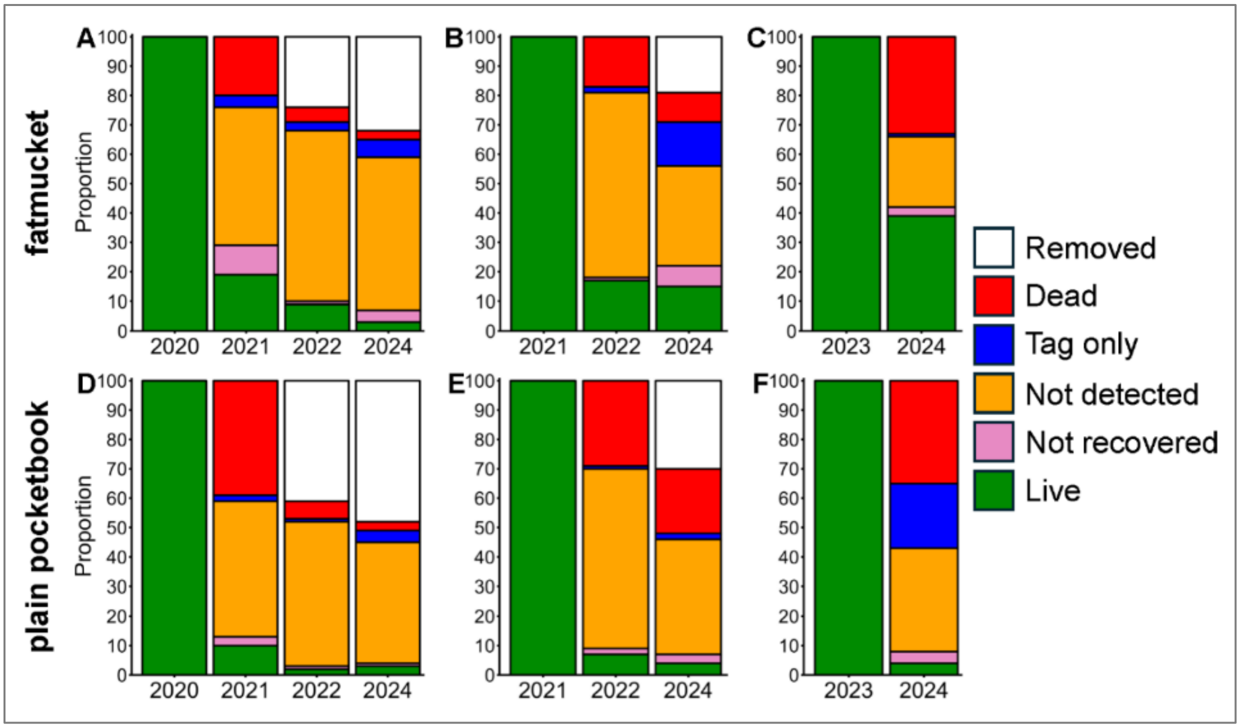

Figure 6. Stacked bar plots showing the fate of PIT tags and mussels from six different cohorts: fatmucket released in 2020 (A), 2021 (B), and 2023 (C), and plain pocketbook released in 2020 (D), 2021 (E), and 2023 (F). The x-axis indicates the initial release year followed by subsequent resampling years. Tags that were scanned but not located are labeled as "Not recovered." "Not detected" indicates that the tag was not scanned during that year's survey. Tags found without an associated shell are classified as "Tag only." Shells from dead individuals and unaccompanied tags were removed from the stream and are shown in white in subsequent bar plots.

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One hundred PIT-tagged plain pocketbook mussels were released each year in 2020 and 2021, and 49 were released in 2023. Of the 2020 cohort, ten were recaptured alive in 2021, two in 2022, and three in 2024 (Table 5, Figure 5). From the 2021 cohort, seven mussels were recovered alive in 2022 and four in 2024. Two mussels of the 2023 cohort were found alive again in 2024. Across all years and cohorts, 116 PIT-tagged mussels have been confirmed dead (47%). One hundred thirty-seven PIT-tags or dead mussels have been removed from the stream (55%), while 112 of the original 300 remain (45%, Figure 6). The number of PIT tags recovered without accompanying shells increased over time following initial restocking in both species (Table 4, Table 5).

Table 5. Tag data for the 2020, 2021, and 2023 PIT-tagged plain pocketbook cohorts. Each cohort includes the release year, followed by subsequent recapture years. Mussel or PIT tags that could not be relocated were recorded as detected but "Not recovered." Detections where only the PIT tag was found with no shell attached were classified as "Tag only." "Not detected" indicates the PIT tag was not detected during that year's survey. The bottom three rows summarize the initial number of tags deployed each year, the number of tags removed from the stream when they were found dead or as tag only, and the final number of tags remaining by cohort and year.

	2020 Plain Pocketbook Cohort				2021 Plain Pocketbook Cohort			2023 Plain Pocketbook Cohort	
Sampling Category	**2020**	**2021**	**2022**	**2024**	**2021**	**2022**	**2024**	**2023**	**2024**
Dead	0	39	6	3	0	29	22	0	17
Live	100	10	2	3	100	7	4	49	2
Not recovered	0	3	1	1	0	2	3	0	2
Not detected	0	46	49	41	0	61	39	0	17
Tag only	0	2	1	4	0	1	2	0	11
Initial tags remaining	100	100	59	52	100	100	70	49	49
Tags removed	0	41	7	7	0	30	24	0	28
Final tags remaining	100	59	52	45	100	70	46	49	21

Discussion and Conclusion

Morphological Variation

The clear separation of LD1 scores between plain pocketbook and fatmucket individuals highlights the potential of LDA to serve as a complementary tool for verifying species designations, especially in cases with morphologically similar species or for non-specialists. It is important to note that nearly all fatmucket and plain pocketbook species labels were confirmed through PIT tags or hatchery glue dots. Without those physical markers, distinguishing between the two species would have been considerably more difficult. Because the true species identity was known for nearly all individuals, we were able to test whether the LDA could effectively separate the species based on three morphometric ratios. In future cases where PIT tags or glue dots have been lost, LDA could serve as a useful quality control tool to verify species identifications. For example, instances in which an individual's LD1 score falls within the distribution of the other species may warrant closer inspection and additional confirmation of its identity (for example, the brown point clearly within the fatmucket distribution, Figure 2).

Mussel Diversity

Improving populations of common mussels may facilitate broader community recovery, including rare or imperiled species (FMCS 2016). Thus, it is important to know which species are present prior to restoration. Mussel surveys conducted in Cub Creek in 2020 prior to reintroductions, and from 2021 to 2024 provided useful baselines for understanding species composition, abundance, and trends following fatmucket and plain pocketbook reintroductions. Species assemblages remained relatively consistent, with mapleleaf, pimpleback, fatmucket, and plain pocketbook mussels remaining the most commonly observed species. Species such as lilliput, white heelsplitter, and yellow sandshell were only detected as shells. This limited presence may reflect historical occurrences and population decline or indicate that current habitat conditions are unsuitable for those species.

Notably, the 2024 survey recorded the highest total number of mussels across all years (N = 909), despite the shorter stream survey sections than in 2020 and 2021. Additionally, there was a large increase in both adult and juvenile giant floaters recorded across years. This may indicate possible local recruitment or improved detection efficiency. Surveys were not time standardized. While the park biologist consistently served as the survey lead, the supporting team varied from year to year. Variability in experience may have contributed to year-to-year fluctuations in mussel counts.

The surveys also offered valuable opportunities for natural history observations that can help to inform future management decisions, including identifying areas with higher mussel densities, what seemed to be preferred substrate composition, and other site characteristics useful for selecting optimal locations for future mussel placement. These observations were equally important for recognizing areas unsuitable for restoration placements.

For instance, in the spring-fed section of Cub Creek, we observed significant erosion and brittle shells near the umbo (Figure 7) across all species present in the section, including mapleleaf, pimpleback, and giant floater. Shells of dead mussels were found with completely eroded holes near the umbo. The sediment in this location is similar to that of other sections of creek. The water temperature in the area is notably colder due to the spring, and there may also be variations in water chemistry or nutrients at the site that could account for observed erosion differences. Although no statistically significant differences in pH or surface temperature were detected in the spring section, the recorded temperature values also likely do not accurately represent the deeper water conditions near the spring outflows. Future studies should further investigate differences in this location that may lead to shell erosion and structural brittleness, which likely increases mortality rates.

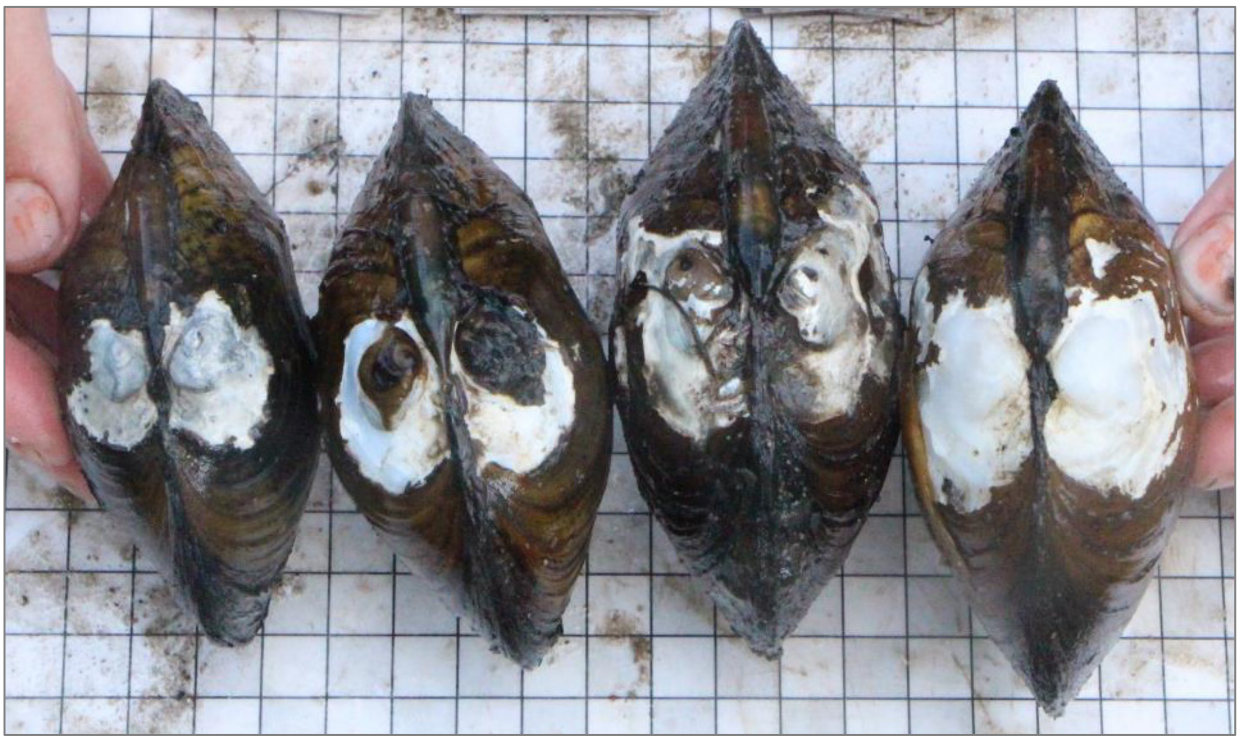

Figure 7. Mapleleaf mussels with deep erosion into the shell near the umbo. Pimpleback and giant floater mussels were also observed with similar erosion.
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Future inventory work should employ a broader range of survey techniques to characterize mussel populations. Dense mussel beds could be measured with transect or quadrat methods. While our survey methods were limited to the more superficial substrate layers, incorporating quadrat excavation surveys in future work could improve detection of juvenile mussels and species that bury deeper within the substrate. Additionally, while the primary objective of this study was to assess current mussel populations, future research could incorporate more detailed environmental measurements to model and predict suitable mussel habitat within streams.

Fatmucket and Plain Pocketbook Reintroductions

Consistent increases in shell size between release and recapture events highlight notable growth among reintroduced mussels. Shell length increased significantly, especially within the first year. In subsequent years, cohorts more than doubled their original size, indicating favorable conditions for surviving mussels.

One challenge in interpreting survival data stemmed from PIT tag retention over time. We observed a noticeable increase in the number of detached PIT tags recovered without associated shells in later survey years and with larger mussels. In some cases, we found tags that had become loose or had fallen off of live mussels, suggesting the adhesion may weaken over time due to physical disturbances or changes in shell morphology as the mussel grows. This tag loss makes it hard to distinguish between mortality and tag detachment, especially when tags are recovered without their corresponding shell. As we found large, healthy individuals with loose or lost tags (the PIT tag cement outline remained, while the tag was gone), this limitation may result in underestimating survival of mature individuals. Improved tag placement methods could help minimize tag loss in future studies (see Tiemann et al. 2025 for best practice guidelines). Future studies should also consider placing both a PIT tag and a secondary shellfish tag to ensure identification if the PIT tag is lost.

In addition to tagged mussels, 200–400 untagged mussels of each species were restocked each year. While they could not be individually tracked, the quantity and status of those found during diversity surveys provided insight into broader survival trends, beyond that of the PIT-tagged individuals. However, the number of surviving untagged individuals is likely much higher than reported, as there may be a detection bias toward dead shells, which are more visible than live, burrowed mussels deep within the substrate.

The surveys also offered insight into the specific characteristics of our stream and helped identify suitable habitats for mussel placement during stocking efforts. In our survey, individuals seemed to be primarily located in sandy or muddy substrates. However, this preference may not reflect broader habitat preferences of these species. At HOME, there may be limited opportunities for them to inhabit their preferred substrates, and these same species might be found in very different habitats in other streams. Additionally, many mussels were found buried high on the bank, likely due to rapid declines in water level shortly after restocking. This increases vulnerability to desiccation and predation.

During surveys, it was common to find shells with evidence of predation. On July 18, 2024, near the end of a PIT tag survey, the reader battery was depleted. Therefore, we chose to leave six tagged mussels near the stream edge in approximately eight inches of water at 4:00 PM, with the intention of returning the following morning to complete scanning and data collection. When we returned at 10:00 AM, all six mussels were moved from their original location, and several were found on the bank as shells. Some still had fresh tissue residue attached in the shell (Figure 8). Raccoons and muskrats are both found in HOME and are known predators of mussels. Raccoon tracks were found in the mud near the original location of the mussels and are the most likely predator. Kabbes and Klocek (2004) noted that virtually all dead shells they collected had suffered from predation by racoons or muskrats. Additionally, raccoon predation may be higher when water becomes shallower as mussels are easily visible from the surface and more easily caught (Johnson et al. 2001). This event demonstrated how quickly the mussels can become prey, and predation likely contributes to a sizeable portion of reintroduction deaths. Future restocking efforts should place mussels in deeper areas to reduce these mortality risks.

Figure 8. PIT-tagged mussels scattered on the bank after a predator removed them from the creek (A), racoon tracks found in mud near where the mussels were placed in the creek overnight (B), and a close-up of the mussels found with their shells broken open and tissue removed (C). Pieces of fresh tissue remained in several of the shells.

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It will also be important to examine the genetic implications of reintroductions. Outbreeding and inbreeding depression, founder effects, and genetic drift may result in the loss of genetic variability from populations which could affect growth, reproductive rate, and ability to deal with environmental stochasticity. Periodic, continued restocking could help introduce artificial genetic diversity and support population resilience, particularly if natural reproduction is low (Jones et al. 2006; Geist et al. 2021).

Although challenges in detection and tag loss complicate survival estimates, the project has established a foundation for long-term success. We have documented the survival of both tagged and untagged mussels, observed significant growth, and gained insights into effective placement strategies and habitat preferences in Cub Creek. These findings suggest that continued restoration efforts in NPS units are both feasible and worthwhile, especially with valuable state partnerships such as the NGPC.

To build on this progress, the next phase of the project should include refining tagging techniques and documenting signs of natural recruitment for fatmucket and plain pocketbook mussels. Cope and Waller (1995) recommend a five-year monitoring cycle to better document growth, recruitment, and reproduction of mussels. Consistent, long-term monitoring will be essential to fully assess trends and evaluate the ongoing status of freshwater mussels in Cub Creek.

Literature Cited

Aldridge, D.C., I.S. Ollard, Y.V. Bespalaya, I.N. Bolotov, K. Douda, J. Geist, W.R. Haag, M.W. Klunzinger, M. Lopes-Lima, et al. 2023. Freshwater mussel conservation: A global horizon scan of emerging threats and opportunities. Global Change Biology 29:575–589.

Bogan, A.E. 1993. Freshwater Bivalve Extinctions (Mollusca: Unionoida): A Search for Causes. American Zoologist 33:599–609.

Bouska, K.L., A. Rosenberger, S.E. McMurray, G.A. Lindner, and K.N. Key. 2018. State-Level Freshwater Mussel Programs: Current Status and a Research Framework to Aid in Mussel Management and Conservation. Fisheries 43:345–360.

Bowles, D.E., J.M. Bolli, and M.K. Clark. 2013. Aquatic invertebrate community trends and water quality at Homestead National Monument of America, Nebraska, 1996–2012. Transactions of the Kansas Academy of Science. 116:97–112.

Box, G.E.P. 1949. A general distribution theory for a class of likelihood criteria. Biometrika 36: 317–346.

Brown, M.B., and A.B. Forsythe. 1974. Robust tests for the equality of variances. Journal of the American Statistical Association 69:364–367.

Cope, W.G., and D.L. Waller. 1995. Evaluation of freshwater mussel relocation as a conservation and management strategy: Regulated Rivers Research & Management. 11:147–155.

Cope, W.G., M.C. Hove, D.L. Waller, D.J. Hornbach, M.R. Bartsch, L.A. Cunningham, H.L. Dunn, and A.R. Kapuscinski. 2003. Evaluation of relocation of Unionid mussels to in situ refugia. Journal of Molluscan Studies 69:27–34.

Dodd, H., and J. Cribbs. 2012. Fish community monitoring at Homestead National Monument of America: 2004–2011 status report. Natural Resource Data Series NPS/HTLN/NRDS—2012/276. National Park Service, Fort Collins, Colorado.

Dunn, O.J. 1964. Multiple Comparisons Using Rank Sums. Technometrics 6:241–252.

Freshwater Mollusk Conservation Society (FMCS). 2016. A national strategy for the conservation of native freshwater mollusks. Freshwater Mollusk Biology and Conservation 19:1–21.

Gangloff, M.M., J.D. Williams, and J.W. Feminella. 2006. A new species of freshwater mussel (Bivalvia: Unionidae), *Pleurobema athearni*, from the Coosa River Drainage of Alabama, USA. Zootaxa 1118: 1 = 43–56.

Geist, J., H. Bayerl, B.C. Stoeckle, and R. Kuehn. 2021. Securing genetic integrity in freshwater pearl mussel propagation and captive breeding. Scientific Reports 11:16019.

Haag, W. 2012. North American freshwater mussels: natural history, ecology, and conservation. Cambridge University Press.

Haag, W.R., and J.D. Williams. 2014. Biodiversity on the brink: an assessment of conservation strategies for North American freshwater mussels. Hydrobiologia 735:45–60.

Hart, M.A., C. Randklev, J. Dickson, N. Ford, B.A. Hernandez, and A. Schwalb. 2016. A literature review of freshwater mussel survey and relocation guidelines. Final report submitted to the Texas Department of Transportation, Project Number 0-6865, Austin, Texas.

Hoke, E. 2011. The Freshwater Mussels (Mollusca: Bivalvia: Unionoida) of Nebraska. Transactions of the Nebraska Academy of Sciences and Affiliated Societies. 32:1–46.

Howard, J.K., and K.M. Cuffey. 2006. The functional role of native freshwater mussels in the fluvial benthic environment. Freshwater Biology 51:460–474.

Inoue, K., A.L. McQueen, J.L. Harris, and D.J. Berg. 2014. Molecular phylogenetics and morphological variation reveal recent speciation in freshwater mussels of the genera *Arcidens* and *Arkansia* (Bivalvia: Unionidae). Biological Journal of the Linnean Society 112:535–545.

Inoue, K., D.M. Hayes, J.L. Harris, and A.D. Christian. 2013. Phylogenetic and morphometric analyses reveal ecophenotypic plasticity in freshwater mussels *Obovaria jacksoniana* and *Villosa arkansasensis* (Bivalvia: Unionidae). Ecology and Evolution 3:2670–2683.

Jennings, S. 1997. Survey notes of national park service units that have unionid resources. National Park Service, St. Croix National Scenic Riverway. Unpublished field notes.

Jennings, S. 1998. Needs in the management of native freshwater mussels in the National Park System NPS/NRWRD/NRTR-97-147. United States Department of the Interior National Park Service. Technical Report.

Johnson, P., A. Farmer, S. Golladay, and W. Michener. 2001. Effects of drought on freshwater mussels and instream habitat in Coastal Plain tributaries of the Flint River, southwest Georgia (July–October, 2000).

Jones, J.W., E.M. Hallerman, and R.J. Neves. 2006. Genetic management guidelines for captive propagation of freshwater mussels (Unionoidea). Journal of Shellfish Research, 25:527–535.

Kabbes, K.C., and R. Klocek. 2004. Urban Stream Restoration and Follow-Up Monitoring Using Mussels as an Indicator of Success in a Stream Project. Protection and Restoration of Urban and Rural Streams. American Society of Civil Engineers, Philadelphia, Pennsylvania, United States. 412–418.

Kruskal, W.H., and W.A. Wallis. 1952. Use of Ranks in One-Criterion Variance Analysis. Journal of the American Statistical Association 47:583–621.

Kurth, J., C. Loftin, J. Zydlewski, and J. Rhymer. 2007. PIT tags increase effectiveness of freshwater mussel recaptures. Journal of the North American Benthological Society 26:253–260.

Lefevre, G., and W.C. Curtis. 1910. Reproduction and parasitism in the Unionidae. Journal of Experimental Zoology 9:79–115

Leis, S.L., and L. Morrison. 2024. Long-term Trends in Prairie Vegetation at Three National Parks 1998–2022. 2024. Natural Resource Report NPS/HTLN/NRR-2024/2623.

Master, L., B. Stein, L. Kutner, and G. Hammerson. 2000. Oxford University Press.

Mueller, L. 1993. Winged mapleleaf mussel and Higgins eye pearly mussel: freshwater mussels threatened with extinction.

National Native Mussel Conservation Committee. 1998. National strategy for the conservation of native freshwater mussels. Journal of Shellfish Research 17:1419–1428.

National Park Service (NPS). 1999. Baseline water quality data inventory and analysis: Homestead National Monument of America. Fort Collins, CO: Technical Report NPS/NRWRD/NRTR-99/213.

Neves, R.J. 1993. A state-of-the-unionids address. Pages 1–10 in KS Cummings, AC Buchanan, and LM Koch, editors. Conservation and management of freshwater mussels. Proceedings of a UMRCC symposium. 12–14 October 1992. St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Newton, T.J., N.A. Johnson, and D.H. Hu. 2023. U.S. Geological Survey science vision for native freshwater mussel research in the United States. U.S. Geological Survey Circular 1511.

Ogle, D.H., J.C. Doll, A.P. Wheeler, and A. Dinno. 2025. FSA: Simple Fisheries Stock Assessment Methods. R package version 0.10.0. https://CRAN.R-project.org/package=FSA

Ohlman, L., B. Sweet, T. Bartels, K. Stoner, S. Schainost, D. Rosenthal, and M. Panella. 2019. Nebraska Freshwater Mussel Restoration Plan (draft).

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Schainost, S. 2016. A Guide to the Freshwater Mussels of Nebraska.

Schainost, S. 2017. Activities related to Rivers and Streams in Nebraska for 2017. North Central Division – American Fisheries Society, Rivers and Streams Technical Committee Summary.

Schneider, R., K. Stoner, G. Steinauer, M. Panella, and M. Humpert. 2011. The Nebraska Natural Legacy Project: State Wildlife Action Plan. 2nd ed. The Nebraska Game and Parks Commission. Lincon, NE.

Schneider, R., M. Fritz, J. Jorgensen, S. Schainost, R. Simpson, G. Steinauer, and C. Rothe-Groleu. 2018. Revision of the Tier I and 2 Lists of Species of Greatest Conservation Need: A Supplement to the Nebraska Natural Legacy Project State Wildlife Action Pan. The Nebraska Game and Parks Commission. Lincoln, NE.

Smith, C.H., N.A. Johnson, J.M. Pfeiffer, and M.M. Gangloff. 2018. Molecular and morphological data reveal non-monophyly and speciation in imperiled freshwater mussels (*Anodontoides* and *Strophitus*). Molecular Phylogenetics and Evolution 119:50–62.

Smith, D.R. 2006. Survey design for detecting rare freshwater mussels. Journal of the North American Benthological Society 25:701–711.

Smith. M.J. 2009. Current distribution of the plain pocketbook mussel (*Lampsilis cardium*) in Nebraska. Master's thesis. University of Nebraska, Kearney.

Sokal, R., and F. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. New York: W.H. Freeman and Company. 451–554.

Strayer, D., J. Downing, W. Haag, T. King, J. Layzer, T. Newton, and S. Nichols. 2004. Changing Perspectives on Pearly Mussels, North America's Most Imperiled Animals. Bioscience 54: 429–439.

Strayer, D.L., and D.R. Smith. 2003. A guide to sampling freshwater mussel populations. American Fisheries Society Monograph No. 8. American Fisheries Society, Bethesda, Maryland.

Strayer, D.L., J. Geist, W.R. Haag, J.K. Jackson, and J.D. Newbold. 2019. Essay: Making the most of recent advances in freshwater mussel propagation and restoration. Conservation Science and Practice 1:e53.

The Nature Conservancy. 1996. Species at risk: annual report card. Arlington, Virginia.

Tiemann, J. S., M. J. Ashton, S. A. Douglass, A. P. Stodala, R. M. Vinsel, and T. J. Newton. 2025. PIT tag application in native freshwater mussels: Case studies across small, medium, and large rivers. Freshwater Mollusk Biology and Conservation 28:71–82.

U.S. Fish and Wildlife Service (USFWS). 2024. ECOS Environmental Conservation Online System. Conserving the Nature of America. Listed U.S. Species by Taxonomic Group - All Invertebrate Animals. https://ecos.fws.gov/ecp/report/species-listings-by-tax-group?statusCategory=Listed&groupName=All%20Invertebrate%20Animals&total=317

Vaughn, C.C. 2018. Ecosystem services provided by freshwater mussels. Hydrobiologia 810:15–27.

Vaughn, C.C., and C.C. Hakenkamp. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology 46:1431–1446.

Vaughn, C.C., and D.E. Spooner. 2006. Unionid mussels influence macroinvertebrate assemblage structure in streams. Journal of the North American Benthological Society 25:691–700.

Watters, G.T., S.H. O'Dee, and S. Chordas. 2001. Patterns of Vertical Migration in Freshwater Mussels (Bivalvia: Unionoida). Journal of Freshwater Ecology 16:541–549.

Wickham, H. 2016. Data Analysis. Pages 189–201 in H. Wickham, editor. ggplot2: Elegant Graphics for Data Analysis. Springer International Publishing, Cham.

Wickham, H., D. Vaughan, and M. Girlich. 2024. tidyr: tidy messy data. https://tidyr.tidyverse.org/

Wickham, H., R. François, L. Henry, K. Müller, and D. Vaughan. 2023. dplyr: a grammar of data manipulation. https://github.com/tidyverse/dplyr

Williams, J., and H. Dodd. 2016. Fish Community status at Homestead National Monument of America 2004–2015. Resource Brief, Heartland Inventory and Monitoring Network of the National Park Service.

Williams, J.D., M.L. Warren, K.S. Cummings, J.L. Harris, and R.J. Neves. 1993. Conservation Status of Freshwater Mussels of the United States and Canada. Fisheries 18:6–22.

Zimmerman, G., and F. Szalay. 2007. Influence of unionid mussels (Mollusca: Unionidae) on sediment stability: An artificial stream study. Fundamental and Applied Limnology 168:299–306.

Appendix A: Survey Section Maps

This appendix presents maps of survey sampling sections for 2020, 2021, 2022, and 2024 (Figures 9–12), as well as density plots of LD1 scores from linear discriminant analysis of morphometric ratios for each year (Figure 13).

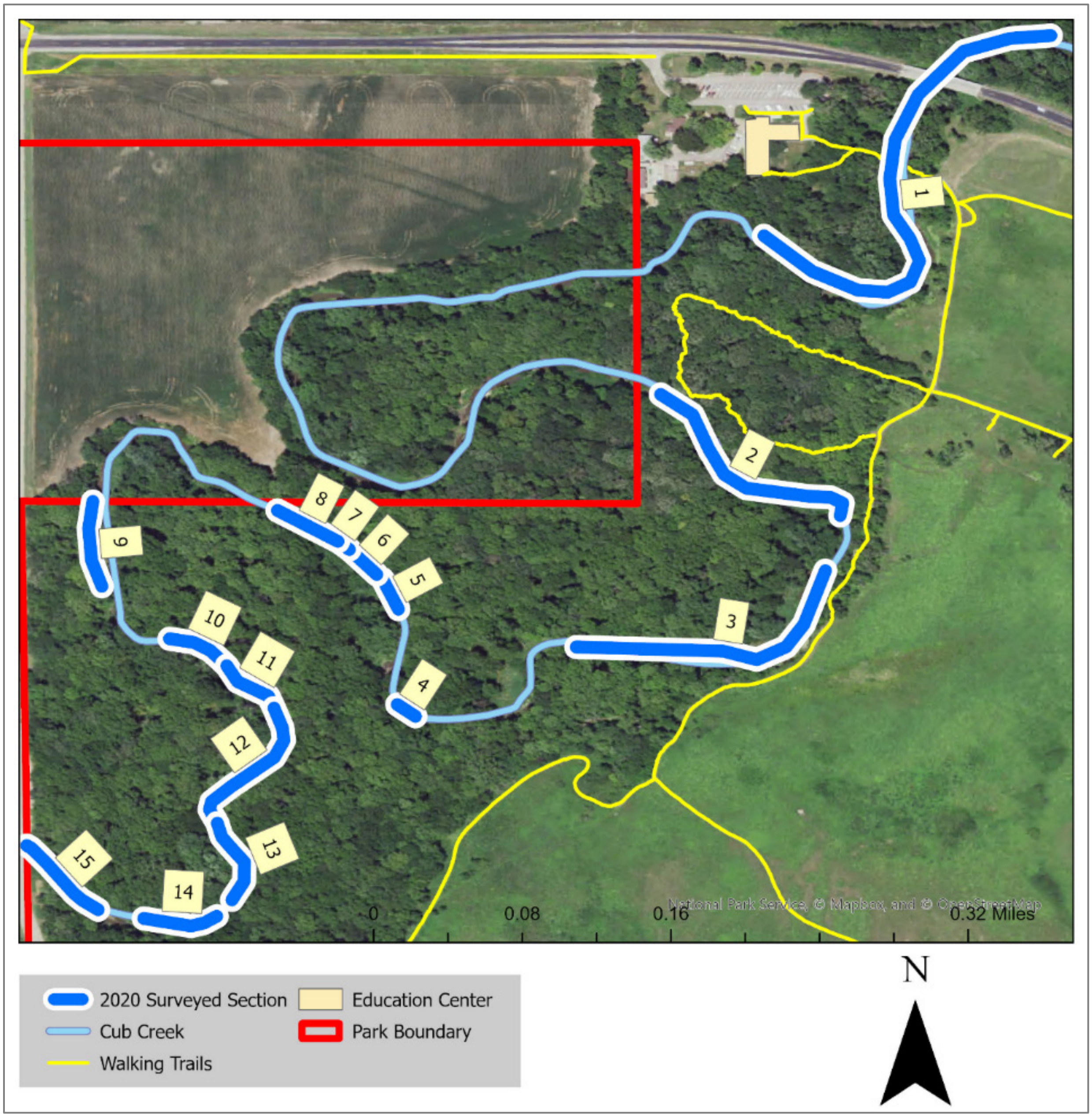

Figure 9. Mussel survey sampling sections of Cub Creek within HOME boundaries in 2020. Cub Creek is shown in blue, with individual sampling sections highlighted as thick blue segments outlined in white. Section numbers are labeled in light yellow boxes above each segment. Walking trails are represented by yellow lines, and the red line delineates the HOME park boundary.
NPS / JESSE BOLLI

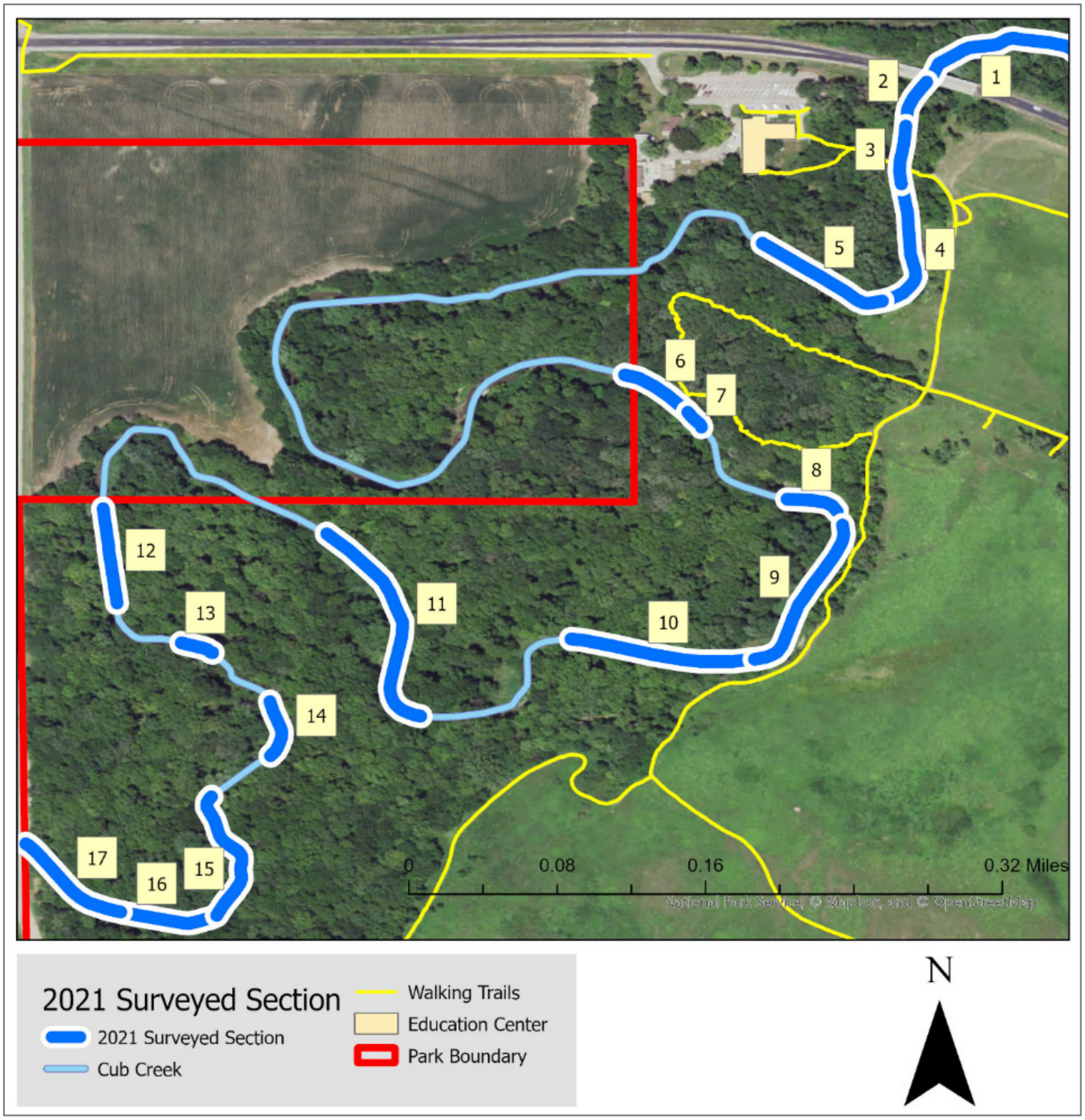

Figure 10. Mussel survey sampling sections of Cub Creek within HOME boundaries in 2021. Cub Creek is shown in blue, with individual sampling sections highlighted as thick blue segments outlined in white. Section numbers are labeled in light yellow boxes above each segment. Walking trails are represented by yellow lines, and the red line delineates the HOME park boundary.

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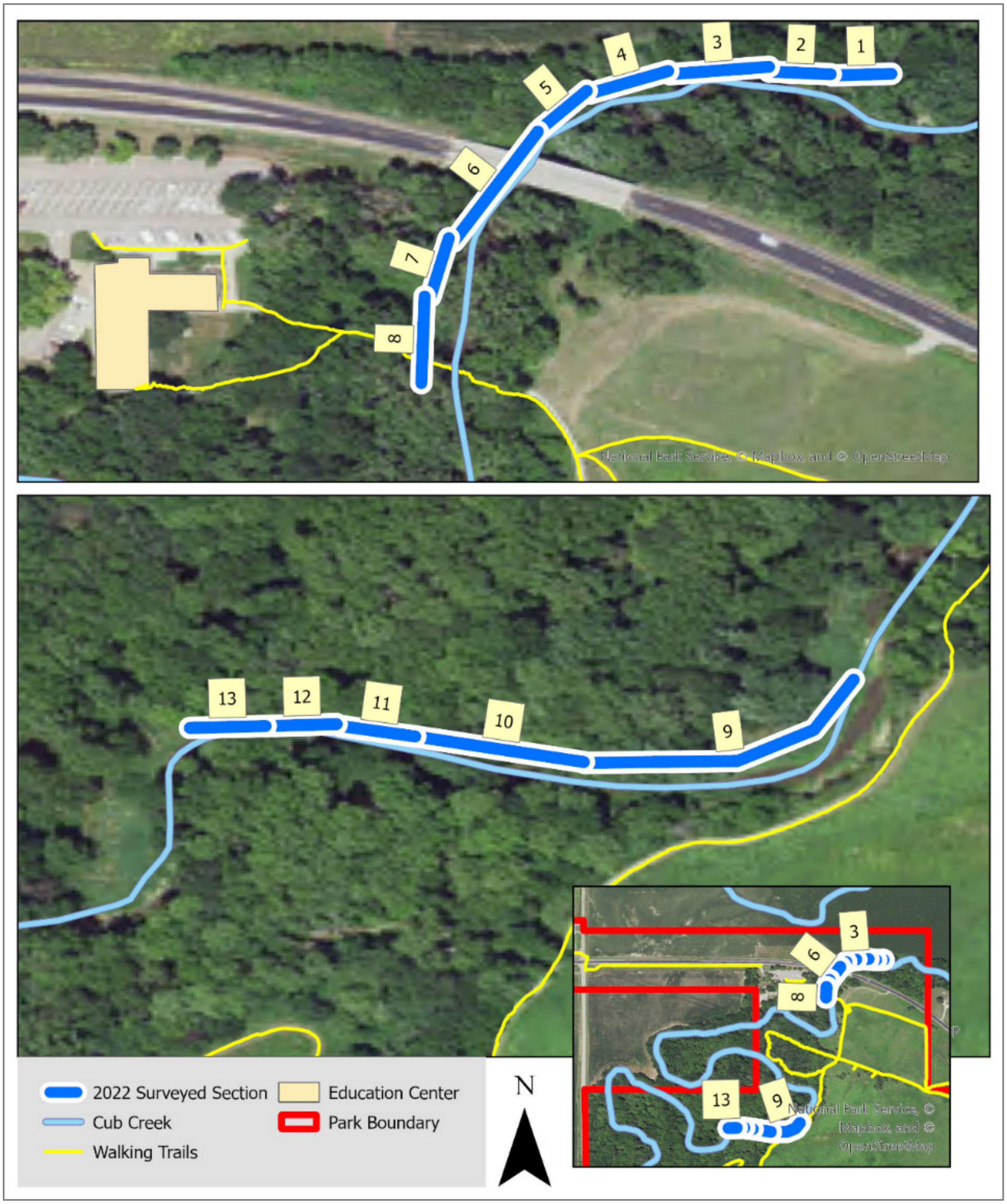

Figure 11. Mussel survey sampling sections of Cub Creek within HOME boundaries in 2022. Cub Creek is shown in blue, with individual sampling sections highlighted as thick blue segments outlined in white. Section numbers are labeled in light yellow boxes above each segment. Walking trails are represented by yellow lines, and the red line delineates the HOME park boundary. The inset image provides a zoomed-out view of sampling locations within the broader creek system.

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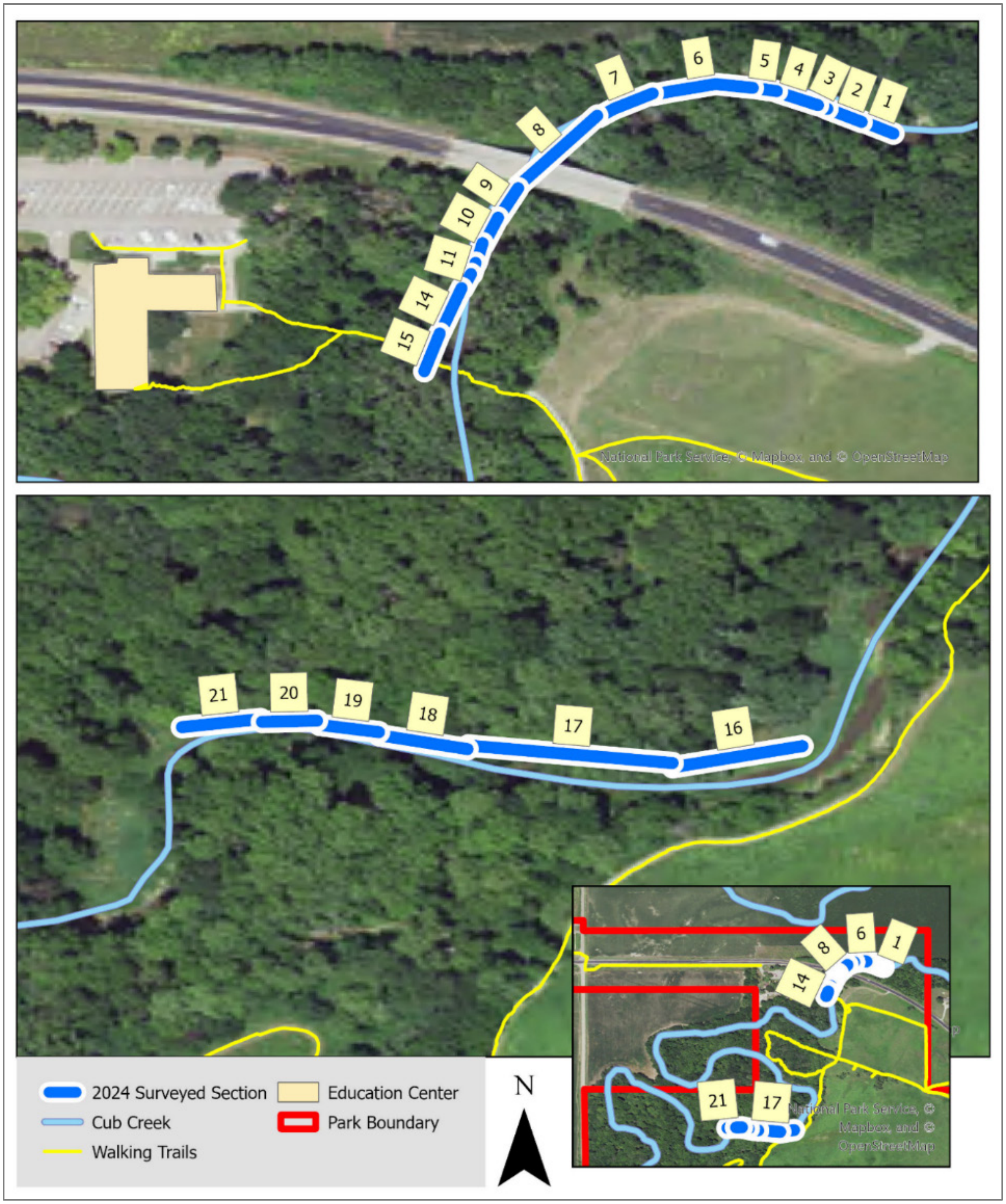

Figure 12. Mussel survey sampling sections of Cub Creek within HOME boundaries in 2024. Cub Creek is shown in blue, with individual sampling sections highlighted as thick blue segments outlined in white. Section numbers are labeled in light yellow boxes above each segment. Walking trails are represented by yellow lines, and the red line delineates the HOME park boundary. The inset image provides a zoomed-out view of sampling locations within the broader creek system.

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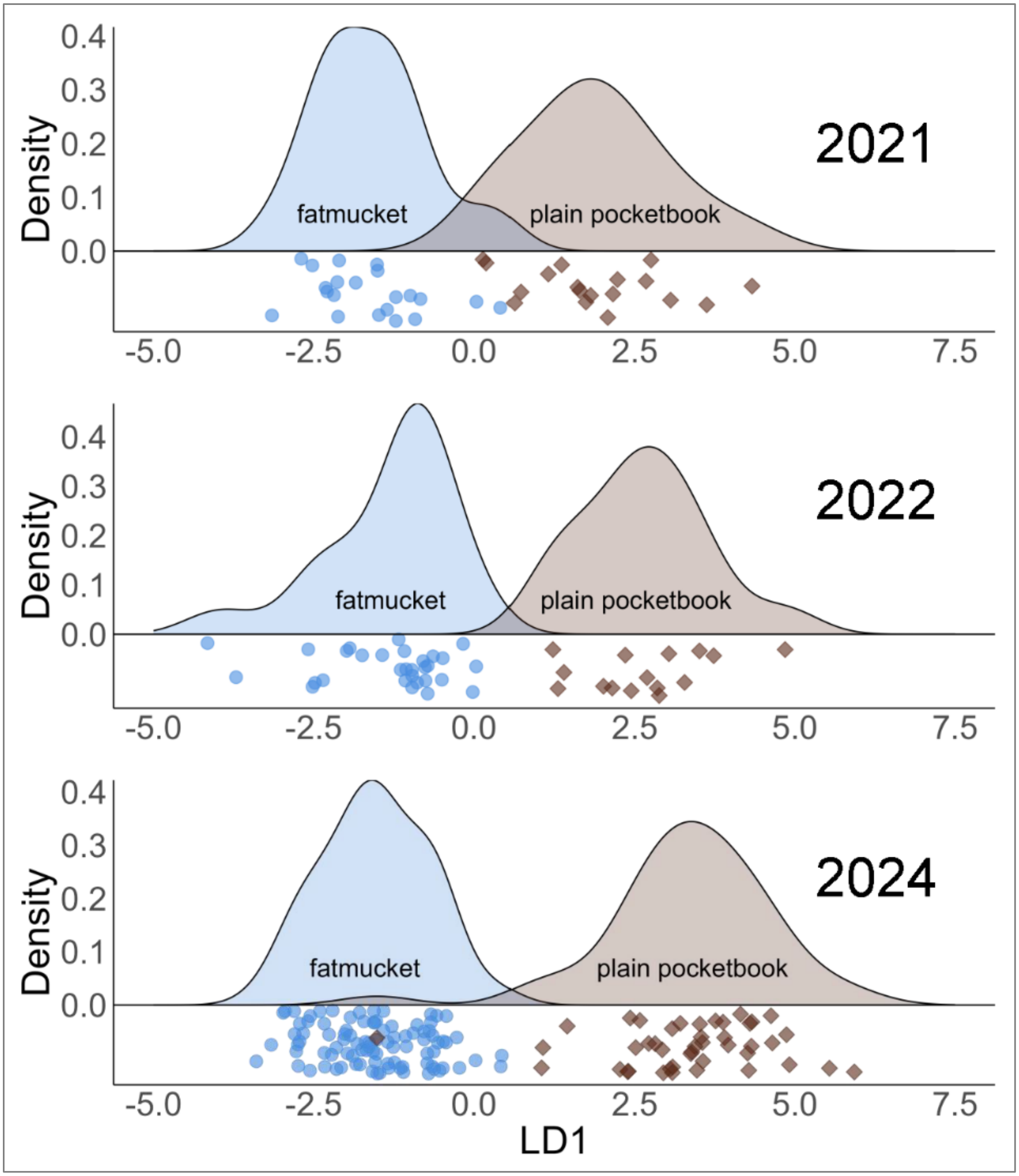

Figure 13. Density plot of LD1 scores from linear discriminant analysis of morphometric ratios of shell height/length, width/length, and width/height for fatmucket (blue circles) and plain pocketbook (brown diamonds) mussels. Jittered points below density plots represent individuals.
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