



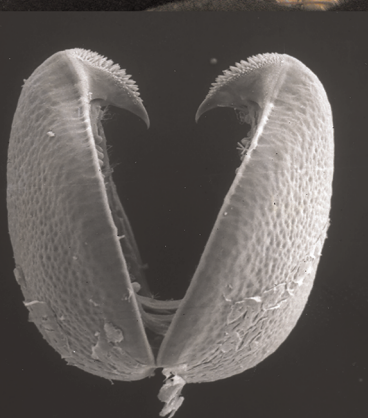

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REGULAR ARTICLE

FINE-SCALE HABITAT AND CO-OCCURRENCE PATTERNS OF FISH, CRAYFISH, AND MUSSELS IN THE MUSKEGON RIVER, MICHIGAN, USA

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ABSTRACT

Although the potential for species interaction at fine spatial scales is high, research on habitat and co-occurrence patterns for multifaunal groups at fine spatial scales is lacking. Video-recorded transect surveys provide the opportunity to examine abundance and distribution of multiple taxa at fine spatial scales to identify patterns in shared habitats. We examined habitat associations and patterns of species co-occurrence for fish, crayfish, and freshwater mussels by using video transect surveys at a site in the Muskegon River, Michigan, USA, in August 2020. Our results suggest that fine-scale habitat characteristics such as depth, substrate, estimated algal density, and siltation influence the distribution and abundance of fish, crayfish, and mussels at our site. Taxa co-occurrence was mostly random across transects, although there was some indication of segregated distribution of crayfish and mussels. Despite lack of strong patterns of transect-scale co-occurrence, we also found that several host fish species co-occurred with mussel species at our site, indicating that potential required life cycle interactions between mussels and host fish could still occur. Continued study of interactions and habitat requirements at fine spatial scales can inform restoration activities and elucidate the environmental and biological filters that influence the distribution of individual organisms and multifaunal communities.

KEY WORDS: assemblages, ecological interactions, multitaxa, macroinvertebrates, video

INTRODUCTION

Spatial scale is an essential consideration for ecological studies, given that organisms interact with each other and their environment differently at different scales (Levin 1992; Hernández 2020; DuBose et al. 2024). Unfortunately, research at fine spatial scales is frequently neglected in the scientific literature (Mehrabi et al. 2014), in part because observations at fine scales do not necessarily translate to larger habitats or ecosystems and may therefore be perceived as having limited value for solving problems that occur across larger scales (Schneider 2001). Despite the focus on larger scales, organisms are most directly impacted by (and conversely, most directly impact) the habitat conditions and species' interactions occurring in their immediate vicinity (Cushman and McGarigal 2004). Furthermore, restoration efforts are often most feasible (although not necessarily most effective) at finer spatial scales (Lake et al. 2007). Hence, understanding fine-scale habitat and species associations is important for management and restoration efforts that aim to promote species' persistence (Banks and Skilleter 2007; Rice et al. 2020).

Research at fine spatial scales has attempted to elucidate—with varying success—the distribution and habitat preferences of taxonomic groups such as freshwater mussels, fish, and crayfish (e.g., Vlach et al. 2009; Manna et al. 2017; Bird et al. 2022). Fine-scale distribution may be influenced by competition, predation or predator avoidance, and resource partitioning across species and taxa (Garvey et al. 1994; Pennock et al. 2018). However, few studies have attempted to quantify fine-scale patterns of co-occurrence and habitat

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associations for all three groups, despite increasing recognition of the importance of cross-taxa interactions in ecological research (Noguiera et al. 2023). For example, fish, crayfish, and mussels are ecosystem engineers and can concurrently influence habitat availability and suitability (Reynolds 2011; Polvi and Sarneel 2017; DuBose et al. 2024). Fish alter habitat through nest digging or sediment disturbance during foraging (Moore 2006). Crayfish can influence base flow and sediment composition in aquatic habitats and impact resource availability for other organisms (Momot 1995; Statzner et al. 2000; Reynolds et al. 2013). Mussels influence nutrient availability, deposit organic material through excretion of pseudofeces, influence sediment stability through burrowing in substrate, and provide shelter and habitat for other organisms (Vaughn et al. 2004; Moore 2006; Zimmerman and de Szalay 2007). In addition, direct interactions of fish, crayfish, and mussel taxa can influence distribution and habitat use. Most freshwater mussels in the order Unionida require host fish during the larval reproductive stage, and at least one species of fish parasitizes freshwater mussels by laying eggs in mussel gills (Haag and Warren 1998; Reichard et al. 2010). Both crayfish and fish have been observed preying on freshwater mussels (Klocker and Strayer 2004; Clark et al. 2022). Some fish species prey on crayfish, and crayfish may eat fish eggs or compete with benthic fish for food or habitat (Garvey et al. 1994; Dorn and Mittelbach 1999).

Efficient methods are needed to quantify the prevalence of cross-taxa interactions; thus, our objectives were to (1) examine fine-scale (i.e., tens of meters) habitat associations for fish, crayfish, and mussels across a gradient of depths; and (2) investigate fine-scale patterns of co-occurrence for these three taxa within a single site in the Muskegon River, Michigan, USA, with comments on the efficacy of video recording for data collection in aquatic environments. We predicted that (1) fine-scale habitat conditions would influence the abundance of fish, crayfish, and mussels, but would be most important for mussels given their relatively sedentary nature; and (2) fish, crayfish, and mussels would have nonrandom patterns of co-occurrence due to important cross-taxa interactions that occur across the groups. Specifically, we predicted that mussels and fish and mussels and crayfish would exhibit aggregated co-occurrence patterns because of mussel-provided ecosystem services such as increased habitat cover and complexity, biodeposition, and nutrient cycling (Vaughn 2018; Hopper et al. 2019). In addition, because mussels often rely on close-up interactions with host fish to complete the mussel life cycle, we predicted that mussels would exhibit aggregated co-occurrence with fish. In contrast, we predicted that fish and crayfish would exhibit segregated co-occurrence patterns because of potential predation or competition for space. Our results can inform restoration activities and provide information, generally lacking, about the distribution of individual organisms and multifaunal communities and cross-taxa interactions.

METHODS

Study Area

We examined fish, crayfish, and mussel communities and microhabitat conditions at a site in the Muskegon River near Paris, Michigan, in August 2020 (Fig. 1). The Muskegon River is an ~341-km river in the Lake Michigan drainage system of the Laurentian Great Lakes (O'Neal 1997). The site was ~70 m wide and was in a rural residential area ~150 km upstream of Lake Michigan, bordered on one bank by a residential lawn and on the other bank by a mixed forest. Substrate at the site was relatively heterogenous, with interspersed sand, pebble, cobble, and boulder across much of the area. Prior observations indicated >10 mussel species, multiple fish species, and at least 1 crayfish species were present at the site (our personal observations). Water temperature at the time of data collection was ~21–22°C and air temperature varied between 24 and 28°C. Total dissolved solids Oakton Instruments (Vernon Hills, IL, USA) at the site were ~250–260 ppm during data collection.

Mussel, Fish, and Crayfish Assemblages

We recorded information about fish and crayfish abundance by using repeated video recordings of 20-m transects ($n = 12$) placed parallel to the river's flow (Fig. 1). We selected areas ~0.25, 0.5, 0.75, and 1 m in depth to capture the range of depths observed at the site. Whenever possible, we selected areas with relatively constant depth across the entire 20-m transect. We established transects by using rebar and rope marked at 1-m intervals. After laying transects, we left the transect location for ~5 min to avoid disturbance. After 5 min, one researcher holding a GoPro (GoPro Inc., San Mateo, CA, USA) recording device (Hero 6 Black or Hero 4 Silver) swam upstream at a speed of ~4 m min^{-1} along the side of the transect closest to the thalweg of the river, passively collecting video data regarding fish and crayfish communities (i.e., not directly seeking out organisms with the camera lens). We attempted to maintain a consistent field of view during data collection, capturing organisms on camera in the water column and near the substrate, resulting in a range of fish size classes. We repeated this process three times (i.e., three runs per transect) at 5-min intervals for each transect, and three different transects were completed for each depth ($n = 36$ recordings total).

After collecting the video data, we laid 1-m^2 quadrats centered at the meter mark every 5 m along each transect (0, 5, 10, 15, and 20 m). We measured water velocity (meters per second) in the center of the quadrat by using a Marsh McBirney Flow-Mate 2000 flowmeter (Marsh-McBirney Inc., Frederick, MD, USA). We also estimated substrate composition within each quadrat based on the Wentworth scale (Wentworth 1922) and recorded macrophyte presence and qualitative estimates of algae (i.e., none, slight [<1-cm-thick accumulation on substrate within the quadrat], medium [1–3-cm-thick accumulation], or high [>3-cm-thick accumulation]) and degree of siltation (i.e., none [>5-m visibility], slight [5- to 1-m visibility), medium (<1-m to 50-cm visibility), high [<50-cm

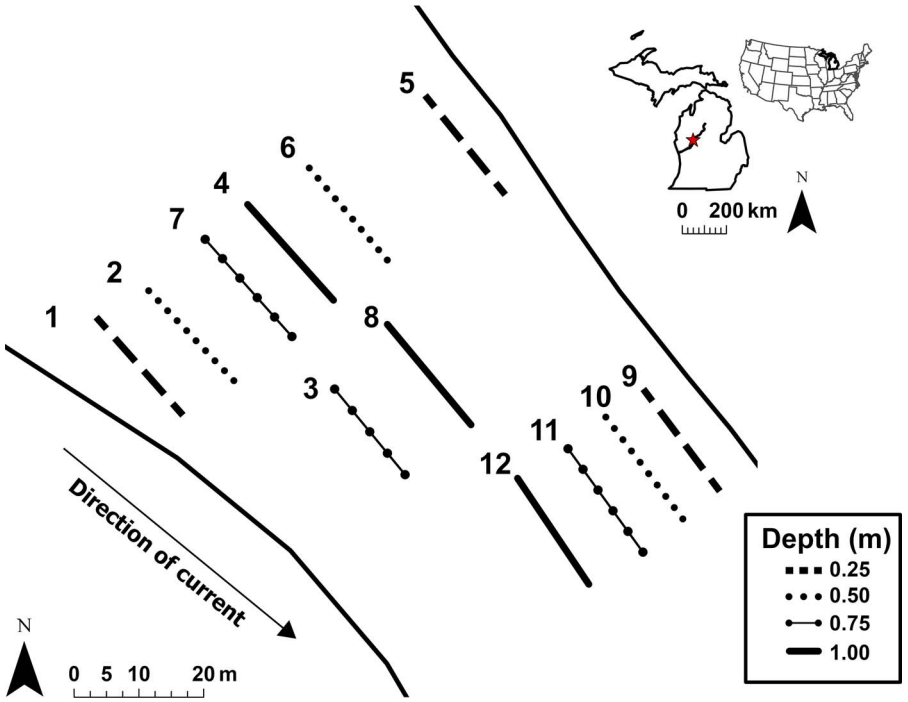

Figure 1. Locations of 12 transects of varying depths sampled for fish, crayfish, and mussels by using video recording and quadrat searches. Transect searches were conducted at a single site (indicated by star) in the Muskegon River, Michigan, USA, in August 2020.

visibility]). We then excavated each quadrat to a depth of ~10 cm and collected, measured (nearest millimeter), and identified (species level) any mussels found in quadrats by using a guidebook of mussel species in Michigan (Mulcrone and Rathbun 2020). We quantified mussel abundance as the cumulative abundance of mussels across quadrats from the same transect. We quantified habitat characteristics by averaging estimates from each quadrat within a transect.

Video Analysis

We analyzed the video recordings of transect runs ($n = 36$) by using BORIS 7.9.15 (Friard and Gamba 2016). Our video recordings ranged 3.3–5.9 min in length, with an average recording time of 4.8 ± 0.1 min. To facilitate recognition of organism presence, we reviewed videos at approximately one third to one fifth the original speed and recorded occurrences of fish and crayfish, for a total viewing time of ~15 min per video. We recorded the timestamp at which individual organisms were observed and identified each individual as a fish or crayfish. Whenever possible (based on image quality), we took screenshots of fish and crayfish that were reviewed by researchers with extensive knowledge of Michigan species to identify organisms to the genus or species level based on a list of species known to occur in the county (Appendix 1; Bailey et al. 2004). Sometimes, we observed mussels on the videos, but because mussels tend to burrow and rarely moved during the timeframe of videos, we used the quadrats (not the videos) to quantify mussel populations.

To enhance the accuracy of our abundance estimates, we reviewed each video three times. One researcher (K.C. Cushway) reviewed all video recordings initially; three additional researchers also rereviewed recordings for comparison (a total of three reviewers per video). We identified 16 additional individual organisms by reviewing videos multiple times after our initial analysis. Within runs, we quantified fish and crayfish abundance as the maximum count of individual observations of organisms (if a fish or crayfish was observed in the same location or moving upstream in the transect, it was counted one time even if it appeared in multiple frames). Hence, if a fish or crayfish moved outside of the camera frame and traveled upstream to another location on the transect, we may have recounted that organism within the same run. We recognize that this method of quantifying abundance may have resulted in double counting of some individuals, but using maximum number of individuals as a metric of abundance has been supported in other video-based studies and may lower sampling variability (Mallet and Pelletier 2014; Bruneel et al. 2022). Across runs, we quantified abundance at a given transect as the maximum number of individuals observed during any single run. For example, in transect 1, we observed 11 crayfish during run 1, 12 crayfish during run 2, and 3 crayfish during run 3, so the abundance of crayfish in transect 1 was set to 12 to account for the likelihood of double counting organisms during consecutive transect runs.

Analysis of Habitat Associations for Fish, Crayfish, and Mussels

We analyzed our data using R 4.2.2 (R Core Team 2022). Given the close spatial proximity of several of our transects within the site, fish or crayfish might have been counted in multiple transects, violating the assumption of independence.

Table 1. Variable groups used in multiple factor analysis, measured at 12 transects at a site in the Muskegon River, Michigan, USA, in August 2020.

Group	Type	Variable
Velocity	Quantitative	Average transect velocity
Depth	Quantitative	Average transect depth
Substrate	Quantitative	% Sand, % pebble, % cobble, % boulder
Algae	Qualitative	Algae
Vegetation	Qualitative	Vegetation presence
Siltation	Qualitative	Degree of siltation

To account for this, we used residual randomization in permutation procedures (RRPP) to assess the influence of fine-scale habitat characteristics on fish, crayfish, and mussel abundance across transects (Collyer and Adams 2018). The RRPP method functions similarly to the 'lm' function in the 'stats' package in R, but constructs empirical distributions over many random permutations ($n = 1{,}000$) to estimate coefficients without requiring the stringent assumptions of a traditional linear regression (Collyer and Adams 2018).

Before conducting RRPP, we ran a multiple factor analysis (MFA) by using the 'FactoMineR' and 'Factoextra' packages in R to better understand the environmental variation explaining differences across transects and to group similar variables (Lê et al. 2008; Kassambara and Mundt 2020). The MFA ordination allows analysis of both quantitative and qualitative data simultaneously using groups of similar variables (Escofier and Pagès 1994). We grouped our data into six groups for the MFA (Table 1) and determined each group's contribution to MFA dimensions. We then used a broken stick model to determine the number of dimensions to retain based on whether a given dimension contributed more than expected to the amount of variation observed in the MFA (Frontier 1976; Jackson 1993). Based on the broken stick method, the expected contribution of a dimension can be calculated as

$$b_k = \sum_{i=k}^{p} \frac{1}{i}$$

where p is equal to the total number of variables used in the analysis and b_k is equal to the size of a given eigenvalue for the kth element of the broken stick model (Jackson 1993).

Following the MFA, we conducted bootstrap selection with the retained dimensions by using the 'FWDselect' package in R to determine what variables to include in our RRPP tests (Sestelo et al. 2015, 2016). Bootstrap selection identifies the number of variables (q) needed to minimize deviance in a model by increasing q one variable at a time and testing the null hypothesis that a given value of q is sufficient to minimize deviance in the model of interest (Sestelo et al. 2016). Using the dimensions selected by bootstrap selection, we used RRPP from the 'RRPP' package in R to run a nonparametric regression to determine how well the selected dimensions (habitat variables) could explain variation in fish, crayfish, or mussel abundance in transects (Collyer and Adams 2018). We conducted Spearman rank correlation tests with Bonferroni correction to determine whether the variables contributing significantly to the selected dimensions were positively or negatively correlated with fish, crayfish, or mussel abundance. We chose Spearman rank correlation because it can handle nonnormal data, and we applied Bonferroni correction to account for using multiple tests on the same dataset (Spearman 1904; Bonferroni 1936).

Analysis of Fish, Crayfish, and Mussel Co-occurrence

To test whether fish, crayfish, and mussel co-occurrence in transects was due to species interactions or random chance, we used randomized co-occurrence null models by using the 'EcoSimR' package in R (Gotelli et al. 2015). These tests work by repeatedly randomly permuting data ($n = 1{,}000$ permutations) in a presence–absence matrix and comparing the observed co-occurrence index with the expected co-occurrence index given random organism occurrence (Gotelli et al. 2015; Santangelo 2019). We used a randomization scheme that maintained the rarity of organismal groups, but allowed each transect equal chances of occurrence given the proximity of our transects ('sim2' algorithm from 'EcoSimR' package; Gotelli 2000). We used a checkerboard score, or "C-score" index, to assess aggregation of fish, crayfish, and mussels across transects. This index uses the concept of checkerboard distributions described by Diamond (1975) to determine whether average checkerboard patterns of species co-occurrence (or in this case taxa) differ from a random distribution (Stone and Roberts 1990). Higher than expected C-scores indicate segregation of organismal groups across transects, whereas lower than expected C-scores indicate aggregation (Gotelli et al. 2015). We constructed randomized null models for all taxa, fish and crayfish only, fish and mussels only, and crayfish and mussels only.

RESULTS

Mussel, Fish, and Crayfish Assemblages

We collected 39 live mussels of 8 different species during surveys (Table 2), with an additional 13 shells representing 5 species, one of which (*Alasmidonta marginata*) was not found alive in quadrats. Mussel density in transects ranged from 0 to 3.8 individuals m^{-2}, with an average of 0.7 ± 1.0 ($\bar{x} \pm \sigma$). Mussels ranged in size from 25 to 114 mm (Appendix 2). Forty-four live fish and 26 live crayfish were observed on video (Fig. 2). We could not identify all crayfish and fish to the species level, but we did identify at least 1 crayfish species (invasive *Faxonius rusticus*) and up to 10 genera of fish present in transects (Table 3; Appendix 1). Most of the individuals we observed were small fish or darters, but we did capture some larger fish (e.g., *Cyprinus carpio*) present in the water column during transect recordings. Across the site (all transects), several potential host fish species co-occurred with

Table 2. Freshwater mussel species collected in 1-m^2 quadrats during transect searches of 12 transects in the Muskegon River, Michigan, USA, in August 2020. Abundance denotes the total number of individuals found in all transects, and transects occupied refers to the specific transects in which each species was found. Species with an asterisk are considered threatened in the state of Michigan (Mulcrone and Rathbun 2020).

Species	Abundance	Transects Occupied
Cambarunio iris	1	1
Eurynia dilatata	17	1, 2, 4, 8, 11
Fusconaia flava	1	7
Lampsilis cardium	2	3, 8
Lampsilis siliquoidea	4	1, 10
Lasmigona costata	1	1
**Ligumia recta*	1	4
Ortmanniana ligamentina	12	1, 2, 3, 4, 9, 11, 12

mussel species (Table 3; Freshwater Mussel Host Database 2017).

Habitat Associations for Fish, Crayfish, and Mussels

The first seven dimensions of our MFA explained ~95% of the total variation across transects. Dimensions 1 and 2 alone explained ~45% of the overall variation, with velocity (dimension 1) and depth (dimension 2) being the primary quantitative variables contributing to these dimensions (Fig. 3). Based on the results of the broken stick model, however, we retained only dimensions 2–7, which explained ~71% of the total variation across transects (Table 4). Although dimension 1 explained ~24% of the overall variation, this was less than the amount of variation that would be expected based on the broken stick model, so it was not retained for further analysis.

Our bootstrap variable selection indicated that algae and transect depth (represented by dimension 3) were sufficient for explaining variation in fish abundance in response to habitat ($q = 1$, $T = 17.59$, $p = 0.566$, deviance = 205.16). Algae and depth explained ~30% of the variation in fish abundance across transects (df = 10, SS = 188.34, $F = 4.28$, $Z = 1.46$, $\Pr(>F) = 0.05$). Depth was negatively correlated with fish abundance, although this relationship was not statistically significant (Appendix 3). Algae, siltation, and substrate (represented by dimension 4) best explained crayfish abundance ($q = 1$, $T = 13.23$, $p = 0.339$, deviance = 40.26), accounting for ~28% of variation in abundance across transects (df = 10, SS = 39.24, $F = 3.83$, $Z = 1.38$, $\Pr(>F) = 0.08$), although the probability of obtaining a larger F value based on the empirical distribution was ~8%. Crayfish abundance tended to be weakly negatively related to sand and boulder substrate and weakly positively related to pebble and cobble substrate, although none of these relationships were statistically significant (Appendix 3). Algae, siltation, and depth (represented by dimension 2) best explained mussel abundance ($q = 1$, $T = 17.66$, $p = 0.531$, deviance = 99.58), accounting for ~35% of the variation in abundance across transects, although the probability of obtaining a larger F value based on the empirical distribution was ~9% (df = 10, SS = 100.15, $F = 5.27$, $Z = 1.55$, $\Pr(>F) = 0.09$). Depth alone was not strongly correlated with mussel abundance (Appendix 3).

Fish, Crayfish, and Mussel Co-occurrence

Based on our randomized co-occurrence null model for all taxa, the C-score for fish, crayfish, and mussel distribution across transects was not significantly different from the randomly simulated C-score (Fig. 4A; Table 5). This result indicated that, on average, the three organismal groups were not significantly aggregated or segregated across transects. Similarly, we found that fish and crayfish and fish and mussels

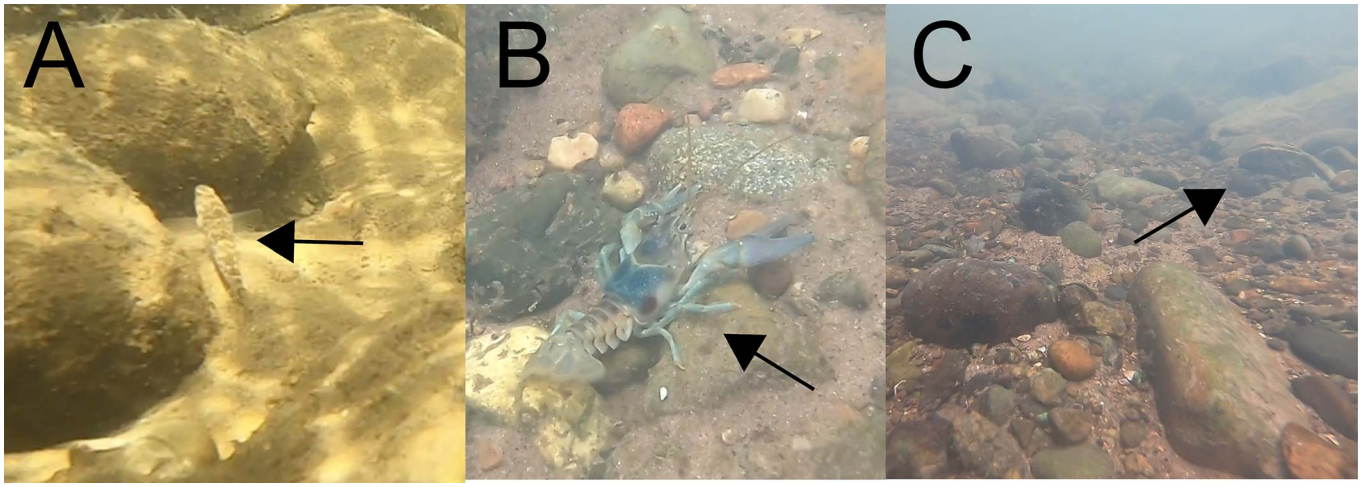

Figure 2. Examples of (A) fish, (B) crayfish, and (C) mussels captured during video-recorded transect surveys of 12 transects at a site in the Muskegon River, Michigan, USA, sampled in August 2020. Note that although mussels were sometimes captured in video recordings, mussels were sampled only by using quadrats placed along transects.

Table 3. Potential host fish species co-occurring with mussel species at a site in the Muskegon River, Michigan, USA, in August 2020. Evidence of host potential was obtained from the Freshwater Mussel Host Database (2017) and included successful infestation or transformation in natural and laboratory settings, including unspecified infestation types. Fish species marked ZT were tested as host fish, but did not support successful transformation of that particular mussel species (Freshwater Mussel Host Database 2017). Species with one asterisk in front of the genus name were identified by experienced researchers from still images obtained using GoPro video recordings along twelve 20-m-long transects at ~0.25-, 0.5-, 0.75-, and 1.0-m depths ($n = 3$ for each depth). Species with two asterisks were also identified as potentially being present at the site and were historically found in the Muskegon River watershed, but not within Mecosta County.

Fish Species			Mussel Species							
Genus	Specific Epithet	Common Name	*Cambarunio iris*	*Eurynia dilatata*	*Fusconaia flava*	*Lampsilis cardium*	*Lampsilis siliquoidea*	*Lasmigona costata*	*Ligumia recta*	*Ortmanniana ligamentina*
**Cyprinus*	*carpio*	Common Carp						X	X	X
**Dorosoma*	*cepedianum*	Gizzard Shad		X				X		
Etheostoma	*caeruleum*	Rainbow Darter	X	X	ZT		ZT	X		
Etheostoma	*exile*	Iowa Darter		X			X			
Etheostoma	*nigrum*	Johnny Darter		X	ZT		X	X		
Micropterus	*salmoides*	Largemouth Bass	X	X	X	X	X	X	X	X
**Neogobius*	*melanostomus*	Round Goby	X				X		X	X
**Notropis*	*atherinoides*	Emerald Shiner			X					
Perca	*flavescens*	Yellow Perch	X	X		X	X	X	X	X
**Percina*	*caprodes*	Logperch	ZT	X	ZT		ZT	X		
Percina	*maculata*	Blackside Darter		X	ZT		ZT	X		
Rhinichthys	*obtusus*	Western Blacknose Dace			X					
Semotilus	*atromaculatus*	Creek Chub	ZT		X		ZT	X		X

co-occurred randomly across transects (Fig. 4B, C; Table 5). Crayfish and mussels exhibited some indication of segregation (i.e., the observed C-score was always higher or equal to the simulated C-score), but this pattern was not statistically significant (Fig. 4D; Table 5).

DISCUSSION

Mussel, Fish, and Crayfish Assemblages

As the technology improves, video recording methods for data collection are becoming increasingly popular (Mallet and Pelletier 2014). Our study demonstrated a unique way to use video analysis to assess habitat associations and co-occurrence of multifaunal groups at fine spatial scales. Although we focused on fish, crayfish, and mussels, our methodology could easily be expanded to include other organisms such as gastropods and other macroinvertebrates, providing a quick and relatively straightforward method of assessing multiple taxa simultaneously. In addition, use of video recordings can decrease the time and training needed to conduct field surveys and help reduce species' misidentification while increasing organism detection (Bruneel et al. 2022). Repeated review of our video recordings (after our initial review) helped us detect an additional 16 organisms and allowed us to consult subject experts for help with crayfish and fish identification.

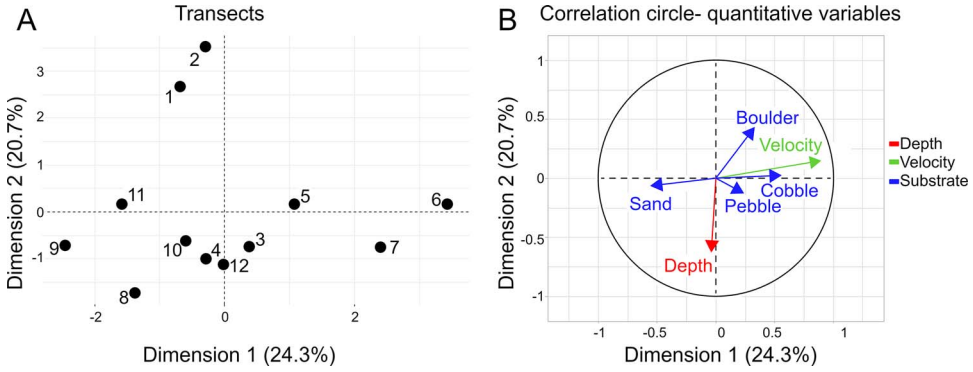

Figure 3. Position of (A) transects and (B) quantitative variables on the primary axes of a multiple factor analysis (MFA) evaluating variation in habitat characteristics across a site in the Muskegon River, Michigan, USA. Transects were sampled for fish, crayfish, and mussels in August 2020. Direction of arrows in B indicate relationships between variables (arrows pointing in opposite directions are negatively correlated), and length of arrows represents the importance of each variable in the MFA. Variables are colored by group.

Table 4. Multiple factor analysis dimensions (along with explained variance) retained for bootstrap variable selection based on the results of a broken stick model. Groups included in each dimension contributed more than expected given uniform contribution from all possible groups (Kassambara 2017).

Dimension	% Variance Explained	Groups Included	Group Contribution to Dimension (%)
2	20.7	Algae	42
		Siltation	35.4
		Depth	17.3
3	15.3	Algae	53.8
		Depth	24.3
4	12	Algae	33.3
		Siltation	31.8
		Substrate	22.5
5	9.2	Algae	52.2
		Siltation	39.3
6	7.9	Substrate	59.2
		Algae	24.2
7	5.6	Algae	47.9
		Vegetation	27.2

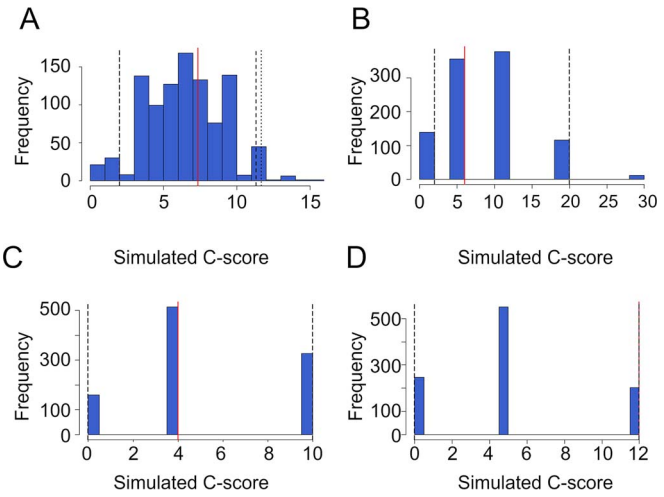

Figure 4. Frequency distribution of simulated checkerboard scores (C-scores) for randomized species co-occurrence null models for (A) fish, crayfish, and mussels, (B) fish and crayfish only, (C) fish and mussels only, and (D) crayfish and mussels only. The red vertical line represents the observed C-score for taxa co-occurrence across 12 transects sampled at a site in the Muskegon River, Michigan, USA, in August 2020. Vertical dashed lines represent one-tailed (long dash) and two-tailed (short dash) 95% confidence intervals. Where only one set of dashed lines is visible, one- and two-tailed confidence intervals were equivalent.

Habitat Associations for Fish, Crayfish, and Mussels

Our results suggest that fine-scale habitat characteristics were related to the abundance and distribution of fish, crayfish, and mussels at our site, although statistical support was rather low for crayfish and mussels. Increased sampling would likely strengthen the statistical power of our results, but even with our small sample sizes, we observed some interesting patterns in taxa occurrence. As we predicted, habitat conditions were most explanatory for mussels. Although mussels can move short distances to seek suitable habitat, they are much less mobile than fish or crayfish and were likely relatively stationary at the timescale we examined (Schwalb and Pusch 2007). By contrast, fish and crayfish can move in and out of suitable areas relatively quickly and could have been influenced by observer presence in transects, which may be why habitat was slightly less important for predicting their abundance (Bruneel et al. 2022).

Estimated algal density appeared as an important factor of the MFA dimensions used in all models, which could be related to trophic interactions and prey density. We identified several species of darters in our transects, and both darters and crayfish prey on macroinvertebrates that eat periphyton (Stelzer and Lamberti 1999). Mussels also influence periphyton abundance through biodeposition and excretion, although the density of mussels observed in our transects was relatively low compared with mussel density in southern streams where these effects have been measured (densities as high as 64 individuals m^{-2} in southern study system; Spooner and Vaughn 2006). In addition, some species of crayfish (including *F. rusticus*), fish (e.g., *C. carpio*), and mussels consume algae and periphyton, which may be why it appeared ubiquitously across all models (Lodge et al. 1994; Matsuzaki et al. 2007; Vaughn et al. 2008). Similarly, degree of siltation was included in influential dimensions for both crayfish and mussels, which may be related to the influence of both taxa on stream bioturbation (Statzner et al. 2000; Vaughn et al. 2004).

Depth was an important factor in the dimensions explaining fish and mussel abundance, whereas substrate was an important factor in the dimension explaining crayfish abundance. Fish abundance tended to decrease with increasing depth, which may be because many of the fish we observed were small darters that use shallow habitats as refuges from predation by larger fish (Schlosser 1987). Mussel abundance was not strongly correlated with depth on its own, which aligns with several studies that suggest complex hydraulic characteristics may be more important for influencing mussel distribution (Allen and Vaughn 2010; Pandolfo et al. 2016). However, mussels in a study in Kentucky were observed preferentially occupying depths between 7 and 30 cm during base flow periods, which falls into the range of observed depths at our study site (Layzer and Madison 1995). Depth preferences may be stronger in southern streams where drought and subsequent stream drying poses a threat to mussels, and species-specific depth preferences may also influence mussel abundance at different depths (Hart 1995; Cushway et al. 2024). Substrate was an important group in the dimension selected for the crayfish model, which aligns with past research suggesting that substrate influences crayfish distribution (e.g., Smith et al. 2019). Many nonburrowing crayfish species prefer cobble or boulder substrates for shelter and as refuge from predation (Smith et al. 2019). We did not find very strong relationships with individual substrate variables, but there

Table 5. Results of randomized species co-occurrence null models (n = 1,000 permutations; Gotelli et al. 2015) for fish, crayfish, and mussels found in 12 transects at a site in the Muskegon River, Michigan, USA, in August 2020. Results include observed (Obs.) and simulated (Sim.) checkerboard scores (C-score); variance of the simulated C-score; upper bound (UB) and lower bound (LB) of one- and two-tailed confidence intervals; p values for upper and lower tails; standard effect size; and the percent of simulated C-scores less than, greater than, or equal to the observed C-score.

	C-Score			Confidence Interval				p Value			% of Permutations		
Taxa	Obs.	Sim.	Sim. variance	One-tailed LB	One-tailed UB	Two-tailed LB	Two-tailed UB	Lower tail	Upper tail	Standard effect size	Sim. < Obs.	Sim. > Obs.	Sim. = Obs.
All	7.3	7	6.7	2	11	2	11.7	0.7	0.41	0.26	59.1	31.3	9.6
Fish/crayfish	6	9.2	30.08	2	20	2	20	0.53	0.86	−0.58	14.2	47.1	38.7
Fish/mussels	4	5.27	12.22	0	10	0	10	0.69	0.85	−0.36	15.5	31.5	53
Crayfish/mussels	12	5.63	17.33	0	12	0	12	1	0.25	1.53	75.2	0	24.8

were weak tendencies for lower crayfish abundances in sandy habitats, which does not provide much cover from predators.

Fish, Crayfish, and Mussel Co-occurrence

Contrary to our expectations, we did not see strong patterns of co-occurrence across taxa, particularly for fish and crayfish or fish and mussels, indicating potential limited cross-taxa interactions. This may be due, in part, to our sampling representing a short snapshot in time. Because most mussels require a host fish to reproduce, fine-scale interactions of fish and mussels are required for successful host infestation and mussel reproduction (Haag 2012). In addition, host fish are often the primary means of mussel dispersal during the glochidial life stage and influence mussel distribution at larger scales, but they also may influence fine-scale distribution if host fish have particular habitat preferences that influence their time spent in specific habitats (which could increase the probability of a juvenile mussel dropping off and settling in that habitat; Schwalb et al. 2015). However, because fish are relatively mobile, there may be stronger patterns of fine-scale co-occurrence over longer time scales and repeated sampling, or with experiments designed more appropriately to detect these types of cross-taxa interactions. Because mussels use several strategies, such as mantle lures or conglutinates that resemble prey items, to attract host fish during spawning, stronger co-occurrence patterns also may be observed during mussel reproductive periods (Haag 2012). We did observe that several host fish species co-occurred with mussels at the site, indicating the potential for interaction to occur, which is essential for completion of the mussel life cycle.

Our results provided some evidence of segregation of mussels and crayfish in transects that could become more apparent with additional sampling. Most research regarding mussel and crayfish interactions have focused on crayfish predation of mussels, which is unlikely to have influenced the individuals in our transects given the size classes observed during sampling (e.g., Klocker and Strayer 2004; Meira et al. 2019). Furthermore, mussels can increase macroinvertebrate prey abundance, stabilize substrate, and provide shelter or habitat which are all beneficial for crayfish (Vaughn 2018). Additional sampling could help clarify this relationship and its potential causes, and whether native crayfish species might exhibit contrasting patterns of co-occurrence with mussels. In addition, our methodology and lack of a control treatment make it difficult to separate the effects of habitat (i.e., shared habitat preferences) from the direct impact of taxa co-occurrence, which could also influence our ability to recognize clear patterns of co-occurrence.

Our use of video recording to capture abundance patterns is particularly suited to understanding co-occurrence and cross-taxa interaction, although we did not observe strong patterns of co-occurrence across fish, crayfish, and mussels. Sampling mobile taxa such as fish and crayfish simultaneously allows investigators to document the presence of organisms at the same point in time and space, which would be difficult or impossible with other sampling methods targeting individual taxa. As a result, this study provides a better understanding of how different organisms may directly share resources in habitats where they are observed together.

CONCLUSION

Although research at fine scales is lacking, the potential for interactions between taxa (and between organisms and their environment) is high at fine scales (Cushman and McGarigal 2004; Mehrabi et al. 2014). Hence, understanding fine-scale distribution patterns in relation to habitat and co-occurring organisms can help clarify important environmental or biological filters acting on multitaxa assemblages occurring in proximity. Our study demonstrated the utility of video-recorded transects for assessing multifaunal habitat and taxa associations, and our methods could be expanded easily to increase the accuracy and scope of riverine studies at fine spatial scales. Even with relatively limited sample sizes, our approach allowed us to detect fine-scale habitat associations for fish, crayfish, and mussels and investigate co-occurrence patterns across taxa. Understanding the patterns that occur at fine spatial scales can help inform management strategies that account for both the habitat requirements and species interactions that influence organism persistence in their immediate environment.

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Appendix 1. Species of fish identified as being present in Mecosta County, Michigan (the county containing the study site; Bailey et al. 2004). Species with one asterisk in front of the genus name were identified by experienced researchers from still images obtained using GoPro video recordings along twelve 20-m-long transects at ~0.25-, 0.5-, 0.75-, and 1.0-m depths ($n = 3$ for each depth) surveyed at a site in the Muskegon River, Michigan, USA, in August 2020. Species with two asterisks also were identified as potentially being present at the site and were historically found in the Muskegon River watershed, but not within Mecosta County. Not all organisms found along transects could be identified due to limitations in visual clarity or organism visibility.

Genus	Specific Epithet	Common Name
Ambloplites	*rupestris*	Rock Bass
Ameiurus	*melas*	Black Bullhead
Ameiurus	*natalis*	Yellow Bullhead
Ameiurus	*nebulosus*	Brown Bullhead
Campostoma	*anomalum pullum*	Central Stoneroller
Catostomus	*commersonii*	White Sucker
Cottus	*bairdii*	Mottled Sculpin
Culaea	*inconstans*	Brook Stickleback
***Cyprinus*	*carpio*	Common Carp
***Dorosoma*	*cepedianum*	Gizzard Shad
Esox	*americanus vermiculatus*	Grass Pickerel
Esox	*lucius*	Northern Pike
Etheostoma	*flabellare flabellare*	Barred Fantail Darter
**Etheostoma*	*caeruleum*	Rainbow Darter
**Etheostoma*	*exile*	Iowa Darter
Etheostoma	*microperca*	Least Darter
**Etheostoma*	*nigrum*	Johnny Darter
Hybognathus	*hankinsoni*	Brassy Minnow
Hypentelium	*nigricans*	Northern Hog Sucker
Ichthyomyzon	*castaneus*	Chestnut Lamprey
Lethenteron	*appendix*	American Brook Lamprey
Lepisosteus	*osseus*	Longnose Gar
Lepomis	*cyanellus*	Green Sunfish
Lepomis	*gibbosus*	Pumpkinseed
Lepomis	*macrochirus*	Bluegill
Lepomis	*peltastes*	Northern Longear Sunfish
Luxilus	*cornutus*	Common Shiner
Margariscus	*nachtriebi*	Northern Pearl Dace
**Micropterus*	*salmoides*	Largemouth Bass
***Neogobius*	*melanostomus*	Round Goby
Nocomis	*biguttatus*	Hornyhead Chub
Nocomis	*micropogon*	River Chub
Notemigonus	*crysoleucas*	Golden Shiner
Notropis	*anogenus*	Pugnose Shiner
***Notropis*	*atherinoides*	Emerald Shiner
Notropis	*dorsalis*	Bigmouth Shiner
Notropis	*heterodon*	Blackchin Shiner
Notropis	*heterolepis*	Blacknose Shiner
Notropis	*rubellus*	Rosyface Shiner
Notropis	*volucellus*	Mimic Shiner
Noturus	*flavus*	Stonecat
Noturus	*gyrinus*	Tadpole Madtom
Oncorhynchus	*mykiss*	Rainbow Trout
**Perca*	*flavescens*	Yellow Perch
***Percina*	*caprodes*	Logperch
**Percina*	*maculata*	Blackside Darter
Phoxinus	*eos*	Northern Redbelly Dace
Pimephales	*notatus*	Bluntnose Minnow
Pimephales	*promelas*	Fathead Minnow
Pomoxis	*nigromaculatus*	Black Crappie
**Rhinichthys*	*obtusus*	Western Blacknose Dace
Salmo	*trutta*	Brown Trout
Salvelinus	*fontinalis*	Brook Trout
Salvelinus	*namaycush*	Lake Trout
**Semotilus*	*atromaculatus*	Creek Chub
Umbra	*limi*	Central Mudminnow

Appendix 2. Length statistics for mussel species collected during transect sampling at a site in the Muskegon River, Michigan, USA, in August 2020. Species were measured to the nearest millimeter by using calipers.

Species	No. of Individuals	Minimum Length (mm)	Mean Length (mm)	Median Length (mm)	Maximum Length (mm)
Actinonaias ligamentina	12	57	89.3	89	107
Cambarunio iris	1	41	41	41	41
Eurynia dilatata	17	43	63.4	63	81
Fusconaia flava	1	56	56	56	56
Lampsilis cardium	2	25	45	45	65
Lampsilis siliquoidea	4	42	57	55.5	75
Lasmigona costata	1	75	75	75	75
Ligumia recta	1	114	114	114	114

Appendix 3. Spearman rank correlations of quantitative habitat variables with fish, crayfish, or mussel abundance observed in transects in the Muskegon River, Michigan, USA, in August 2020. The α value for tests for crayfish was corrected to 0.01, with Bonferroni correction due to multiple comparisons.

Taxa	Habitat Variable	S	*r*	*p*
Fish	Depth	434.84	−0.52	0.08
Crayfish	Sand	342.69	−0.20	0.54
	Pebble	225.36	0.21	0.51
	Cobble	271	0.05	0.87
	Boulder	316.59	−0.11	0.74
Mussels	Depth	280.91	0.018	0.96

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REGULAR ARTICLE

AGE, ASYMPTOTIC SIZE, AND GROWTH CONSTANTS OF EAST TEXAS FRESHWATER UNIONID MUSSELS

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ABSTRACT

Age and growth information helps researchers better understand how freshwater mussels (Bivalvia: Unionida)—among the most imperiled aquatic fauna worldwide—cope with environmental change. Shell thin sectioning is the primary method for estimating age and growth. Using a low-speed saw, a thin, radial cross-section of shell is taken and then mounted on an unfrosted microscope slide and read using a dissecting microscope. Thin sectioning can be time intensive, and species-specific issues, such as crowded annuli, can complicate efforts to provide accurate estimates. To date, only 69 of the approximately 300 North American species have age and growth information. Texas illustrates this issue perfectly; population-specific growth estimates are available for only 6 of the 52 species known to occur in the state. For the remaining species, information is either unavailable or inferred from closely related congeners or populations outside the state. This is problematic because incorrect inferences about age and growth can lead to erroneous assumptions about a species' life history, which could result in management and conservation actions that, at best, waste resources, and at worst, lead to population declines. We thin-sectioned eight different mussel species, including three species of conservation concern, then estimated growth parameters using von Bertalanffy growth curves. Our work more than doubled the number of Texas species with age and growth information from populations within the state. We found that growth serves as a good proxy for species position along a continuum contrasting higher growth and shorter lifespans versus lower growth and longer lifespans. Our results should be useful for making inferences about how species respond to environmental change.

KEY WORDS: Age, growth, Texas, freshwater mussel, von Bertalanffy, life history, unionid

INTRODUCTION

Freshwater mussels (Bivalvia: Unionida) are among the most imperiled aquatic fauna worldwide (Lydeard et al. 2004; Strayer et al. 2004; Lopes-Lima et al. 2018; Ferreira-Rodríguez et al. 2019; Böhm et al. 2021). In the USA, where they reach their greatest diversity, an estimated 65% of the approximately 300 described species have some conservation designation (Williams et al. 1993, 2008; Lydeard et al. 2004; Strayer et al. 2004; Haag and Williams 2014). Efforts to mitigate the decline of freshwater mussels are complicated by significant information gaps, such as species life-history strategies (Haag 2012). Life-history strategies provide a framework for classifying and comparing species on the basis of features that represent an optimization of trade-offs between growth, survival, and reproduction to maximize fitness to specific environments (MacArthur and Wilson 1967; Pianka 1970; Stearns 1992; Winemiller 2005; Kozlowski 2006; Albaladejo-Robles et al. 2023). Of these, growth has been shown to be an indicator of the position or "speed" of a species along the *r/K* continuum (Haag and Rypel 2011), which

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contrasts species on the basis of their investment in offspring and different population-regulation mechanisms (MacArthur and Wilson 1967; Pianka 1970; Stearns 1992; Winemiller 2005; Kozlowski 2006; Albaladejo-Robles et al. 2023).

According to *r*/*K* selection theory, fast-growing or *r*-selected species are adapted to density-independent influences, such as disturbance events, and they possess a suite of attributes (i.e., short lifespans, small bodies, and high fecundity) that allows them to recover quickly (MacArthur and Wilson 1967; Pianka 1970; Stearns 1992; Haag 2012; Albaladejo-Robles et al. 2023). In contrast, slow-growing or *K*-selected species are adapted to density-dependent influences, such as competition and predation, and are unable to recover quickly from disturbance events (MacArthur and Wilson 1967; Pianka 1970; Stearns 1992; Winemiller 2005; Haag 2012; Albaladejo-Robles et al. 2023). For this reason, slow-growing species are usually considered more sensitive to environmental changes, whereas fast-growing species are considered more tolerant (Albaladejo-Robles et al. 2023). *r/K* selection theory is the basis for more complex life-history models (e.g., competitive–stress tolerant–ruderal [C-S-R] or equilibrium–periodic–opportunistic [E-P-O]; Grime 1977, 1979; Southwood 1977; Winemiller and Rose 1992; Haag 2012), but these models require additional life-history information, which is often unavailable for rare or understudied species. In Texas, life-history data for many species remain incomplete, presenting challenges in applying more complex life-history models. Critical parameters, such as age at maturity and reproductive effort, are frequently unknown for numerous taxa, limiting the ability to accurately categorize species within frameworks like the C-S-R or the E-P-O models. Thus, *r/K* selection theory provides a straightforward and useful framework for evaluating how species may cope with environmental change and for assessing extinction risk and sensitivity to management and conservation actions at the population level, especially when only age and growth are known (Hastie et al. 2000; Haag 2012; Albaladejo-Robles et al. 2023).

Freshwater mussels deposit annual growth rings, hereafter annuli, in their shells, which can be used to estimate age, growth, and longevity (Neves and Moyer 1988; Haag and Commens-Carson 2008). Thin sectioning is the primary method for identifying annuli. Using a low-speed saw, a thin, radial cross-section of shell is taken and then mounted on an unfrosted microscope slide. Independent observers then use a dissecting microscope to count the annuli to determine age and measure the distance between annuli to estimate annual growth (Neves and Moyer 1988; Haag and Commens-Carson 2008; Haag and Rypel 2011). This method can be time intensive and species-specific issues, such as crowded annuli, can complicate efforts to provide accurate estimates. To date, only 69 of approximately 300 North American species have age and growth information available, and much of what is known is drawn from a limited number of species from a narrow geographic area (Haag and Rypel 2011; Moore et al. 2021; Hopper et al. 2023). Texas is a perfect illustration of the lack of age and growth data. Currently, information is available for only 6 (*Amblema plicata*, *Fusconaia mitchelli*, *Lampsilis bracteata*, *Pleurobema riddellii*, *Pustulosa necki*, and *Pustulosa petrina*) of the 52 species known to occur in the state (Dudding et al. 2020; Ford et al. 2020; DuBose et al. 2022; de Moulpied et al. 2024). For the remaining species, age and growth information is either unavailable or is inferred from populations outside of the state or from closely related congeners (Randklev et al. 2023), neither of which may provide accurate data. Growth can vary across populations of the same species within the same river system (Sansom et al. 2016), between river systems (Haag and Rypel 2011), and latitudinally (DuBose et al. 2022). For example, Sansom et al. (2016) suggest that variations in discharge patterns may drive growth differences within river systems. Haag and Rypel (2011) highlight the role of water chemistry in influencing shell growth, whereas Dubose et al. (2022) found that water temperature plays a significant role in determining growth rates. These findings emphasize the need for population-specific data when applying life-history models, as broad generalizations on the basis of related species or geographically distant populations could lead to misclassifications.

We aimed to address the lack of life-history information for mussels by quantifying observable age, asymptotic size, and the Brody growth constant *K*. Our specific objectives were to (1) estimate age and *K* using thin sectioning for eight species, including several species of conservation concern; (2) compile age and growth information for other species in Texas using literature reports; and (3) describe patterns of longevity across phylogenetic groups and across and within species.

METHODS

Study Sites

We collected freshwater mussels at four sites across Texas in the Brazos River, the Sabine River, Village Creek, and the Lower Neches Valley Authority (LNVA) Canal within the Neches River basin (Fig. 1). The Brazos River is formed at the confluence of the Salt and Double Mountain forks in Stonewall County. The basin drains 117,870 km^2 and flows 1,509 km through Texas before entering the Gulf of Mexico (Brazos River Authority 2024). Near the collection site, the climate is subtropical humid (Texas Water Development Board 2012), with an air temperature range of 4.4–34.4°C and predominately agricultural land use (Griffith et al. 2007). The river near the collection site is slow flowing and generally silty with occasional visible bedrock.

The Sabine River drains a total area of 25,267 km^2 from its origin in Hunt County, Texas to the Gulf of Mexico (Texas Commision on Environmental Quality[TCEQ] 2002). The basin is heavily forested and is largely used for pasture, timber production, and livestock and poultry production (Griffith et al. 2007). The climate is predominately humid and

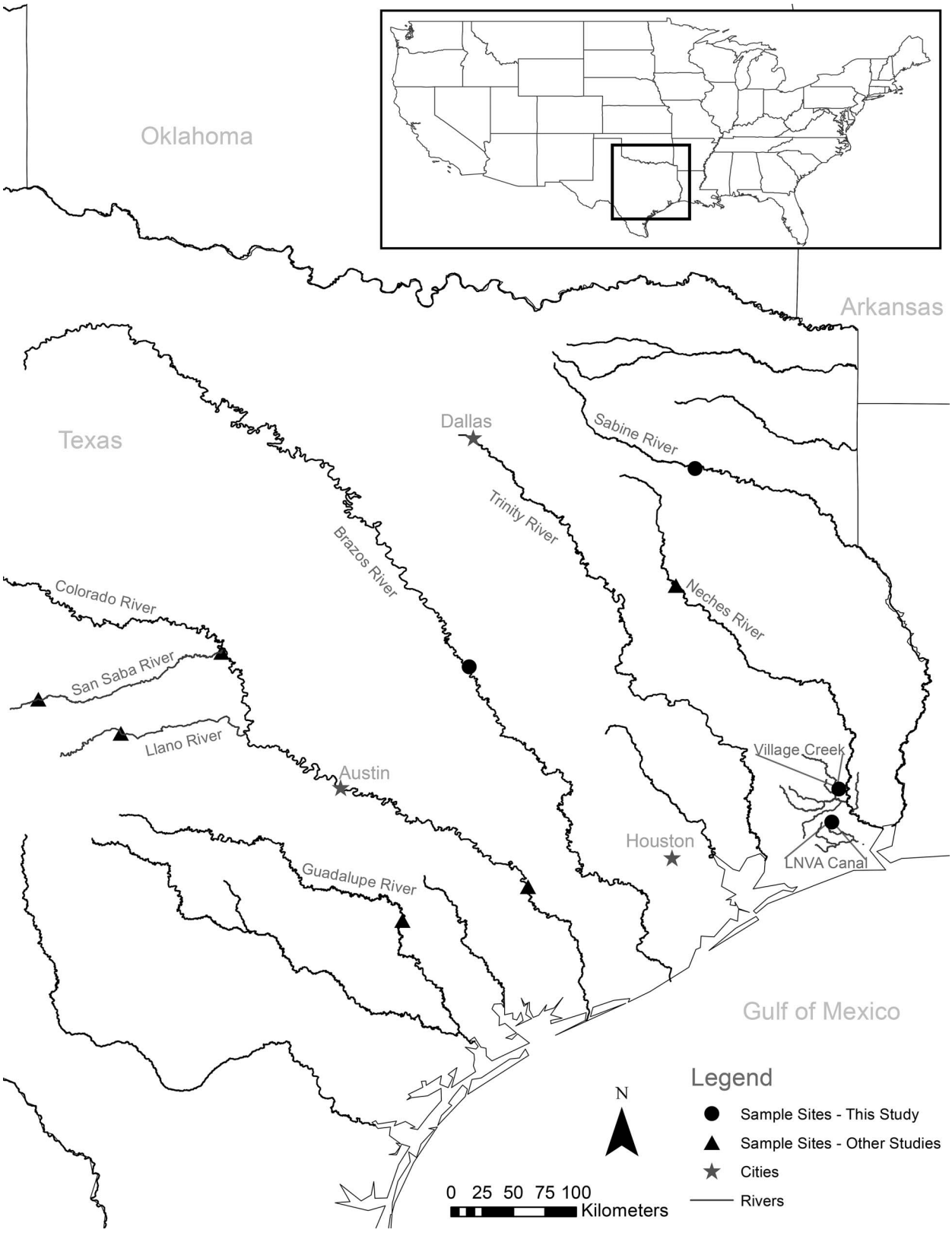

Figure 1. Collection sites for original data for this study and other studies where age and growth have been analyzed in Texas.

subtropical with an average annual rainfall of 1,016 mm (Sabine River Authority 2021) and an average annual air temperature range of 1.1–34.4°C near the collection site (Griffith et al. 2007). The river near the site can be flashy and the streambed is composed of cobble and gravel.

The Neches River originates near Colfax, Texas and flows for 669 km into Sabine Lake, an estuary of the Gulf of Mexico. Its watershed in southeast Texas drains 26,676 km^2 (Harrel and Hall 1991; Lower Neches Valley Authority 2024). We collected at two sites within the Neches River watershed, the LNVA canal and Village Creek; the climate for both is subtropical humid (Texas Water Development Board 2012). The LNVA Canal is in a predominately agricultural area and is used to supply water for local industry; it has a very fine silt substrate (Harrel and Hall 1991; Griffith et al. 2007). Rainfall in the area ranges from 939.8 to 1,447.8 mm annually, and air temperature ranges from 5.6 to 33.3°C. Village Creek is unregulated and is a major tributary of the lower Neches River (Khan et al. 2019). Mean annual rainfall for Village Creek is 193.8–1,473.2 mm and air temperature ranges from 4.4 to 33.3°C (Griffith et al. 2007). Near the sampling site, the river is generally slow flowing with a sandy substrate (Texas Parks and Wildlife Department [TPWD] 2016).

Study Species

The species analyzed in this study were *Pustulosa nodulata*, *Pustulosa pustulosa*, and *Quadrula quadrula* within the Quadrulini tribe, *Fusconaia askewi* and *Pleurobema riddellii* within the Pleurobemini tribe, and *Lampsilis teres*, *Truncilla donaciformis*, and *Truncilla macrodon* within the Lampsilini tribe. Of these species, *F. askewi*, *Pl. riddellii*, and *T. macrodon* are considered state threatened (TPWD 2010, 2020); at the federal level, *Pl. riddellii* has been proposed for listing, and *T. macrodon* is currently listed, as threatened under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service [USFWS] 2023, 2024). At each site, we surveyed a search area not exceeding ~150 m^2 both tactilely and visually for a total of 4 person-hours. We identified all live mussels to species and retained a subset of our focal species for thin sectioning. An effort was made to include a broad range of sizes to ensure a comprehensive representation of the age classes present in the population. We collected a total of 16 *Pl. riddellii*, 15 *Pu. nodulata*, 15 *Pu. pustulosa*, 10 *Q. quadrula*, and 15 *T. donaciformis* from the LNVA Canal and 16 *F. askewi* from Village Creek. We also collected 16 *Pu. pustulosa* and 10 *L. teres* from the Sabine River and 22 *T. macrodon* from the Brazos River in Central Texas.

Preparation and Interpretation of Shell Thin Sections

We prepared individuals for analysis by creating thin sections following Haag and Commens-Carson (2008). Specifically, shells were cleaned and dried, then one uncut valve was set in epoxy resin (craft resin creative liquid) on popsicle sticks. Once the resin was fully cured, we cut radial thin sections along the dorsal–ventral axis with a Buehler IsoMet 1000 precision cutter low-speed saw equipped with a diamond wafering blade (12.7 mm). Thin sections were mounted on glass microscope slides with Crystalbond 509 clear mounting adhesive (ThermoFisher Scientific) and sanded with progressively finer grits of wet-sanding blocks until annuli were clearly visible under light microscopy with an Olympus stereo microscope. We identified annuli for each thin section following Haag and Commens-Carson (2008). Specifically, a putative annulus must extend from the umbo through the periostracum without interruption and terminate through the prismatic layer. Juveniles were not available at our sample locations and so we back-calculated age and length for younger age classes. This was accomplished by aligning annuli that had been marked on the thin section with the raw edge of the corresponding cut valve to identify annuli on the outside of the shell. Once identified, these external annuli were then matched to the corresponding annuli on the uncut valve. Maximum length (i.e., the longest antero-posterior distance) of each external annulus on the uncut valve was then measured to estimate size at age for back-calculated ages. We also took the maximum length of the uncut valve and the observed age from the thin section and used this as the true length at age of the individual. Thus, we had a back-calculated length at age for each visible external annulus and a true length at age for when the individual was collected. We validated age estimates by using two independent readers. We reconciled any discrepancies by repeating the reading process until a consensus was reached or we removed the thin section from the data set. Outliers, that is, extreme ages relative to shell length, were identified by fitting growth curves (see below for details) with prediction intervals. Age estimates outside of the prediction intervals were reread and either included if the new estimate fell within the prediction interval or omitted if age–length discrepancies could not be reconciled. For the latter, if an unreconciled estimate was a back-calculated measurement, only that estimate was removed. If the unreconciled estimate was a true measurement, then the whole individual, including corresponding back-calculated estimates, was removed from the data set.

Growth and Longevity Estimates

To characterize growth and longevity for our focal species we fit the von Bertalanffy growth function (Ricker 1975) to length-at-age data

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

where L_t is the mean length at a given age (t in years); L_∞ is the asymptotic length, which represents the maximum mean length (mm) for a population; K is the Brody growth constant (yr^{-1}), which describes how quickly length approaches L_∞, and t_0 is the theoretical time in which $L = 0$ (Buckmeier et al. 2017). We also generated pseudo-R^2 values to gauge the fit of the data to the growth function. von Bertalanffy growth curves were fit to each population using the FSA (version 0.9.1; Ogle et al. 2022) and ntools (version 1.0.2; Baty et al.

Table 1. Population growth parameters for all species analyzed in this study derived from fitted von Bertalanffy growth curves. N = sample size; water body = sample collection site; pseudo-R^2 = coefficient of determination; L_∞ = predicted mean maximal length for the population; K = Brody growth constant; t_0 = theoretical time at which $L = 0$; and max age = maximum observed age. SE and 95% confidence intervals (CIs) are provided for each parameter estimate. LNVA = Lower Neches Valley Authority.

Species	Water Body	N	Pseudo-R^2	L_∞	L_∞ SE	CI Lower	CI Upper	K	K SE	CI Lower	CI Upper	t_0	t_0 SE	CI Lower	CI Upper	Max Age
Fusconaia askewi	Village Creek	9	0.96	56.81	1.50	54.36	59.95	0.19	0.02	0.14	0.24	−0.74	0.37	−1.64	−0.18	21
Lampsilis teres	Sabine River	9	0.97	139.78	2.82	134.68	145.74	0.34	0.03	0.28	0.39	0.39	0.13	0.12	0.62	13
Pleurobema riddellii	LNVA Canal	14	0.97	59.52	2.15	56.14	64.47	0.16	0.02	0.12	0.20	−1.26	0.32	−1.96	−0.74	18
Pustulosa nodulata	LNVA Canal	15	0.96	48.60	1.52	45.98	51.98	0.21	0.02	0.17	0.25	−0.43	0.16	−0.80	−0.15	15
Pustulosa pustulosa	Sabine River	15	0.95	65.91	2.40	61.72	71.22	0.13	0.02	0.10	0.16	−1.41	0.39	−2.31	−0.76	26
Pustulosa pustulosa	LNVA Canal	15	0.95	56.85	3.07	51.96	64.04	0.13	0.02	0.09	0.17	−1.42	0.41	−2.28	−0.75	20
Quadrula quadrula	LNVA Canal	10	0.96	53.02	2.10	49.66	58.20	0.16	0.02	0.12	0.21	−1.02	0.41	−1.94	−0.36	16
Truncilla donaciformis	LNVA Canal	10	0.95	41.19	2.16	38.17	46.72	0.25	0.04	0.17	0.33	−0.26	0.31	−0.96	0.18	10
Truncilla macrodon	Brazos River	22	0.96	57.82	3.63	52.73	66.84	0.20	0.03	0.14	0.26	−0.41	0.21	−0.91	−0.07	10

2015) packages in R (version 4.2.1; R Core Team 2022). To evaluate variation within populations of the same species, we compared growth parameters between the two populations of *Pu. pustulosa* for which we generated original data in this study. This was done by visually comparing confidence intervals (CIs) on K, L_∞, and t_0 values and concluding that nonoverlapping CIs are statistically significant.

Literature Review of Age and Growth

Using peer-reviewed literature reports, we compiled estimates of the von Bertalanffy growth parameters L_∞, K, and t_0 for species that occur within Texas (Hanson et al. 1988; Stoeckel et al. 1996; Morris and Corkum 1999; Christian et al. 2000; Anthony et al. 2001; Haag and Rypel 2011; Daniel and Brown 2014; Sansom et al. 2016; Dudding et al. 2020; Ford et al. 2020; DuBose et al. 2022; Hopper et al. 2023; de Moulpied et al. 2024). We included original data from this study plus reports for other species that are endemic to Texas or range within the state. For the latter, age and growth information is taken from populations that occur outside of Texas.

RESULTS

Age and Growth Variation among Species

We found K and L_∞ to vary across the eight species of Texas freshwater mussels we evaluated (Table 1). *Pustulosa pustulosa* from the Sabine River had the lowest growth constant with a K value of 0.13 (95% CI 0.10, 0.14) and *L. teres* from the Sabine River had the highest growth constant with a K value of 0.34 (95% CI 0.28, 0.39). Maximum observed age also varied with *Pu. pustulosa* from the Sabine River, being the longest-lived species at 26 yr and *T. macrodon* from the Brazos River being the shortest-lived species at 10 yr. Comparing growth parameters among major phylogenetic groups shows the Lampsilini tribe with the highest values for K (mean and range 0.26, 0.20–0.34 yr^{-1}) and both the largest (*L. teres*, 139.78 mm) and smallest (*T. donaciformis*, 41.19 mm) values for L_∞ in the study. The Quadrulini tribe had the lowest values for K (0.16, 0.13–0.21 yr^{-1}) and L_∞ ranged from 48.60 mm (*Pu. nodulata*) to 65.90 mm (*Pu. pustulosa*, Sabine River population). The Pleurobemini tribe, which included *Pl. riddellii* and *F. askewi*, had intermediate K values (0.17, 0.16–0.19 yr^{-1}) and L_∞ estimates of 59.52 mm and 56.81 mm, respectively.

Variation Among Populations

We compared two populations of *Pu. pustulosa* from the Sabine River in East Texas and the LNVA Canal in Southeast Texas. *Pustulosa pustulosa* from the Sabine River and the LNVA Canal had similar K values (i.e., 0.13), indicating slow growth for both subpopulations. However, L_∞ was higher for the subpopulation in the Sabine River (i.e., 65.91 mm) compared with the LNVA Canal subpopulations (i.e., 56.85 mm). The CIs for these estimates overlap (Table 1), which indicates the estimates are not significantly different. Maximum observed age for the Sabine River population was 26 yr, whereas the maximum observed age for the LNVA Canal population was 20 yr.

Literature Review of Growth and Longevity

We compiled a data set on freshwater mussel age and growth for 29 species from 70 populations (Table 2). The species included in this review represent the following genera: *Amblema*, *Fusconaia, Glebula*, *Lampsilis*, *Lasmigona*, *Leaunio*, *Megalonaias*, *Obliquaria*, *Plectomerus*, *Pleurobema*, *Potamilus*, *Pustulosa*, *Pyganodon*, *Quadrula*, *Toxolasma*, and *Truncilla* within the tribes Amblemini, Lampsilini, Pleurobemini, and Quadrulini, and represent a little over half of the species known to occur within Texas. Within this review we found that K ranges from 0.04 yr^{-1} (*Fusconaia flava*, Mountain Fork River, Arkansas; Sansom et al. 2016) and 0.04 (*Megalonaias nervosa*, St. Francis River, Arkansas; Christian et al. 2000) to 1.01 yr^{-1} (*Toxolasma parvum*, Davis Lake, Mississippi; Haag and Rypel 2011) and L_∞ ranges from

Table 2. Summary of (median or mean) growth parameter information for species of freshwater mussel known to occur in Texas. L_∞ = predicted mean maximal length for the population; K = Brody growth constant; t_0 = theoretical time at which $L = 0$; A_{max} = maximum observed age; L_{max} = measured maximum length; and N = number of individuals.

Species	Site	K	L_∞	t_0	A_{max}	L_{max}	N	Source
Amblema plicata	Little Tallahatchie River, MS	0.21	109	0.75	18	101.3	37	Haag and Rypel 2011
A. plicata	Sipsey River, AL	0.07	109.2	−0.34	54	114.7	11	Haag and Rypel 2011
A. plicata	White River, AR	0.09	138	0.83	25		22	Christian et al. 2000
A. plicata	Ouachita River, AR	0.13	87	−0.34	25		50	Christian et al. 2000
A. plicata	Guadalupe River, TX	0.12	92.4	−2.29	13		6	DuBose et al. 2022
A. plicata	Colorado River, TX	0.18	99.9	−0.97	23		9	DuBose et al. 2022
A. plicata	Amite River, LA	0.11			39	122.3	77	Daniel and Brown 2014
Fusconaia askewi	Village Creek, TX	0.19	56.81	−0.74	21		9	This study
Fusconaia flava	Little River, AR	0.06	92.11		29		3	Sansom et al. 2016
F. flava	Mountain Fork River, AR	0.04	89.16		64		3	Sansom et al. 2016
F. flava	Mulberry River, AR	0.26	74.9	−1.12			25	Stoeckel et al. 1996
Fusconaia mitchelli	Guadalupe River, TX	0.23	56.43	−0.12	15		54	Dudding et al. 2020
Glebula rotundata	Amite River, LA	0.05			34	164.5	69	Daniel and Brown 2014
Lampsilis bracteata	Llano River, TX	0.19	61.4	−0.19	13		24	de Moulpied et al. 2024
L. bracteata	San Saba River, TX	0.21	61.52	−0.13	12		24	de Moulpied et al. 2024
Lampsilis cardium	Iroquois River, IL	0.21	131.6	−1.72	10		8	DuBose et al. 2022
L. cardium	Mississippi River, MN	0.65	104.8	−1.64	10		1	DuBose et al. 2022
L. cardium	Mississippi River, MN	0.44	106.2	−1	13		3	DuBose et al. 2022
Lampsilis hydiana	Mulberry River, AR	0.47	83	0.64			70	Stoeckel et al. 1996
L. teres	Sipsey River, AL	0.41	108.2	0.34			7	Haag and Rypel 2011
Lampsilis teres	Sabine River, TX	0.34	139.78	0.39	13		9	This study
L. teres	St. Francis River, AR	0.57	132.8	0.61			22	Haag and Rypel 2011
L. teres	Amite River, LA	0.32			14	121.7	66	Daniel and Brown 2014
Lasmigona complanata	Ontario, Canada; grassy		136.59				40	Morris and Corkum 1999
L. complanata	Ontario, Canada; forested		153.61				45	Morris and Corkum 1999
Leaunio lienosus	Amite River, LA	0.21			20	77.0	89	Daniel and Brown 2014
L. lienosus	Kettle Creek, MS	0.40	70.1	0.18	11	72.4	6	Haag and Rypel 2011
L. lienosus	Sipsey River, AL	0.78	49.8	0.50	5	54.3	8	Haag and Rypel 2011
Megalonaias nervosa	Sipsey River, AL	0.09	165.8	−1.50	38	168.3	3	Haag and Rypel 2011
M. nervosa	St. Francis River, AR	0.04	217.8	−14.33	41		48	Christian et al. 2000
M. nervosa	Cache River, AR	0.08	239.2	−1.07	43		38	Christian et al. 2000
Obliquaria reflexa	Licking River, KY	0.37	55.1	0.55	15	60.9	15	Haag and Rypel 2011
O. reflexa (female)	Sipsey River, AL	0.16	50.7	−0.72	23	54	12	Haag and Rypel 2011
O. reflexa (male)	Sipsey River, AL	0.25	50.7	0.23	21	53	17	Haag and Rypel 2011
O. reflexa	Amite River, LA	0.23			19	59.6	89	Daniel and Brown 2014
Plectomerus dombeyanus	Amite River, LA	0.27					99	Daniel and Brown 2014
P. dombeyanus	Leaf River, MS	0.26	125	0.46	17	134	10	Haag and Rypel 2011
P. dombeyanus	Pearl River, MS	0.15	136.9	−1.88	38	144.5	8	Haag and Rypel 2011
Pleurobema riddellii	Neches River, TX	0.11	69.9			60.9	55	Ford et al. 2020
P. riddellii	LVNA Canal, TX	0.16	59.53	−1.26	18		14	This study
Potamilus fragilis	Licking River, KY	0.60	98.9	0.40	7	119.3	10	Haag and Rypel 2011
P. fragilis	St. Francis River, AR	0.72	141	0.19	4	137	115	Haag and Rypel 2011

Table 2, continued.

Species	Site	K	L_∞	t_0	A_{max}	L_{max}	N	Source
Potamilus purpuratus (male)	St. Francis River, AR	0.81	148.1	1.16	10	149.3	4	Haag and Rypel 2011
P. purpuratus (female)	St. Francis River, AR	0.49	120	0.25	9	129.4	15	Haag and Rypel 2011
P. purpuratus	Amite River, LA	0.08			32	204.3	76	Daniel and Brown 2014
Pustulosa necki	Guadalupe River, TX	0.14	55.49	−1.64	13		54	Dudding et al. 2020
Pustulosa nodulata	LNVA Canal, TX	0.21	48.60	−0.43	15		15	This study
Pustulosa petrina	Colorado River, TX	0.07	94.43	−2.18	22		16	de Moulpied et al. 2024
P. petrina	Llano River, TX	0.09	76.44	−1.5	17		19	de Moulpied et al. 2024
P. petrina	San Saba River, TX	0.10	55.03	−1.98	29		16	de Moulpied et al. 2024
Pustulosa pustulosa	Sabine River, TX	0.13	65.91	−1.41	26		15	This study
P. pustulosa	LVNA Canal, TX	0.13	56.85	−1.42	20		15	This study
P. pustulosa	Licking River, KY	0.14	86.2	0.37	39	88.2	17	Haag and Rypel 2011
P. pustulosa	Little Tallahatchie River, MS	0.08	72.4	−2.88	48	79.8	174	Haag and Rypel 2011
Pyganodon grandis	Kettle Creek, MS	0.31	123.2	0.03	9	127.5	9	Haag and Rypel 2011
P. grandis	Pearl River, MS	0.66	129	0.62	11	134.4	9	Haag and Rypel 2011
P. grandis	Wabana Lake, MN	0.03	112.0				55	Anthony et al. 2001
P. grandis	Narrow Lake, AB	0.26	74.2	0.94	12	75	618	Hanson et al. 1988
P. grandis	Ontario, Canada		135.13				200	Morris and Corkum 1999
Quadrula quadrula	LNVA Canal, TX	0.16	53.02	−1.02	16		10	This study
Q. quadrula	Ozark Lake, AR	0.10	120	−0.03	17		49	Christian et al. 2000
Q. quadrula	Lake Dardenelle, AR	0.90	99.4	−0.88	24		49	Christian et al. 2000
Quadrula verrucosa (male)	Sipsey River, AL	0.15	104.1	0.34	37	107.4	19	Haag and Rypel 2011
Q. verrucosa (female)	Sipsey River, AL	0.13	127.8	1.14	21	117.1	7	Haag and Rypel 2011
Q. verrucosa	Little River, AR	0.11	125.44		15		3	Sansom et al. 2016
Q. verrucosa	Little River, AR	0.10	111.67		34		4	Sansom et al. 2016
Toxolasma parvum	Davis Lake, MS	1.01	24	0.02	5	26.7	15	Haag and Rypel 2011
Toxolasma texasiense (male)	Kettle Creek, MS	0.56	42.3	0.18	8	43.7	5	Haag and Rypel 2011
T. texasiense (female)	Kettle Creek, MS	0.29	35.7	−0.56	11	35.8	2	Haag and Rypel 2011
Truncilla donaciformis	Licking River, KY	0.46	40.3	0.29	8	46.8	14	Haag and Rypel 2011
T. donaciformis	LNVA Canal, TX	0.25	41.19	−0.26	10		10	This study
Truncilla macrodon	Brazos River, TX	0.20	57.82	−0.41	10		22	This study
Truncilla truncata	Licking River, KY	0.24	65.8	0.05	10	65.3	14	Haag and Rypel 2011
T. truncata	St. Croix River, MN	0.16	50.2	−0.53	18	50.6	3	Haag and Rypel 2011

24 mm (*To. parvum*, Davis Lake, Mississippi; Haag and Rypel 2011) to 239.20 mm (*M. nervosa*, Cache River, Arkansas; Christian et al. 2000).

All tribes represented in our review showed considerable variation in longevity. Maximum observed age for Quadrulini ranged from 13 yr (*Pu. necki*, Guadalupe River, Texas; Dudding et al. 2020) to 48 yr (*Pu. pustulosa*, Little Tallahatchie River, Mississippi; Haag and Rypel 2011). Pleurobemini age had the most variability and ranged from 15 yr (*F. mitchellii*, Guadalupe River, Texas; Dudding et al. 2020) to 64 yr (*F. flava*, Mountain Fork, Arkansas; Sansom et al. 2016). The only Amblemini species reviewed was *A. plicata*, which had a reported observed age range of 13 yr (Guadalupe River, Texas; DuBose et al. 2022) to 54 yr (Sipsey River, Alabama; Haag and Rypel 2011). Maximum observed age for the Lampsilini tribe ranged from 5 yr (*Leaunio lienosus*, Sipsey River, Alabama and *To. parvum*, Davis Lake, Mississippi; Haag and Rypel 2011) to 23 yr (*Obliquaria reflexa*, Licking River, Kentucky; Haag and Rypel 2011).

The Brody growth constant, K, also showed considerable variation between and within the four tribes represented by this data set. Pleurobemini had the smallest range and the lowest K values of the four tribes, ranging from 0.04 yr^{-1} (*F. flava*) to 0.11 yr^{-1} (*Pl. riddellii*). It is worth noting that *F. flava* has also been reported to have a much higher K value of 0.26 yr^{-1} (Stoeckel et al. 1996). Next fastest was Quadrulini,

which ranged from 0.04 yr^{-1} (*M. nervosa*) to 0.90 yr^{-1} (*Q. quadrula*). Only one Amblemini species was reviewed as part of this study, *A. plicata*, with a *K* value range of 0.07 yr^{-1} to 0.21 yr^{-1}. Lampsilini had both the highest growth constant and widest range, from 0.05 yr^{-1} (*Glebula rotundata*) to 1.01 yr^{-1} (*To. parvum*).

DISCUSSION

In this study we provided growth parameters for eight mussel species across four rivers in Texas. Of the species we evaluated, three previously lacked information on age and growth and four had estimates for populations outside of the state. Further, three of the species are of conservation concern (*F. askewi*, *Pl. riddellii*, and *Tr. macrodon*). Brody growth constants were highly variable, showing a gradient of growth rates and lifespans that generally can be correlated to fast and slow end points of the *r/K* continuum for unionids. Last, we compiled age and growth information for 29 species using original data and literature reports.

We found that age and growth varied across species and observed that species with smaller growth constants had higher maximum observed ages. This corroborates findings from previous studies (i.e., Haag and Rypel 2011) and suggests that growth constants serve as a good proxy for position along the *r/K* continuum. For example, in this study *Pu. pustulosa* had the lowest growth constant, 0.13 yr^{-1}, and was the longest-lived (20 to 26 yr) species we examined on the basis of maximum observed age. In contrast, *La. teres* had the highest growth, 0.34 yr^{-1}, more than double that of *Pu. pustulosa,* and was among the shortest-lived (13 yr) of the species we examined. Taken together, this would indicate that *Pu. pustulosa* is likely a slow or *K*-selected species, and should have higher fitness in stable, productive habitats, and *La. teres* is likely a fast or *r*-selected species and is adapted to rapid colonization and persistence in disturbed and unstable habitats (Haag 2012). Similarly, *Tr. donaciformis* is also likely an *r*-selected species with high growth (0.25 yr^{-1}) and short lifespan (10 yr; Haag and Rypel 2011; Haag 2012). For *Q. quadrula*, *Pl. riddellii*, and *F. askewi*, growth constants suggest they are likely positioned near the *K* end point, whereas *Pu. nodulata* and *Tr. macrodon* likely fall near the *r* end point.

We found that maximum observed age and the growth constant did not vary among populations for *Pu. pustulosa*, which is not unexpected given the geographic proximity and similarity in climate between sample locations. Nearly identical growth parameters were found for *La. bracteata* (Texas fatmucket) from the Llano and San Saba rivers of the Colorado River basin, which are close together (de Moulpied et al. 2024). In a review of age and growth for mussels in North America, similar growth estimates were noted between populations in the same river system for several species (Haag and Rypel 2011). Both studies also found instances where *K* varied among populations of the same species. For example, *K* varied across three populations of *Pustulosa petrina* (Texas pimpleback) in three different river systems in the Colorado River basin (de Moulpied et al. 2024). The authors hypothesized that the variation in *K* could be the result of differences in extreme flooding between sample locations. Similarly, *Quadrula asperata*, now *Pustulosa kieneriana*, had differences in *K* for different populations, suggesting the differences represent alternative fitness-maximizing life-history strategies (Haag and Rypel 2011). Taken together, these findings indicate that growth parameters are not always portable across populations even if they are near one another, and they also suggest that differences may arise because of local disturbances or polymorphisms that represent divergent life histories (Haag and Rypel 2011; Sansom et al. 2016).

Our estimates of longevity and growth were different across phylogenetic groups, corresponding with differences in life history. Specifically, we found that, on average, species in the Lampsilini tribe had the highest *K* value, followed by Pleurobemini and then Quadrulini. Variation in longevity across the three tribes follows a similar pattern such that species in the Lampsilini tribe have shorter lifespans compared with species in Pleurobemini and Quadrulini (Haag and Rypel 2011). According to *r/K* theory, species with fast growth and short lifespans are expected to occur in more variable environments compared with species with slower growth that are long lived (Pianka 1970; Haag 2012). These relationships suggest that age and growth are broadly constrained by phylogeny (Haag and Rypel 2011), which is not unexpected given that other traits also show the same patterns. For example, Quadrulini species generally reach maturity later, have lower fecundity, and use a broadcasting strategy to infect host fish with their larvae (Haag 2012; Neemuchwala et al. 2023), whereas Lampsilini species generally mature young, have higher fecundity, and use lures to attract a specific fish (Barnhart et al. 2008; Haag 2012).

Although we successfully estimated growth parameters for eight species in East Texas, 41 species have yet to be evaluated within the state. The results of this study show that growth parameters may be transferable within species, but phenotypic plasticity does exist. Although this variation could complicate efforts to predict how species may respond to environmental change or specific management actions, a general understanding of whether a species is *r*- or *K*-selected can still guide managers in their decision-making. That said, more information is needed on age and growth within and across species—not just in Texas but for freshwater mussels everywhere. Research efforts should continue focusing on generating age and growth information but also validate those estimates using mark–recapture and statistical methods such as cross-dating (Rypel et al. 2008). These methods allow for the formation of long-term growth chronologies that can be used to validate new age estimates. Also, more information is needed on the relationships between growth and reproduction, which would facilitate the application of more complex life-history models. Future studies should focus on characterizing fecundity and age of maturation in conjunction with estimating age and growth. The former can be obtained by collecting

gravid females and then enumerating the number of larvae (i.e., glochidia) following established protocols (e.g., Haag and Staton 2003).

Patterns of age and growth are fundamental to understanding species life history, which provides a framework for understanding how mussels may respond to environmental change or management and conservation actions (Hastie et al. 2000; Albaladejo-Robles et al. 2023). For example, species with high growth rates and reduced longevity are found more often in habitats that experience streambed disturbance during high-flow events compared with species with lower growth rates and longer lifespans. These associations have been used to develop a conceptual model (Randklev et al. 2019) to predict mussel assemblage structure on the basis of extreme flooding. The growth parameters we estimated for our eight focal species could be incorporated into this model to provide greater insight into how these species and others may respond to changes in the flow regime. This type of information is critical because future climate and land-use change in Texas may increase droughts and floods (Griffith et al. 2007; Jiang and Yang 2012; Nielsen-Gammon et al. 2020) and understanding how mussels respond to these events is critical for ensuring conservation strategies are tailored to the life history of target species.

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REGULAR ARTICLE

IMPACTS OF PROPAGATION ON POPULATION GENETICS OF THE THREERIDGE MUSSEL *AMBLEMA PLICATA* (SAY, 1817)

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ABSTRACT

Freshwater mussels provide many ecosystem services, but over the past century, they have become among the most imperiled taxa in the world. Often, efforts to restore mussel populations have included the propagation and release of juveniles. We utilized microsatellites to compare the genetic diversity of propagated mussels to the source population from which the broodstock was derived. Three wild-fertilized female threeridge mussels (*Amblema plicata*) sourced from the Cedar River watershed in Minnesota were used as broodstock. We then genetically characterized a sample from the source population, a subsample of the juvenile cohort directly after transformation (Juv-0Y), and another subsample of the juvenile cohort after 1 yr of being raised in the hatchery (Juv-1Y). After correcting for sample sizes, the Juv-0Y sample set contained the greatest allelic richness, followed by the source sample set and then the Juv-1Y sample set. All three sample sets exhibited alleles that were not shared with other samples sets, henceforth referred to as "private alleles." Private alleles in Juv-0Y and Juv-1Y indicated the dams (mothers) were likely fertilized by males living upstream of the source population, outside of the sampling effort of this study. High levels of multiple paternity were observed in the juveniles from both subsamples. In total, 89 juveniles were estimated to have been sired by 58 males, increasing the amount of genetic variability in the population. Analyses indicated the Juv-1Y samples were produced nearly entirely from a single dam, indicating that differential mortality in the hatchery reduced the amount of genetic variability in the released population. The Juv-1Y sample was significantly differentiated from the source, suggesting the juvenile population did not fully represent the source population. This study highlights the importance of genetic monitoring of mussels in hatchery environments to maximize the genetic diversity of the propagules that are released.

KEY WORDS: freshwater mussel, genetic, multiple paternity, selection, microsatellite

INTRODUCTION

Freshwater mussels (Bivalvia: Unionoida; hereafter referred to as "mussels") provide important ecological services in riverine habitat building and nutrient cycling (Spooner and Vaughn 2006). As freshwater ecosystems have been altered and degraded by human activity in past centuries, mussel populations have declined steeply (Strayer and Dudgeon 2010). Historical overharvesting continues to affect mussels today, with current populations greatly reduced and highly fragmented (Lopes-Lima et al. 2014).

Environmental stressors and low population densities can negatively impact mussel reproduction before they cause mortality in adults (Haag and Rypel 2011). Successful reproduction requires the presence of suitable fish hosts for the parasitic larval stage of the mussel life cycle. This parasitic stage is likely the primary driver of mussel dispersal, with species distributions tied to host-fish movement patterns (Schwalb et al. 2013). Therefore, the parasitic stage also facilitates connectivity of species metapopulations (Modesto et al. 2018). After metamorphosis from glochidium (larva) and detachment from the host, a juvenile mussel is still nearly microscopic in size and highly vulnerable to environmental stressors. Fluctuations in water quality, chemicals, poor physical conditions, and

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predators are much more likely to kill mussels in their first few years of life than in adulthood (Wang et al. 2007; Brim-Box and Mossa 2016).

Conservation managers have turned to propagation as a means of augmenting freshwater mussel populations and dispersing mussels throughout their historic ranges (Lopes-Lima et al. 2014). Most practitioners collect gravid female mussels from the wild to begin propagation, so this system relies on an already established breeding population (Hoftyzer et al. 2008). It also requires knowledge of the fish species utilized as hosts by each mussel species, while circumventing the potential problems of host-fish absence and environmental stressors preventing larval attachment (Modesto et al. 2018). In laboratory propagation, juveniles are raised in the hatchery during the most vulnerable period of life, often for at least a year (Carey et al. 2015). After this period, the older, larger juvenile mussels are released in the wild to mature and to contribute to breeding populations (in the case of augmentation) or to establish new breeding populations (in the case of reintroduction) (Hoftyzer et al. 2008).

Since the early 2000s, millions of produced mussels of dozens of species have been released throughout the United States (Jones et al. 2006). The release of propagated mussels could have unintended consequences for wild populations (Jones et al. 2006; Hoftyzer et al. 2008; McMurray and Roe 2017). Capturing a limited number of gravid females to propagate juveniles to serve as a new population may result in a population with lowered genetic variability. Moreover, as the new population reproduces, inbreeding depression is possible (O'Grady et al. 2006). A highly variable family size being used to source the new population may lead to a lowered effective population size, thus making inbreeding more likely or increasing the rate of genetic drift (Lande and Barrowclough 1987). The geographical location from which parent mussels are collected and the location of the juvenile release is important to consider as well. If mussels are propagated and then released to interbreed with a separate extant wild population, the dilution of locally adapted alleles could result in outbreeding depression, though the likelihood of this is low based on the recency (<500 yr ago) of most mussel population fragmentation and the relative similarity of habitats that populations of the species continue to inhabit (Neves 2004; Frankham et al. 2011). It is also possible that the hatchery can alter the gene pool of a produced population. Some juvenile mortality is expected within the hatchery; however, if hatchery mortality is correlated to specific genotypes in the hatchery, it could bias the genetic diversity of the propagated juveniles and potentially introduce maladapted genotypes into wild populations (Neves 2004).

We sought to document what, if any, differences arise between the source population from which gravid female mussels are drawn and the propagated juvenile mussels produced in a hatchery. Threeridge mussels *Amblema plicata* (Say, 1817) are a common, widespread species in the American Midwest (Elderkin et al. 2007), but they are listed as a Species of Greatest Conservation Need (SGCN) in the state of Iowa (Iowa Department of Natural Resources 2015). A previous study examining the genetic structure of threeridge mussel populations found low among-population structure compared to within-population structure, especially within a single river drainage, leading to the conclusion that it was appropriate to release mussels produced from within the same watershed (Elderkin et al. 2007).

The Minnesota Department of Natural Resources propagated threeridge mussels from a population from the upper Cedar River watershed in southern Minnesota. Our first objective was to determine if the propagated juvenile population was genetically representative of the source population, thereby decreasing chances for inbreeding effects. Our second objective was to determine if there was a significant reduction in juvenile genetic diversity from the start to the end of residency in the hatchery. Our results add to the growing body of information on the genetic impacts of propagation and aid propagation practitioners who are concerned with preserving the genetic diversity of their target species while enhancing opportunities for recovery.

METHODS

Sample Collection and Data Generation

We collected nondestructive samples from 50 threeridge mussels in the upper Cedar River near Lansing, Minnesota, using buccal swabs, which were then stored in ethanol (the "source" sample set). We found three of the mussels to be gravid, and these gravid females were used for propagation at the Center for Aquatic Mollusk Programs in Lake City, Minnesota. Hatchery staff infested individual walleye (*Sander vitreus*) host fish with the glochidia from a single dam, or mother. After transformation and dropping from their hosts, 20 juveniles from each dam were collected and preserved in ethanol for a total of 60 juveniles (the "Juv-0Y" sample set). Staff combined the remaining juveniles and reared them at the hatchery for 1 yr. On July 22, 2020, staff collected 50 more juveniles and preserved them in ethanol (the "Juv-1Y" sample set). General guidelines suggest sampling 25 to 30 individuals from each population to obtain accurate allele frequencies and estimates of genetic diversity, though this may not capture all rare alleles from the population (Hale et al. 2012). The "juvenile population" refers to all the juvenile mussels that were produced, while the "released juvenile population" is the juvenile population after its release into the lower Cedar River in Iowa. As the Juv-1Y sample set was collected after 1 yr in the hatchery, immediately before juveniles were released, it alone is used to make inferences about the released juvenile population.

Genomic DNA was isolated from the buccal swabs of the source population using Qiagen's Puregene Buccal Cell Core Kit A following the "DNA Purification from a Buccal Brush" protocol (Qiagen). Genomic DNA was isolated from the whole Juv-0Y samples using Chelex 100 Resin (BioRad)

Table 1. Loci amplified in *Amblema plicata*, presented with repeating motif and number of alleles (N_a) total (all sample sets), N_a in the Source set, N_a in the Juv-0Y set, and N_a in the Juv-1Y set. — indicates no value reported because locus was eliminated from the study.

Locus name	Motif	N_a total	N_a source	N_a Juv-0Y	N_a Juv-1Y
Anec101	CATC	10	7	No data	7
Anec103	CATC	—	—	—	—
Anec114	CATC	22	17	9	15
Anec117	CATC	—	—	—	—
Anec122	CATC	—	—	—	—
Anec126	TAGA	38	27	14	16
Anec130	CATC	13	7	9	8
Anec144	TAGA	—	—	—	—
Anec103	TAGA	—	—	—	—
Aned104	TAGA	13	9	10	10
Anec106	TAGA	14	12	9	9
Aned108	TAGA	10	8	9	6
Aned126	TAGA	25	22	10	14
Aned132	TAGA	6	4	3	4
Aned134	TAGA	—	—	—	—
Aned140	TAGA	16	14	7	6

following a modified version of the protocol (Singh et al. 2018). Tissue samples were taken from the Juv-1Y samples, and genomic DNA was isolated using the QIAamp DNA mini kit according to the "Tissue" protocol (Qiagen). All extracted DNA was quantified using a Nanodrop ND1000 spectrophotometer and stored at 4°C. We used 16 microsatellite markers developed for the fat threeridge (*Amblema neislerii*; Díaz-Ferguson et al. 2011) to genotype the mussel samples collected (Table 1).

We conducted polymerase chain reaction (PCR) amplification using the BIOLASE PCR kit (Bioline, Boston, MA). Each 10 μL reaction contained 6.6 μL of sterile deionized water, 1 μL of Biolase NH_4 reaction buffer (10×), 0.6 μL of $MgCl_2$ (50 mM), 0.8 μL of dNTP's (2.5 mM each), 0.1 μL of M13 labeled forward primer (20 mM), 0.1 μL of reverse primer (20 mM), 0.05 μL of M13 labeled oligo (20 mM), 0.05 μL of Biolase DNA Taq polymerase (5 U/μL), and 1 μL of template DNA (approximately 2 ng/μL). Reactions were completed in Eppendorf Master Cycler thermocyclers under the following conditions: 95°C/5 min; (94°C/30 sec, touchdown beginning at 56°C and dropping by 0.6°C per cycle/1 min, 72°C/30 sec) × 11; (94°C/30 sec, 55°C/1 min, 72°C/30 sec) × 25; 72°C/20 min. A negative control without mussel DNA was performed with each reaction. PCR products were visualized on 1.5% agarose gels against a 100 bp DNA ladder to confirm the success of the reactions and to ensure the negative control showed no contamination. We sent products to the Iowa State University DNA Facility to determine allele sizes with capillary electrophoresis on an Applied Biosystems 3500 Genetic Analyzer.

Data Analysis

We scored raw data with the software Gene Marker (Version 3.0.1). We checked all loci for null alleles with MICROCHECKER (Van Oosterhout et al. 2004). Loci with possible null alleles were excluded from the data set because their presence can bias genetic analyses (Selkoe and Toonen 2006). We used GenePop version 4.7.5 (Rousset 2008) to perform Hardy-Weinberg exact tests. Exact *P* values were estimated with the Markov chain method according to the following parameters: dememorization number was 1,000, batches set to 100, and 1,000 iterations per batch. GenePop was also used to check for linkage disequilibrium within and among sample sets.

We used GenAlEx 6.5 (Peakall and Smouse 2006) to calculate statistics of genetic diversity including sample size, which was adjusted for missing data by subtracting proportionally for each missing locus (i.e., subtracting 0.10 for one missing locus as data were collected for 10 loci per individual), number of alleles, effective number of alleles, Information Index (also known as Shannon's index), observed heterozygosity, expected heterozygosity, unbiased heterozygosity, and fixation index. HP-Rare 1.0 (Kalinowski 2005) was used to calculate allelic richness and private allelic richness using rarefaction to account for unequal sample sizes between sample sets. We used GenePop 4.7.5 (Rousset 2008) to calculate pairwise F_{ST} values between the sample sets and to calculate genetic differentiation for each pair with an exact G-test. We used GenAlEx to conduct an analysis of molecular variance (AMOVA) and to visualize genetic distances between the three sample sets by generating a principal coordinates analysis (PCoA) based on a covariance matrix with data standardization. In this instance, the covariances were standardized by subtracting the mean and dividing by the standard deviation, resulting in each element representing the correlation between two variables instead of their raw covariance. We used Colony 2.0.6.6 (Wang and Jones 2010) to analyze both parentage and sibship using a full-pedigree likelihood method. We set parameters to full likelihood (FL) with male and female polygamy and ran analysis for a medium length of time. We knew and preassigned the dams of the Juv-0Y samples. The data set used for this study is available on Dryad (https://doi.org/10.5061/dryad.08kprr5cp).

RESULTS

Sample Collection and Data Generation

We successfully genotyped 45 out of 50 threeridge mussels collected from the source population, including the three dams. For the Juv-0Y subsample (immediately after transformation), we successfully genotyped 41 out of 60 collected juveniles; for the Juv-1Y subsample (after 1 yr of being raised in the hatchery), we successfully genotyped 48 out of 50 collected juveniles.

We eliminated 3 of the 16 microsatellite loci due to poor amplification (Anec117, Anec144, Aned103) and 3 others

Table 2. Summary statistics by population. Mean and standard error over all loci for each population. *N* is sample size (with missing data subtracted from original sample size), N_a is number of alleles (averaged across all loci), N_e is number of effective alleles (averaged across all loci), *I* is Information Index, H_o is observed heterozygosity, H_e is expected heterozygosity, uH_e is unbiased expected heterozygosity, F_{IS} is fixation index.

Pop	*N*	N_a	N_e	*I*	H_o	H_e	uH_e	F_{IS}
Source								
Mean	42.1	12.7	5.955	1.933	0.754	0.784	0.793	0.045
SE	0.862	2.329	0.975	0.18	0.067	0.042	0.042	0.061
Juv-0Y								
Mean	21.2	8	3.464	1.421	0.667	0.64	0.654	−0.06
SE	2.529	1.238	0.531	0.186	0.078	0.078	0.079	0.054
Juv-1Y								
Mean	48	9.5	3.31	1.462	0.769	0.651	0.657	−0.183
SE	0	1.319	0.437	0.13	0.055	0.047	0.047	0.022

(Anec103, Anec122, Aned134) for returning larger-than-expected numbers of homozygotes for most allele size classes, indicating the possible presence of null alleles. We retained the 10 remaining microsatellite loci for analysis: Anec101, Anec114, Anec126, Anec130, Aned104, Aned106, Aned108, Aned126, Aned132, and Aned140. Across all 10 loci, we identified a total of 173 alleles, with the number of alleles per locus ranging from 7 to 38. No genotypes from Anec 101 were successfully called for the Juv-0Y set (Table 1).

Data Analysis

Hardy-Weinberg equilibrium.—The source population sample evinced a deficit of heterozygotes at locus Anec130 ($P = 0.0000$, S.E. $= 0.0000$) and locus Aned132 ($P = 0.0222$, S.E. $= 0.002$) and an excess of heterozygotes at locus Aned104 ($P = 0.0474$, S.E. $= 0.0074$). Overall, the source population sample was heterozygote deficient ($P = 0.0119$, S.E. $= 0.0038$). Overall, the Juv-0Y sample was found to be within Hardy-Weinberg equilibrium, and the Juv-1Y sample was found to exhibit an excess of heterozygotes ($P = 0.0000$, S.E. $= 0.0000$). Across all samples, linkage disequilibrium was detected at 4 out of 45 locus pairs (8% of pairs): Anec101 and Anec114 ($P = 0.029798$), Anec126 and Aned106 ($P < 1.54\text{e-}06$), Anec126 and Aned126 ($P < 1.59\text{e-}06$), and Aned106 and Aned126 ($P < 4.82\text{e-}07$). Because the purpose of this study was to compare samples from a population and a group of offspring from that population, and not to investigate population substructure, we retained all loci for the study.

Summary statistics, heterozygosity, and private alleles.—The sizes of the different sample data sets were adjusted for each locus with missing data (Table 2 and Fig. 1). The source set contained the greatest number of alleles averaged across all loci, followed by the Juv-1Y set. Across all loci, the Juv-0Y set averaged only 1.5 fewer alleles than the Juv-1Y set, despite having half of the adjusted sample size. After rarefaction was conducted to make comparisons of populations with

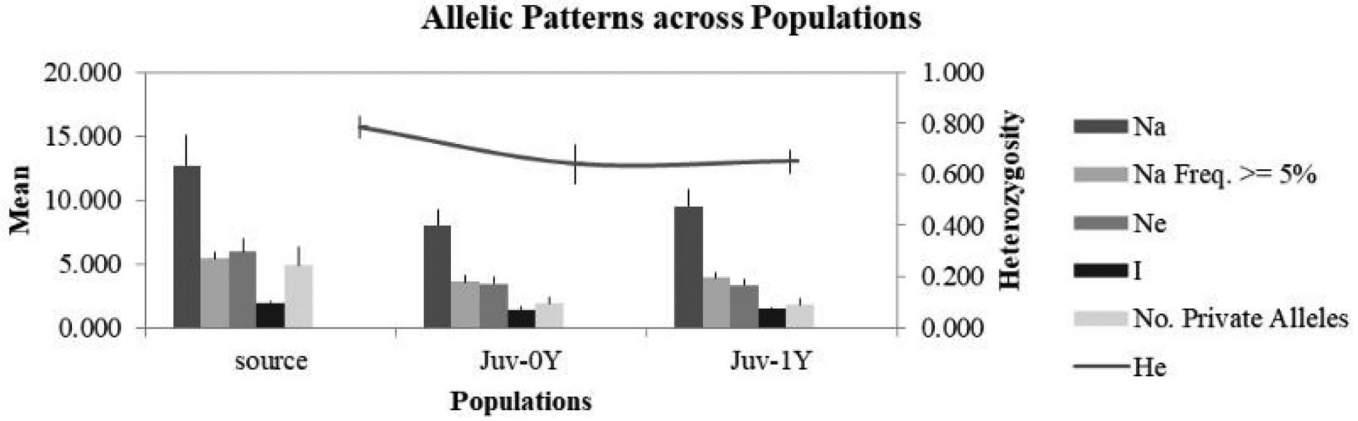

Figure 1. Allelic patterns by population. The labels on the left *x* axis correspond to the bar graph representing the mean values for each population of each variable. N_a is number of alleles, N_a Freq. $\geq 5\%$ is the number of different alleles with a frequency greater than or equal to 5%, N_e is the number of effective alleles, No. Private Alleles is the number of private alleles in the population. The labels along the right *x* axis correspond to the line chart representing expected heterozygosity (H_e) for each population.

Table 3. Rarefied allelic richness per locus, by population.

	Source	Juv-0Y	Juv-1Y
Average across all loci	5.33	6.01	4.07
Anec101	4.44	3.62	3.69
Anec114	6.64	5.15	6.01
Anec126	7.57	5.16	4.80
Anec130	4.65	4.43	3.54
Aned104	5.21	5.36	4.12
Aned106	5.27	3.89	3.91
Aned108	4.80	4.93	4.07
Aned126	6.74	4.46	4.43
Aned132	2.59	2.66	2.11
Aned140	5.35	4.11	4.01

different sample sizes more meaningful, the Juv-0Y set had the greatest allelic richness, followed by the source set, and then the Juv-1Y set (Table 3).

The Information Index (I) indicated that the source set ($I = 1.933$) was the most genetically diverse, while the Juv-0Y ($I = 1.421$) and Juv-1Y ($I = 1.462$) sets exhibited similar diversity. Observed heterozygosity (H_o) was 0.754 in the source set, decreased to 0.667 in the Juv-0Y set, and increased to 0.769 in the Juv-1Y set. H_o was slightly lower than H_e (expected heterozygosity) in the source set, resulting in an F_{IS} value of 0.045. In the Juv-0Y set, H_o was slightly higher than H_e, resulting in an F_{IS} value of -0.06. In the Juv-1Y set, H_o was higher than H_e, resulting in an F_{IS} value of -0.183.

All sets of samples exhibited private alleles. In the source set, 49 private alleles were exhibited in 33 out of 45 individuals (73%). In the Juv-0Y set, 19 private alleles were exhibited by 20 out of 41 individuals (49%). In the Juv-1Y set, 18 private alleles were exhibited by 15 out of 48 individuals (31%). An additional three alleles were found in both the Juv-0Y and Juv-1Y sets that were not found in the source set, resulting in a total of 40 alleles found in the juvenile sets that were not found in the source set. After rarefaction, the source set had the greatest number of private alleles, followed by the Juv-0Y set; the Juv-1Y set had the fewest private alleles (Table 4).

Table 4. Rarefied private allelic richness per locus, by population.

	Source	Juv-0Y	Juv-1Y
Average across all loci	1.89	1.07	0.87
Anec101	0.00	No data	0.00
Anec114	2.27	0.78	1.83
Anec126	4.76	2.04	1.85
Anec130	1.52	1.44	0.51
Aned104	1.18	1.56	0.84
Aned106	1.95	1.27	1.14
Aned108	0.99	1.13	0.37
Aned126	3.28	1.23	1.35
Aned132	0.64	0.69	0.27
Aned140	2.30	0.61	0.56

Table 5. Pairwise population F_{ST} values. All values are significantly different from $F_{ST} = 0$.

	Source	Juv-0Y
Juv-0Y	0.0254	
Juv-1Y	0.0625	0.0615

Population comparison.—All sample sets were significantly differentiated (Table 5). The source and Juv-0Y sets were the most similar, followed by the two juvenile sets. The source and Juv-1Y sets were the most genetically distinct from each other. AMOVA results indicated a global F_{ST} of 0.149 ($P = 0.001$) with 15% of molecular variation detected among populations, 10% among individuals, and 75% within individuals.

We used principal coordinate analysis to visualize genetic similarity within and between groups (Figs. 2, 3). The Juv-0Y sample set was the least tightly clustered sample set, while the Juv-1Y set was the most tightly clustered. The source set was located between the Juv-0Y and Juv-1Y sets and overlapped somewhat with each. There was little overlap between Juv-0Y and Juv-1Y. Axis 1 explained 23.06% of variation, Axis 2 explained 6.06% of variation, and Axis 3 explained 4.82% of variation.

Multiple paternity.—Parentage analysis revealed high levels of multiple paternity in all three broods. In the combined Juv-0Y and Juv-1Y sets (89 juveniles total), 56 juveniles were assigned to dam D191 and were sired by at least 33 males, 17 juveniles were assigned to dam D231 and sired by at least 11 males, and 13 juveniles were assigned to dam D185 and sired by at least seven males. No male sired more than three juveniles, and no male sired juveniles from more than 1 dam.

Differential mortality by dam.—The juveniles experienced high levels of mortality in the hatchery, with less than half of all juveniles surviving between the sampling events immediately after transformation and 1 yr later. Juv-0Y samples were

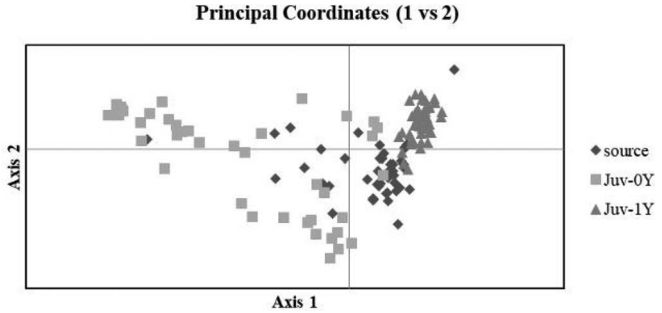

Figure 2. Principal coordinates analysis (PCoA) via covariance matrix with data standardization.

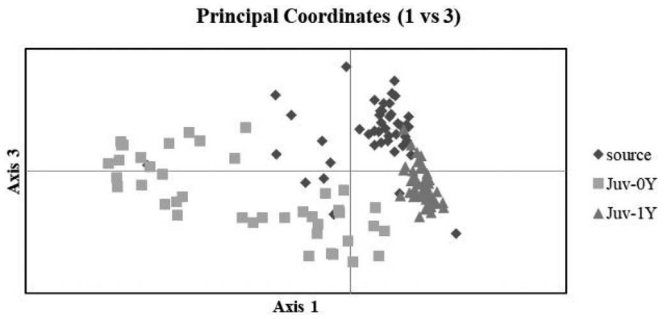

Figure 3. Principal coordinates analysis (PCoA) via covariance matrix with data standardization.

purposely taken proportionately from each of the three dams. Of the 41 samples successfully genotyped, 11 (27% of Juv-0Y) were from dam D191, 17 (41% of Juv-0Y) were from dam D231, and 13 (32% of Juv-0Y) were from dam D185 (Fig. 4). After obtaining the Juv-0Y subsample, the remaining juveniles were pooled and raised together. Analysis of the Juv-1Y set found that 45 out of 48 (94% of Juv-1Y) juveniles in the subsample were the offspring of one dam, D191 (Fig. 5). Two juveniles (4% of Juv-1Y) were from dam D231, and one juvenile (2% of Juv-1Y) was from dam D185.

DISCUSSION

Captive propagation can be an important tool for the conservation of rare species, and in some instances, it may have prevented the extirpation of populations (Hebdon et al. 2004). However, it can significantly impact the genetic structure and evolutionary trajectory of target populations (Waples and Drake 2004; McMurray and Roe 2017). Thus, while the propagation of freshwater mussels has the potential to greatly aid the conservation of the growing number of species that are imperiled, care must be taken to ensure that release of

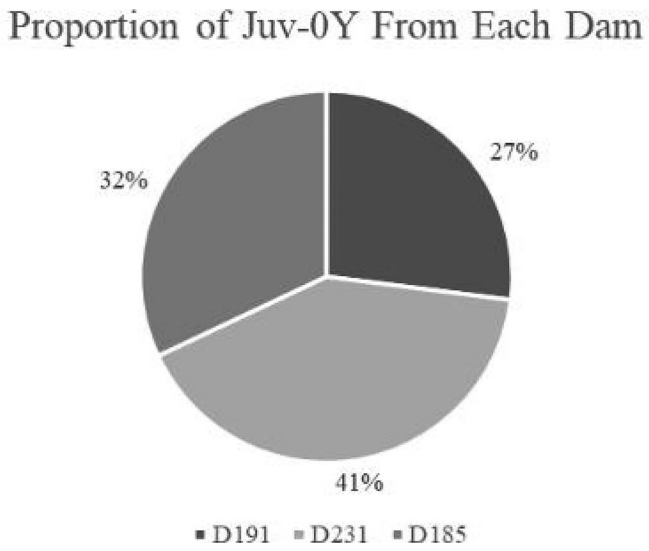

Figure 4. Proportion of Juv-0Y individuals from dams D185, D191, and D231.

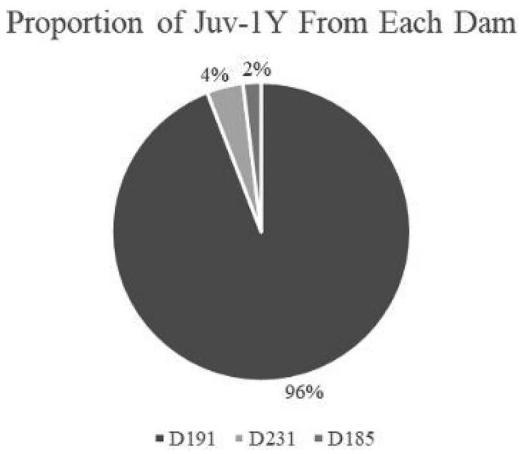

Figure 5. Proportion of Juv-1Y individuals from dams D185, D191, and D231.

propagated juvenile mussels maximizes the benefits and minimizes the risks to the target species/populations. Our examination of the genetic characteristics of propagated juvenile threeridge mussels and their source population provides insight into how propagation of mussels can be improved.

The ratio of H_o to H_e in the source population was comparable to nine other threeridge mussel populations analyzed using the same set of microsatellite markers (Olson and Vaughn 2020) and may be typical for this species. The source population exhibited a deficit of heterozygotes, although heterozygosity was well within the range observed for a variety of other freshwater mussel species (Inoue et al. 2015; Paterson et al. 2015; Chong et al. 2016; Schwarz and Roe 2022). Low levels of heterozygosity might indicate high relatedness of individuals in the source population, with potential occurrences of inbreeding (Harmon and Braude 2010).

The Juv-1Y sample exhibited excess heterozygosity, as would be consistent with a recent reduction in population size (Barker et al. 2009). Mortality in the hatchery, as in any bottleneck event, could have reduced the number of alleles more than it reduced the overall measured heterozygosity (in the sense of Nei's [1987] gene diversity), thus leaving the signature of excess heterozygosity (Piry et al. 1999). Populations naturally recover from genetic bottlenecks through immigration, connectivity with other populations, and mutation giving rise to new alleles (McEachern et al. 2011; Jangjoo et al. 2016). Our results indicate that augmented populations might require multiple infusions of genetic diversity either naturally via gene flow or through additional introductions.

All sample sets displayed a substantial number of private alleles, and the combined juvenile populations exhibited 40 alleles not detected in the source population (19 in Juv-0Y, 18 in Juv-1Y, 3 in both Juv-0Y and Juv-1Y). This observation could be evidence for fertilization of female mussels by sires located upstream of the source population. Male freshwater mussels broadcast sperm into the water column to be filtered by females (Haag 2012). In a study of the population structure of the plain pocketbook mussel *Lampsilis cardium* Rafinesque,

1820, a male mussel was found over 16 km upstream of the dam it fertilized (Ferguson et al. 2013). Dispersal of sperm over long distances allows for gene flow between spatially distinct mussel beds within a watershed. We found 49 alleles in the source population that were not represented in either juvenile set, while the juvenile sets combined had 40 alleles that were not represented in the source population. The Juv-0Y sample set was significantly different from the source population based on alleles present, but the two sets were similar in terms of genetic variability, with the Juv-0Y exceeding the source population in rarefied allelic richness. The three dams successfully produced a cohort of juveniles that were representative of the genetic variability of the source population based on allelic richness. However, the sample sets were significantly different based on the identity of the alleles exhibited. Private alleles were present in 20 out of 41 Juv-0Y juveniles, indicating nearly half of the juveniles were sired by males outside of the source sample. The sum of private alleles within those 20 juveniles, across all loci, was 19.

Parentage analyses in this study found high levels of multiple paternity, with broods of 56, 19, and 14 juveniles being fertilized by 33, 15, and 10 sires, respectively. Multiple paternity has been observed before in other species of freshwater mussels. Ten broods of the triangle sail mussel *Sinohyriopsis cumingii* (Lea, 1852) composed of 23 to 29 offspring each were fertilized by 2 to 4 males (Bai et al. 2012), while nearly every juvenile was found to be fertilized by a different male in 15 broods of *M. margaritifera* (Wacker et al. 2019). In the largest brood, 43 juveniles were sired by at least 32 different males. Multiple paternity increases the genetic variability of juveniles from a single dam. Reproductive methods differ between unionid species; multiple paternity, although phylogenetically widespread, has not been documented in every species, nor always to the same degree when observed (Bai et al. 2012; Ferguson et al. 2013; Hewitt et al. 2018; Wacker et al. 2019; Garrison et al. 2021). Therefore, the evidence available for a particular species must be considered when determining the minimum number of females needed to produce a genetically representative juvenile population. Our multiple paternity results highlight the importance of collecting female mussels that were fertilized in the wild, rather than fertilizing females in a hatchery setting. Wild fertilization allows the chance for many male mussels upstream of the female to sire juveniles, maximizing genetic variability in each brood.

The unexpected finding that nearly all the mussels sampled from the released cohort were from a single dam and the high mortality in the hatchery indicates the possibility that mortality in the hatchery biased the produced juveniles in favor of a single dam. The highly variable rate of survival reduced the genetic variability initially captured in the produced juvenile population and contributed to significant genetic differentiation between the source population and Juv-1Y. Frequently, propagation has been found to alter population genetic variability and structure in both bivalve and fish species (Heath et al. 2003; Osborne et al. 2006; Carlson et al. 2007; Hornick and Plough 2019; Geist et al. 2021). The alteration of the selective pressures faced by juveniles raised in the hatchery, either by removing selection present in the wild and/or by inducing artificial selection, can promote maladaptive traits and reduce fitness in subsequent generations (Heath et al. 2003). In some bivalve species, propagation has been shown to successfully produce representative populations when following practices such as controlling for even contributions from brood stock and contributing to new populations via broods from multiple years (Hornick and Plough 2019; Geist et al. 2021). When guidance from the literature is unavailable, the resources to conduct genetic studies should be included in the cost of propagation to ensure that the latter is providing more benefits than harm to populations in the long term.

Freshwater mussel populations commonly exhibit low effective population sizes (N_e) compared to total population sizes (N). Analysis of nine beds of threeridge mussels in Oklahoma found ratios of effective population size to total population size (N_e/N) ranging from 0.002 to 0.219, with a mean of 0.071 (Olson and Vaughn 2020). Frankham (1995) reviewed data for 102 species and found mean estimates of N_e/N ranging from 0.10 to 0.11. A small N_e makes inbreeding more likely and means the population would be likely to lose genetic diversity more quickly through genetic drift (Lande and Barrowclough 1987). The source population of threeridge mussels in the Cedar River was not thoroughly surveyed, so N_e/N data were unavailable for this study, but we recommend that estimates of N_e should be conducted in the future to develop a baseline for freshwater mussel species. Uneven family size, as was observed in the produced juveniles in this study, can reduce N_e, so the population may be at risk of the adverse effects that come with low N_e (McMurray and Roe 2017). It is possible to mitigate problems of low N_e/N by equalizing family size—i.e., by releasing equal numbers of juveniles from different broods (Harmon and Braude 2010; McMurray and Roe 2017).

Threeridge mussel populations from 10 river drainages in the central United States exhibited little genetic structure between beds within the same river drainage (Elderkin et al. 2007). Many studies of mussel species have reported similar findings, assuaging concerns of outbreeding depression resulting from translocating propagated mussels within a river (Ferguson et al. 2013; Galbraith et al. 2015; Jones et al. 2015; Inoue and Berg 2017). However, some genetic structure between upstream and downstream beds of threeridge mussels in the Little River in Oklahoma was found (Olson and Vaughn 2020). A study examining the Texas hornshell mussel *Popenaias popeii* (Lea, 1857) in the Black River of New Mexico also detected genetic structuring within the river (Inoue et al. 2015). Our project used the breeding population of threeridge mussels found closest to the release site within the same watershed to produce juveniles, and it is recommended that future propagation efforts do the same.

Our study revealed changes in the genetic diversity and variation between the source population and the population of juveniles produced for introduction. Reductions in genetic diversity during the propagation process offset the addition of genetic variation due to multiple paternity. Moving forward, the propagation of freshwater mussels will undoubtedly play a role in their conservation. We encourage hatchery managers to embrace a perspective that includes the preservation of genetic diversity as well as the production of juvenile mussels. Maintaining genetic variation in mussel populations will help maintain the adaptive potential of these species in a changing environment.

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REGULAR ARTICLE

MUSSEL INVENTORY AND POPULATION DEMOGRAPHICS OF THE FEDERALLY ENDANGERED *POTAMILUS CAPAX* (GREEN 1832) IN THE LOWER WABASH RIVER, ILLINOIS AND INDIANA

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ABSTRACT

The Wabash River is a key component of the freshwater mussel biodiversity of the Ohio River Basin. The basin historically supported approximately 75 mussel species, but currently only 30 are thought to be extant in the mainstem. Though the basin was historically well surveyed, the limited number of recent studies have primarily been small, and the last basin-wide survey effort is already over a decade old. This situation is problematic given that several rare species were historically present and populations of the federally endangered *Potamilus capax* may remain. We surveyed 46 sites within the lower Wabash River (river mile 0.0 to 117.0) to characterize mussel assemblages and distributional patterns. In total, we located 996 live mussels of 23 species. The assemblage was dominated by *Obliquaria reflexa, P. capax, Potamilus fragilis* and *Potamilus ohiensis*, and shell-length frequencies indicated ongoing recruitment of several species, including *P. capax* (multiple size classes). We did not find any evidence of major changes in species distributions or occurrences compared to other recent surveys but we did note a shift from species with equilibrium life-history strategies to species with opportunistic strategies moving downriver. Though the lower Wabash River appears to remain a sanctuary for *P. capax* and other smooth-shelled species, many species that were present historically remain absent.

KEY WORDS: Wabash River, *Potamilus capax*, Indiana, Illinois, mussels, unionid

INTRODUCTION

The Wabash River is a major tributary to the Ohio River, marks the border between Illinois and Indiana, and is an important component of the natural resources of both states due to its diversity of fish, freshwater mussels (Bivalvia: Unionida), and other wildlife and plant species (Fisher 2006; Simon 2006; Stodola et al. 2014). Over 70 native mussel species have been documented in the basin, including several currently listed as either Endangered or Threatened under the US Endangered Species Act, along with several candidates and various Illinois and Indiana state-listed species (Table 1). Unfortunately, mussels in the basin are in decline, and only about 30 species remain extant within the mainstem (Table 1; Fisher 2006; Tiemann et al. 2007; Illinois Endangered Species Protection Board 2020; Indiana Division of Fish and Wildlife 2020). Most of the aforementioned rare/listed species appear to be restricted to tributaries, though occasionally, individuals are found in the mainstem (Fisher 2006; Stodola et al. 2014). One exception is the federally endangered *Potamilus capax*, which can be found throughout the lower Wabash River and is often locally abundant and dominant (Fisher 2006; Stodola et al. 2014).

The mussel fauna of the Wabash River basin has been surveyed regularly over the past 100 years. The first comprehensive basin inventory was completed in 1881 (Stein) and updated by Call, Blatchely, Daniels, Goodrich, van der Schalie, and others (Fisher 2006). Between 1987 and 1991, Cummings et al. (1992) surveyed 100 locations. Fisher (2006) compiled data from various Indiana Department of Natural Resources surveys conducted between 1995 and 2006, along with existing survey information. A more recent survey was conducted by the Illinois Natural History Survey (INHS) and Illinois Department of Natural Resources (ILDNR) in 2011 and 2012, albeit primarily at previously sampled locations (Tieman et al. 2012). Other recent surveys consist of an update on mussel distribution

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Table 1. Freshwater mussel (Bivalvia: Unionida) species previously reported from the Wabash River Basin in Indiana (Fisher 2006) and Illinois (Stodola et al. 2014; ILDNR 2024; INHS 2024), their current state and federal status, and their historical and recent distributions. Abbreviations: C = Candidate species, E = Endangered, EX = extirpated, H = historically reproducing but now absent, L = species still reproducing, R = reintroduced, SSC = State Special Concern, T = Threatened, X = Extinct. Dates indicate the period a species was last found extant. Species names are adjusted to reflect current nomenclature.

				Indiana		Illinois	
Species	Federal Status	IL Status	IN Status	Mainstem	Tributary	Mainstem	Tributary
Actinonaias ligamentina	–	–	–	L	L	1977–1999	2000–2013
Alasmidonta marginata	–	–	–	L	L	–	2000–2013
Alasmidonta viridis	–	–	SSC	–	L	–	2000–2013
Amblema plicata	–	–	–	L	L	2000–2013	2000–2013
Anodontoides ferussacianus	–	–	–	–	L	Pre-1950	2000–2013
Arcidens confragosus	–	–	–	H	L	2000–2013	2000–2013
Cambarunio iris	–	E	SSC	H	L	–	2000–2013
Cumberlandia monodonta	E	E	X	EX	EX	Pre-1950	–
Cyclonaias nodulata	–	–	–	L	L	2000–2013	2000–2013
Cyclonaias pustulosa	–	–	–	L	L	2000–2013	2000–2013
Cyclonaias tuberculata	–	T	–	L	L	1977–1999	2000–2013
Cyprogenia stegaria	E	E	–	H	L	1977–1999	Pre-1950
Ellipsaria lineolata	–	T	–	H	L	1977–1999	Pre-1950
Elliptio crassidens	–	E	SSC	H	L	2000–2013	–
Epioblasma flexuosa	X	–	X	EX	EX	–	–
Epioblasma obliquata	E	–	E	EX	EX	Pre-1950	–
Epioblasma personata	X	–	X	EX	EX	–	–
Epioblasma propinqua	X	–	X	EX	EX	–	–
Epioblasma rangiana	E	E	E	EX	EX	Pre-1950	R
Epioblasma sampsonii	X	–	X	EX	EX	–	–
Epioblasma torulosa	E	–	X	EX	EX	Pre-1950	–
Epioblasma triquetra	E	E	E	H	L	Pre-1950	2000–2013
Eurynia dilatata	–	E	SSC	H	L	Pre-1950	2000–2013
Fusconaia flava	–	–	–	L	L	2000–2013	2000–2013
Fusconaia subrotunda	T	–	X	EX	EX	Pre-1950	–
Hemistena lata	E	–	X	EX	EX	Pre-1950	–
Lampsilis abrupta	E	E	X	EX	EX	Pre-1950	–
Lampsilis cardium	–	–	–	L	L	2000–2013	2000–2013
Lampsilis fasciola	–	E	SSC	L	L	–	2000–2013
Lampsilis hydiana	–	–	–	–	–	Pre-1950	2000–2013
Lampsilis ovata	–	–	SSC	L	L	Pre-1950	–
Lampsilis siliquoidea	–	–	–	L	L	1950–1976	2000–2013
Lampsilis teres	–	–	–	L	L	2014–2018	2000–2013
Lasmigona complanata	–	–	–	L	L	2014–2018	2000–2013
Lasmigona compressa	–	–	–	–	L	–	2000–2013
Lasmigona costata	–	–	–	L	L	Pre-1950	2000–2013
Leaunio lienosus	–	–	SSC	–	L	–	2000–2013
Ligumia recta	–	–	SSC	L	L	Pre-1950	2000–2013
Megalonaias nervosa	–	–	–	H	L	2000–2013	2000–2013

Table 1, continued.

				Indiana		Illinois	
Species	Federal Status	IL Status	IN Status	Mainstem	Tributary	Mainstem	Tributary
Obliquaria reflexa	–	–	–	L	L	2014–2018	2000–2013
Obovaria olivaria	–	–	–	L	L	2000–2013	2000–2013
Obovaria retusa	E	–	X	EX	EX	Pre-1950	–
Obovaria subrotunda	T	–	E	H	L	Pre-1950	1977–1999
Paetulunio fabilis	E	–	E	H	L	–	2000–2013
Plethobasus cicatricosus	E	–	X	EX	EX	Pre-1950	–
Plethobasus cooperianus	E	E	X	EX	EX	–	–
Plethobasus cyphyus	E	E	E	H	L	1977–1999	–
Pleurobema clava	E	E	–	H	L	Pre-1950	2000–2013
Pleurobema cordatum	–	E	SSC	H	L	1977–1999	Pre-1950
Pleurobema plenum	–	–	E	EX	EX	Pre-1950	–
Pleurobema rubrum	–	–	X	EX	EX	Pre-1950	1977–1999
Pleurobema sintoxia	–	–	–	L	L	Pre-1950	2000–2013
Potamilus alatus	–	–	–	L	L	2014–2018	2000–2013
Potamilus capax	E	E	E	L	L	2014–2016	1977–1999
Potamilus fragilis	–	–	–	L	L	2014–2018	2000–2013
Potamilus leptodon	E	E	X	EX	EX	Pre-1950	–
Potamilus ohiensis	–	–	–	L	L	2014–2018	2000–2013
Ptychobranchus fasciolaris	–	E	SSC	H	L	Pre-1950	2000–2013
Pyganodon grandis	–	–	–	L	L	2000–2013	2000–2013
Quadrula fragosa	E	–	–	EX	EX	Pre-1950	–
Quadrula quadrula	–	–	–	L	L	2014–2018	2000–2013
Reginaia ebenus	–	E	SSC	H	L	2000–2013	2000–2013
Sagittunio subrostratus	–	–	–	–	L	Pre-1950	2000–2013
Simpsonaias ambigua	C	E	SSC	H	L	Pre-1950	2000–2013
Strophitus undulatus	–	–	–	L	L	Pre-1950	2000–2013
Theliderma cylindrica	T	E	E	H	L	Pre-1950	2000–2013
Theliderma metanevra	–	T	–	L	L	2000–2013	2000–2013
Toxolasma lividum	–	E	SSC	H	L	Pre-1950	2000–2013
Toxolasma parvum	–	–	–	–	L	2014–2018	2000–2013
Toxolasma texasiense	–	–	SSC	–	L	2000–2012	2000–2013
Tritogonia verrucosa	–	–	–	L	L	2014–2018	2000–2013
Truncilla donaciformis	–	–	–	L	L	2014–2018	2000–2013
Truncilla truncata	–	–	–	L	L	2014–2018	2000–2013
Uniomerus tetralasmus	–	–	–	–	L	Pre-1950	2000–2013
Utterbackia imbecillis	–	–	–	L	L	2000–2013	2000–2013
Utterbackiana suborbiculata	–	–	–	–	L	2014–2015	2000–2013
Venustaconcha ellipsiformis	–	–	–	–	–	Pre-1950	2000–2013
Total Species				66	75	65	58

in the Illinois portion of the basin by INHS based on previously collected data and some limited new data (Stodola et al. 2014), and a few small unpublished surveys conducted by state agencies (ILDNR 2024; INHS 2024).

Despite substantial historical efforts in the Wabash River basin, the most recent comprehensive surveys are more than a decade old. Additionally, previous efforts often have used only shallow-water sampling techniques, limiting surveys to

shallow areas and potentially biasing data. As a result, current inferences regarding the health of mussels in the Wabash River basin, including species' conservation status and long-term viability, may be outdated and/or incorrect. Accurately assessing a species' health and status and effectively managing it require reliable, precise, and current abundance and distribution data (Huang et al. 2011). Thus, updated information on the status and health of mussels in the basin is needed to manage and protect them. We used a multimethod sampling approach to evaluate the current diversity, distribution, assemblage structure, health, and viability of the freshwater mussel (hereafter mussel) fauna in the lower Wabash River.

METHODS

Study Area

The Wabash River, located in the midwestern United States, is the third-largest tributary of the Ohio River (Fig. 1). From its headwaters in Fort Recovery, Ohio, the river flows approximately 810 km to its confluence with the Ohio River at the southern end of the Indiana-Illinois border near New Haven, Illinois. The basin drains an area of approximately 85,300 km^2 with a mean annual discharge of 1,000 m^3/s (Pyron et al. 2020). Though several reservoirs are on its tributaries, only one is on the mainstem (near Huntington, Indiana, at river km 662), and the lower portion is the longest undammed river reach east of the Mississippi River (Pyron et al. 2020).

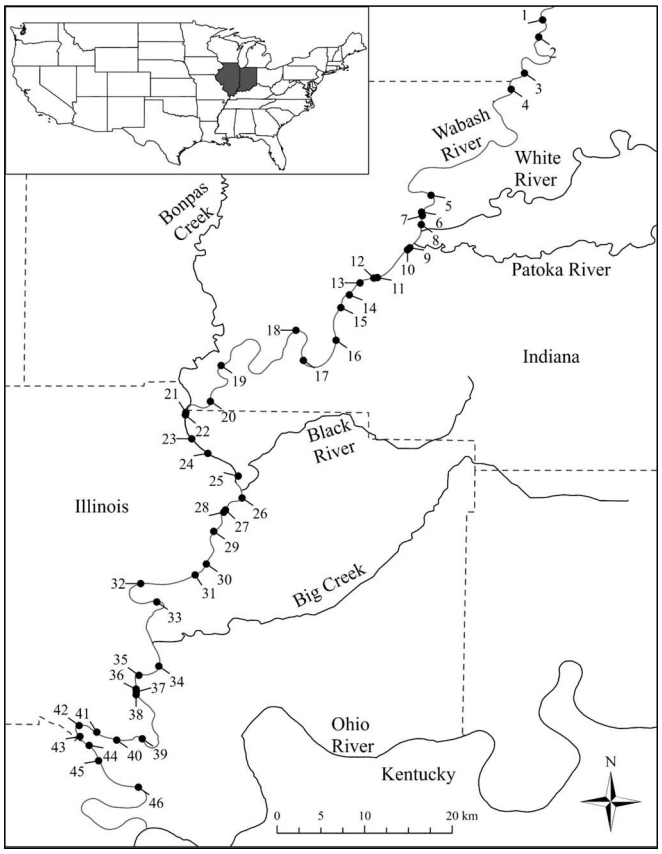

Figure 1. Location of our 46 survey sites within the lower Wabash River.

Survey Methods

Between August 22, 2021, and August 28, 2022, we conducted a multimethod mussel study within the lower Wabash River in conjunction with the ILDNR. We surveyed 46 sites in the lower Wabash River, beginning from just upstream of Mt. Carmel, Illinois (Lawrence County, Illinois), to just upstream of the confluence with the Ohio River (Gallatin County, Illinois; Fig. 1). For this study, we considered the lower Wabash River to be between navigation mile marker 0.0 and 117.0 in Lawrence, Wabash, White, and Gallatin Counties in Illinois and Knox, Gibson, and Posey Counties in Indiana.

The ILDNR selected the survey sites, which consisted of locations where previous survey efforts had occurred or where ILDNR wanted to determine if mussel assemblages and/or federally listed mussel species and other rare species occur. Our goal was to locate mussel assemblages and characterize population demographics (e.g., density, age class structure, etc.), with a particular focus on state and federally listed species. To meet these study goals, and in coordination with ILDNR, we developed a systematic multimethod survey approach, combining three different sampling methods (i.e., timed searches, transects, and quadrats).

Timed searches.—For each site, we delineated a 300-m linear length of the river as the survey area. Initially, surveyors sampled for mussels for a combined one person-hour. Sampling was spatially distributed across the entire river, with one-third of search effort spent equally along each bank (extending 10 to 15 m riverward), and midchannel. Following this, we examined abundances and species richness from each section, and spent five additional person-hours surveying the section with the greatest abundance and richness (resulting in a total of six person-hours per site). If it was not apparent which section had the highest abundance and species richness (i.e., if one section had high abundance of a single species while another had lower abundances but higher species richness), then search effort was evenly split between each section. If no live or fresh dead mussels were located, no additional survey effort beyond the initial person-hour occurred. After the six person-hours of sampling, all mussels were returned to the approximate collection location after processing and before additional transect and quadrat surveys.

Transects.—Based on the results of timed searches, sites 9, 15, 16, 18, 20, 23, 25, 26, 31, 32, 33, 37, 45, and 46 were selected for additional transect surveys. Site selection was based on several factors, including mussel abundance and species richness from timed searches, *P. capax* abundance, and spatial placement of the site within the study area. At each site, we placed nine 100-m transects perpendicular to river flow. Sites 18, 20, 26, 32, 37, and 46 contained the highest abundances of *P. capax* and/or other species in timed

searches and were assigned an additional nine transects. Transects began at the upstream end of the 300-m site along the bank with the greatest mussel abundance and species richness. Transects ($n = 180$) were placed every 10 m moving downstream and were subdivided into 10-m intervals. Mussels and data were collected for each 10-m interval ($n = 1{,}800 \times$ 10-m intervals). All mussels were returned to the approximate collection location after processing and prior to quadrat surveys.

Quadrats.—We conducted quadrat surveys at all sites where transect sampling occurred. We used a systematic sampling design with one random start and 0.25 m^2 quadrats (Strayer and Smith 2003). We excavated by hand the substrate from each quadrat to a depth of approximately 15 cm and sieved it through a 6.4 mm mesh to collect any mussels (Vaughn et al. 1997; Obermeyer 1998). We sampled 12 quadrats at each site, but as with transect samples, sites 18, 20, 26, 32, 37, and 46 were assigned 12 additional quadrats ($n = 146 \times 0.25$ m^2 quadrats; overall area surveyed = 60 m^2).

Mussel sampling.—We searched sites visually and tactilely, which provides the most accurate results for mussel species richness, evenness, and abundance (Hornbach and Deneka 1996; Vaughn et al. 1997). We surveyed using wading or snorkeling techniques in shallow water (depths $\leq$ 1 m) and SCUBA techniques in deeper habitat. Generally, we first conducted a visual search, followed by a tactile search, where we disturbed the substrate to a depth of about 5 cm to dislodge buried mussels and to move obstructions, such as woody debris or large rocks. After tactile searches, we conducted an additional visual search to collect exposed mussels. We kept mussels submerged in water until survey efforts and processing were complete. All mussels were returned to their approximate collection location after processing.

We identified and measured shell length (nearest 0.1 mm) of all live mussels and took photographic vouchers. We collected, identified, and enumerated fresh dead shells. Species located as weathered and relic dead shell were recorded but not enumerated. Shells were considered fresh dead if both valves were present, the nacre lustrous, the hinge flexible, and the periostracum intact. Fresh dead shells were recorded as a positive species occurrence. We measured shell length of all fresh dead shells of federally listed species. We visually assessed the general substrate composition at each site but did not quantify substrate particle size.

Data Analysis

Individual survey method analyses.—We used timed search data (combined for each site) to calculate CPUE (mussels/person-hour) estimates. To determine if horizontal mussel distribution (i.e., from bank to midchannel) was even, we ran two regression analyses using log transformed total mussel abundance and species richness from each 10-m transect interval (n=1,800) as response variables and distance from the bank as the predictor variable. We used data from quadrat samples to calculate density (mussels/m^2).

Comparison between sampling methods.—We ran three ANOVAs to test for significant differences in (1) average shell lengths within species, (2) total mussel abundance, and (3) species richness among the three different survey methods. We ran a Tukey's post hoc test to determine the pairwise differences between survey methods. We used data only from the 14 sites where all sampling methods were employed.

All survey methods.—We combined data from all samples of each survey method type to provide a total species list, total species abundance and relative abundance (species abundance/total abundance), and we assigned mussels life-history strategies (opportunistic, periodic, or equilibrium) following Haag (2012) and Moore et al. (2021). For species without a published life-history strategy, we assigned strategies based on those of similar species (e.g., species from the same genus) and our knowledge of the respective species' behaviors (Table 2). We used our total abundances and species richness to calculate Shannon-Wiener species diversity (H') and Pielou's evenness (J') indices for each site. To examine changes in the mussel community along the river, we ran eight regression analyses using abundance, species richness, CPUE, H' index, J' index, and relative abundance of each of the three life-history strategies at each site as response variables and site location as the predictor variable. We defined site location as the distance moving upstream from the confluence with the Ohio River and calculated distance using the U.S. National Hydrography dataset to trace the entire length of the Wabash River. We then plotted site locations in ArcGIS and used the "Locate Feature" geoprocessing tool to generate distance by locating where the site intersected the river polyline.

The proportion of fresh dead shell in an assemblage was used as an index of recent mortality, which can indicate recent stress events (Dunn et al. 2020). We calculated the percentage of recent mortality (number fresh dead/[number fresh dead + number live]) for all species using data from all survey methods. Pooling data from all sampling sites and methods, we tested for differences in average shell length between live and fresh dead *P. capax* using an ANOVA.

We used shell length as a proxy for age to assess recent recruitment for each species. Mussels $\leq$ 40 mm in length were considered recent recruits (Obermeyer 1998; Smith and Crabtree 2010; Ford et al. 2023) for all species except *P. capax, Toxolasma parvum*, *Truncilla donaciformis*, and *T. truncata*. Due to its large maximum size (approximately 150 mm; Peck et al. 2014), *P. capax* recruits were considered those $\leq$50 mm in length (Wentz et al. 2009), and due to their small maximum size and age of maturity, recruits of *T. parvum, T. donaciformis*, and *T. truncata* were considered those $\leq$30 mm in length (Haag 2012). Shell length-frequency histograms were created for the six most-abundant species. These histograms were used to identify individual recruitment cohorts and to assess the viability of these species.

Table 2. Summary of freshwater mussel (Bivalvia: Unionida) data from the lower Wabash River using timed search, transect, and quadrat sampling methods. Abbreviations: n = total number of mussels, RA = relative abundance (percent representation of a species in the assemblage), CPUE = catch-per-unit-effort (mussels/person-hour), Rec. = proportion of recruits, Density = mussels/m^2, Occ. = percent occurrence (percentage of sites occupied by a species), and Mort. = proportion of recent mortality. No recruits were located in the quadrat samples. A dash (–) indicates a species was not detected.

	Timed Search				Transect			Quadrat			All Methods		
Species	*n*	RA	CPUE	Rec.	*n*	RA	Rec.	*n*	RA	Density	Occ.	Rec.	Mort.
Equilibrium Species													
Amblema plicata	24	2.7	0.1	–	–	–	–	–	–	–	6.5	–	4.0
Cyclonaias nodulata	8	0.9	<0.1	–	–	–	–	–	–	–	15.2	12.5	–
Cyclonaias pustulosa	31	3.4	0.2	–	–	–	–	–	–	–	15.2	12.9	–
Megalonaias nervosa	4	0.4	<0.1	–	–	–	–	–	–	–	8.7	–	20.0
Quadrula quadrula	79	8.8	0.4	10.1	6	6.6	16.7	–	–	–	43.5	10.7	4.5
Reginaia ebenus	7	0.8	<0.1	–	–	–	–	–	–	–	6.5	–	22.2
Theliderma metanevra	4	0.4	<0.1	–	–	–	–	–	–	–	8.7	–	–
Tritogonia verrucosa	53	5.9	0.3	–	1	1.1	–	–	–	–	34.8	–	5.3
Total	210	23.3	1.0		7	7.7	–	–	–	–			
Opportunistic Species													
Lampsilis teres	2	0.2	<0.1	–	–	–	–	–	–	–	4.3	–	50.0
Lasmigona complanata	2	0.2	<0.1	–	–	–	–	–	–	–	4.3	–	–
Potamilus alatus	12	1.3	0.1	–	3	3.3	–	–	–	–	21.7	–	16.7
Potamilus capax	139	15.4	0.7	–	24	26.4	–	3	60.0	0.05	47.8	1.8	60.2
Potamilus fragilis	86	9.6	0.4	20.7	24	26.4	–	1	20.0	0.02	52.2	20.7	32.3
Potamilus ohiensis	125	13.9	0.6	15.2	18	19.8	–	–	–	–	60.9	13.3	23.1
Pyganodon grandis	2	0.2	<0.1	–	1	1.1	–	–	–	–	4.3	–	25.0
Toxolasma parvum	1	0.1	<0.1	–	–	–	–	–	–	–	2.2	–	–
Truncilla donaciformis	7	0.8	<0.1	100.0	2	2.2	50.0	–	–	–	8.7	88.9	18.2
Truncilla truncata	13	1.4	0.1	100.0	1	1.1	–	–	–	–	15.2	21.4	17.6
Utterbackia imbecillis	1	0.1	<0.1	–	–	–	–	–	–	–	2.2	–	50.0
Utterbackiana suborbiculata	DS	–	–	–	–	–	–	–	–	–	2.2	–	100.0
Total	390	43.3	1.9		73	80.2		4	80.0	0.07			
Periodic Species													
Lampsilis cardium	3	0.3	<0.1	–	–	–	–	–	–	–	2.2	–	–
Lampsilis ovata	1	0.1	<0.1	–	–	–	–	–	–	–	2.2	–	–
Obliquaria reflexa	255	28.3	1.2	20.4	9	9.9	44.4	1	20.0	0.02	47.8	21.1	2.6
Obovaria olivaria	41	4.6	0.2	7.3	2	2.2	50.0	–	–	–	23.9	9.3	4.4
Total	300	33.3	1.2		11	12.1		1	20.0	0.02			
Total	900	90.4	4.3		91	9.1		5	0.5	0.08			

RESULTS

Overall Results

We surveyed approximately 13.8 linear km of the Wabash River. Across all sites, substrate consisted primarily of stable silt (16.9%) and sand (74.8%), with some small amounts of gravel (7.6%), cobble (0.3%), boulder (0.1%), and clay (0.3%) along the banks. Gravel and cobble were more prevalent at upstream sites (Sites 1–8), and substrate became primarily sand downstream of the confluence with the White River (downstream of site 8; Fig.1). Substrate in the center of the channel consisted entirely of loose gravel and/or sand.

We detected 996 live mussels of 23 species (Table 2), approximately 30.7% of the historical mussel assemblage known from the basin. Live mussels were found at 33 sites (71.7%), and the total number of live mussels ranged from 0 to 118 per site (mean ± SE: 21.7 ± 4.2). *Obliquaria reflexa* was the most abundant species, comprising 26.6% of all

mussels collected. The federally endangered *P. capax* and the nonlisted *Potamilus ohiensis* and *Potamilus fragilis* were the second-, third-, and fourth-most abundant species comprising 16.7%, 14.4%, and 11.1% of total mussels, respectively (Table 2).

Species richness ranged from 0 to 13 (mean ± SE: 4.4 ± 0.6) species per site. Patterns of ubiquity varied by species, and only five species were widely distributed across the lower basin, occurring at ≥ 20 sites: *P. ohiensis* ($n = 28$), *P. fragilis* ($n = 24$), *P. capax* ($n = 22$), *O. reflexa* ($n = 21$), and *Quadrula quadrula* ($n = 20$). Most species ($n = 17$) occurred at ≤ 10 sites each. Three species were represented by a single live individual: the Indiana species of special concern *Lampsilis ovata* (Site 10), the nonlisted *T. parvum* (Site 20), and *Utterbackia imbecillis* (Site 6). Species with an opportunistic life-history strategy represented the greatest relative abundance (46.9%) and species richness (11 live species) within the assemblage, followed by periodic species (31.3%, 4 live species). Opportunistic species were the dominant life-history strategy at > 50% of sites and were found at all sites with live mussels. Both equilibrium and periodic life histories were primarily represented by a single species at a site.

Total mussel abundance and CPUE significantly decreased moving downstream toward the confluence with the Ohio River. Species richness, species diversity (H'), and species evenness (J') also declined but not statistically significantly (Figs. 2a–e). In addition, the relative abundance of species with equilibrium and periodic life histories, respectively, significantly declined moving downstream (Figs. 2f and h). Conversely, opportunistic life histories significantly increased moving downstream (Fig. 2g). Our findings indicate a shift from species with periodic and equilibrium life histories to primarily opportunistic species in a downstream direction.

Fresh dead shell material was found for 17 species but primarily consisted of *P. capax*, *P. fragilis*, and *P. ohiensis* (93.0% of fresh dead). One species, *Utterbackiana suborbiculata* (Site 37), was represented as fresh dead shell material only (Table 2). Mortality of *P. capax* was 58.8%, and average fresh dead shells were significantly longer (ANOVA: $F_{1,401} = 13.339$, $P < 0.05$) than live individuals (Fig. 3).

We observed numerous size classes for several species, and 13.1% of collected mussels were recruits, primarily *O. reflexa* ($n = 56$), *P. fragilis* ($n = 23$), and *P. ohiensis* ($n = 19$). Three *P. capax* recruits as well as several individuals from the subsequent size class were located, and small (<70 mm) *P. capax* were located at nine sites. Shell length-frequency-plots of the six most abundant species exhibited a general bell-shaped curve, indicating annual recruitment (Figs. 4a–f). All six exhibited unimodal shell length-frequency distributions except *P. ohiensis* (Fig. 4c) and *P. fragilis* (Fig. 4d), which exhibited two distinct modal peaks indicating two cohorts.

Timed Searches (46 Sites)

We spent a total of 211 person-hours conducting timed searches (Tables 2 and 3) at the 46 sites. The majority of mussels ($n = 900$; 90.4%) and all 23 species were detected in timed searches (Table 2). *Obliquaria reflexa*, *P. capax*, *P. ohiensis*, and *P. fragilis* were the most abundant species, and several species were found only in timed searches. Overall CPUE was 4.3 mussels/person-hour and ranged from 0 to 19.7 mussels/person-hour (mean ± SE: 3.3 ± 0.7 mussels/person-hour) per site. CPUE was highest for sites 6, 8, 13, and 32, respectively (Table 3).

Transects (14 Sites)

We located 91 live mussels of 11 species in transect samples. These consisted primarily of *P. capax*, *P. fragilis,* and *P. ohiensis*, all opportunistic species. Across the 1,800 10-m transect intervals, mussels were located significantly more often (Regression: $R^2 = 0.623$, $P = 0.007$; Fig. 5a) and with greater species richness (Regression: $R^2 = 0.652$, $P = 0.005$; Fig. 5b) within the first 10 m from the bank. The majority of mussels (> 80%) and all 11 species were found in the first 10 m from the bank. More than 90% of *P. capax* were found in the first two intervals (Fig. 5a).

Quadrats (14 Sites)

Across the 60 m^2 surveyed via quadrat sampling, we located only five live mussels of three species. Overall, density (0.08 mussels/m^2) was low and ranged from 0.00 to 0.33 mussels/m^2 (mean ± SE: 0.12 ± 0.04 mussels/m^2; Table 3) per site. Density was highest for *P. capax* (Table 2), which was the only species with more than a single individual located in quadrats.

Comparison Between Sampling Methods

Mussel abundance (ANOVA: $F_{2,135} = 20.03$, $P < 0.001$) was significantly affected by survey methods, with abundance greater in timed searches than transect (Tukey: $P < 0.001$) or quadrat samples (Tukey: $P < 0.001$). Species richness (ANOVA: $F_{2,135} = 43.85$, $P < 0.001$) was also significantly affected by survey methods, with greater richness in timed searches than transect (Tukey: $P < 0.001$) or quadrat samples (Tukey: $P < 0.001$). Average *P. ohiensis* shell lengths were significantly different among survey methods (ANOVA: $F_{1,79} = 13.697$, $P < 0.001$), and were significantly longer in transect samples than in timed searches (Tukey post hoc tests significant at $P < 0.001$). Shell length was not significantly affected by survey method in any other species (Table 4).

DISCUSSION

Building on previous research (Fisher 2006; Stodola et al. 2014), this study presents the results of a large-scale survey of the diversity, distribution, and population structure of mussels in the lower Wabash River. Recent surveys recorded approximately 30 mussel species in the mainstem Wabash River, and the lower Wabash River appears to support about

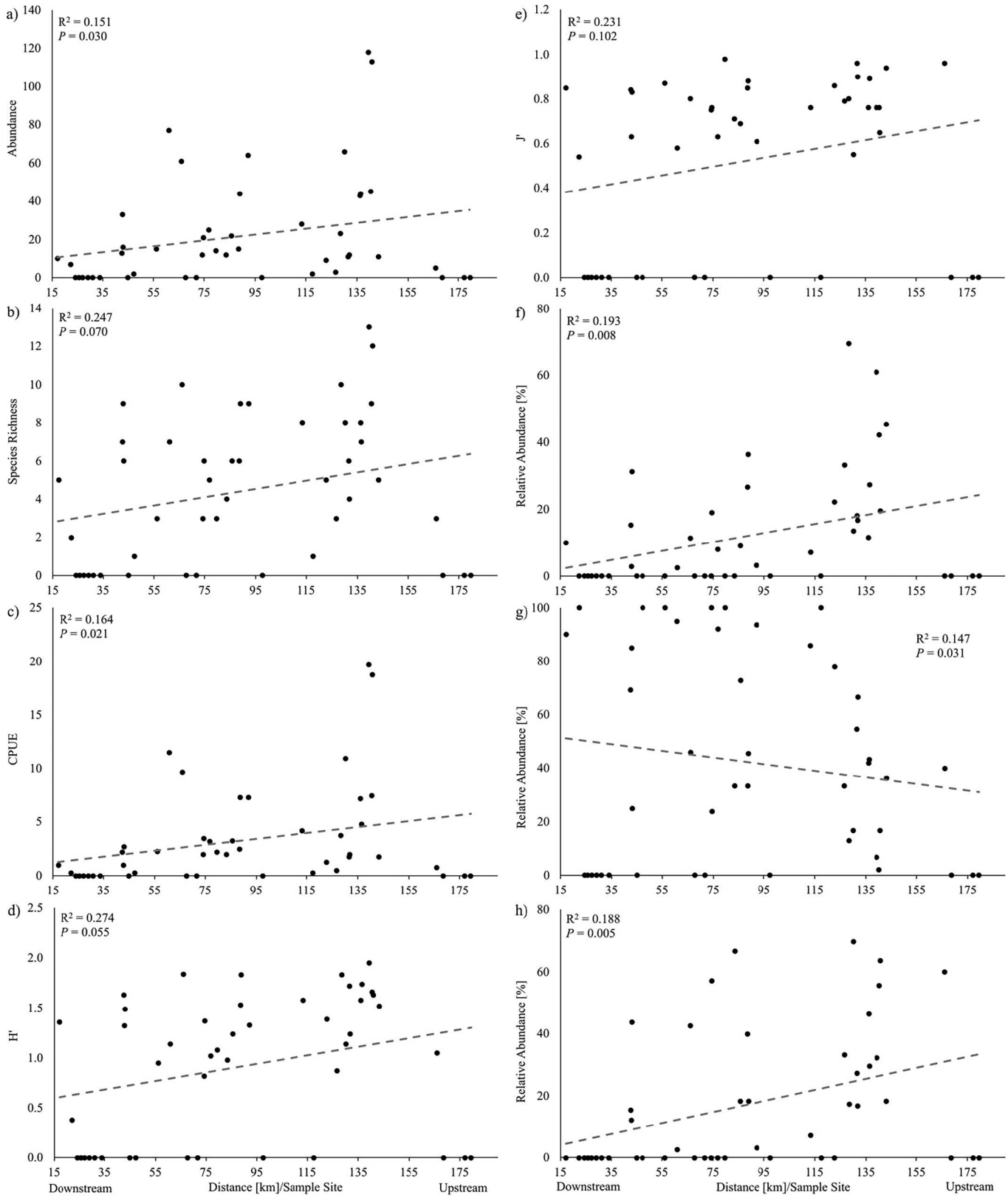

Figure 2. Scatterplots and corresponding regression lines and statistics for (a) total mussel abundance, (b) catch-per-unit-effort (CPUE = mussels/person-hour), (c) species richness, (d) species diversity (H'), (e) species evenness (J'), and relative abundance of mussel species with (f) equilibrium, (g) opportunistic, and (h) periodic life-history strategies per site with increasing distance (km) from the confluence with the Ohio River. Site 46 is the closest to the confluence with Ohio River, and Site 1 is the farthest.

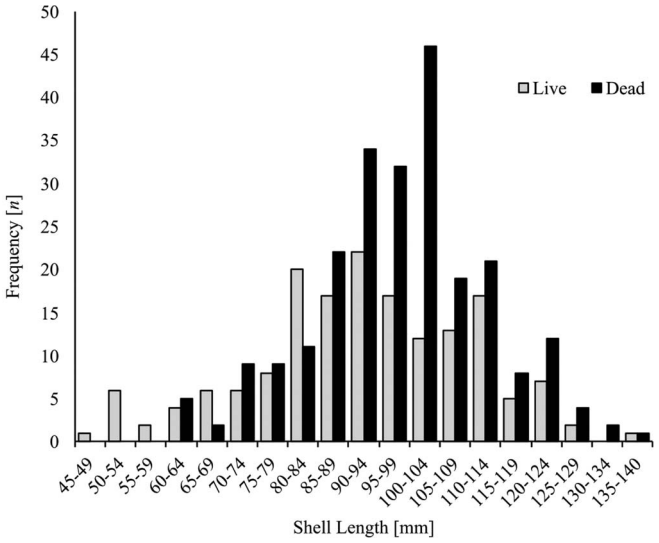

Figure 3. Shell length-frequency plots of live and dead *Potamilus capax* found at our 46 survey sites in the lower Wabash River.

75% of these (Fisher 2006; Stodola et al. 2014; ILDNR 2024; INHS 2024), though they account for only about a third of the mussel species historically known from the basin. Our results are similar to those of Fisher (2006) and Stodola et al. (2014) in terms of the species detected and their distribution across the lower Wabash River. For example, *O. reflexa, P. alatus, P. ohiensis,* and *Q. quadrula* were previously highly ubiquitous and remain so, while historically rare species, such as *Megalonaias nervosa* and *Reginaia ebenus*, remain rare (Fisher 2006; Tieman et al. 2012; Table 2). That said, differences in sampling methods, survey effort, and reporting limit comparability with our study. Our length-frequency plots indicated multiple size classes for several species, and the presence of small (< 70 mm) *P. capax* and other species is encouraging and indicates ongoing recruitment (Fig. 4a–f). Unfortunately, no recruits of *M. nervosa* and *R. ebenus* were located, and both species are thought to be functionally extirpated from the mainstem (Fisher 2006; Tieman et al. 2012).

In general, abundance, CPUE, and species richness declined moving downstream (Fig. 2), and mussels were widely spatially distributed with limited chance of detection beyond the bank (Figs. 5a, b). More than 90% of mussels were found in the timed searches when surveyors could search large areas of a site and focus on areas where mussels were located (i.e., the banks). Usually, mussel abundances and species richness increase with increasing river size, as larger waterbodies typically have more and varied available habitat and can support dense and diverse mussel aggregations (Haag 2012; Ford et al. 2016). Conversely, small streams have limited habitat and support few and scattered aggregations with fewer species (Atkinson et al. 2012; Haag 2012; Ford et al. 2016). The limited and decreasing species richness and abundances of mussels in the lower Wabash River more closely resembled aggregations in smaller stream habitats rather than those in medium/large streams, likely due in part to the habitat homogeneity found throughout much of our study area. This pattern is especially prominent following Site 8, after which population demographic variables shifted and declined, and the assemblage makeup changed (Table 3; Fig. 2a–h).

Following the confluence with the White River, a distinct substrate shift occurred. The White River inputs large amounts of fine sediment into the basin (Pyron et al. 2020), and the substrate shifted from a gravel, sand, and silt mixture to almost exclusively sand/silt, a habitat typically more often utilized by smooth-shelled opportunistic species (Haag 2012). Species with opportunistic life histories tend to have traits that facilitate colonization and survival in more oligotrophic, degraded, or unsuitable habitats (i.e., shifting silt/sand, unconsolidated gravel). Conversely, equilibrium species tend to have traits that favor stable and suitable habitats, while periodic species are intermediate in their traits (Haag 2012). As expected with this habitat change to a silt/sand substrate, the mussel community in our study area shifted from mostly sculptured species with equilibrium and periodic life-history strategies to primarily smooth-shelled, opportunistic species (Table 3). Of species with opportunistic life-history strategies found in our study, 100% of *P. capax*, 100% of *P. alatus*, 93.7% of *P. fragilis*, and 88.1% of *P. ohiensis* were located downstream of Site 8. These are silt/sand preferring species, with smooth and globose shell morphologies that enhance movement in fine substrates (Watters 1994; Haag 2012). Many opportunistic species also utilize multiple common fish species as hosts, enabling them to spread quickly and easily (Haag 2012). Seven of the opportunistic species detected in this study (*Pyganodon grandis* and all *Potamilus* and *Truncilla* species) are known to use *Aplodinotus grunniens* as a host, an abundant and widespread fish species in the Wabash River (Jacquemin et al., 2015). Interestingly, *O. reflexa* and *Amblema plicata* have also been found to use *A. grunniens*, and this may explain their relatively high abundance despite non-opportunistic life-history strategies (Freshwater Mussel Host Database, 2017).

Primarily, mussels were located on gently sloping banks along the edges of sand bars (Fig. 5a, b) and were rarely found in the loose sand/gravel substrates midchannel, likely due to the substrates' instability, which precludes mussels from anchoring (Haag 2012). When mussels were present in the midchannel, abundance and CPUE were low, aggregations were absent, and individuals were widely dispersed (Fig 5a, b). The most practical survey method was to walk along banks looking for movement trails, which could be followed. Mussels would often be buried at the end of trails, some of which terminated out of water. Mussels out of water would often be buried to the water table, possibly enabling them to remain moist until water levels rose (Gough et al. 2012; Galbraith et al. 2015; Mitchell et al. 2018). We found several fresh dead shells with scratches

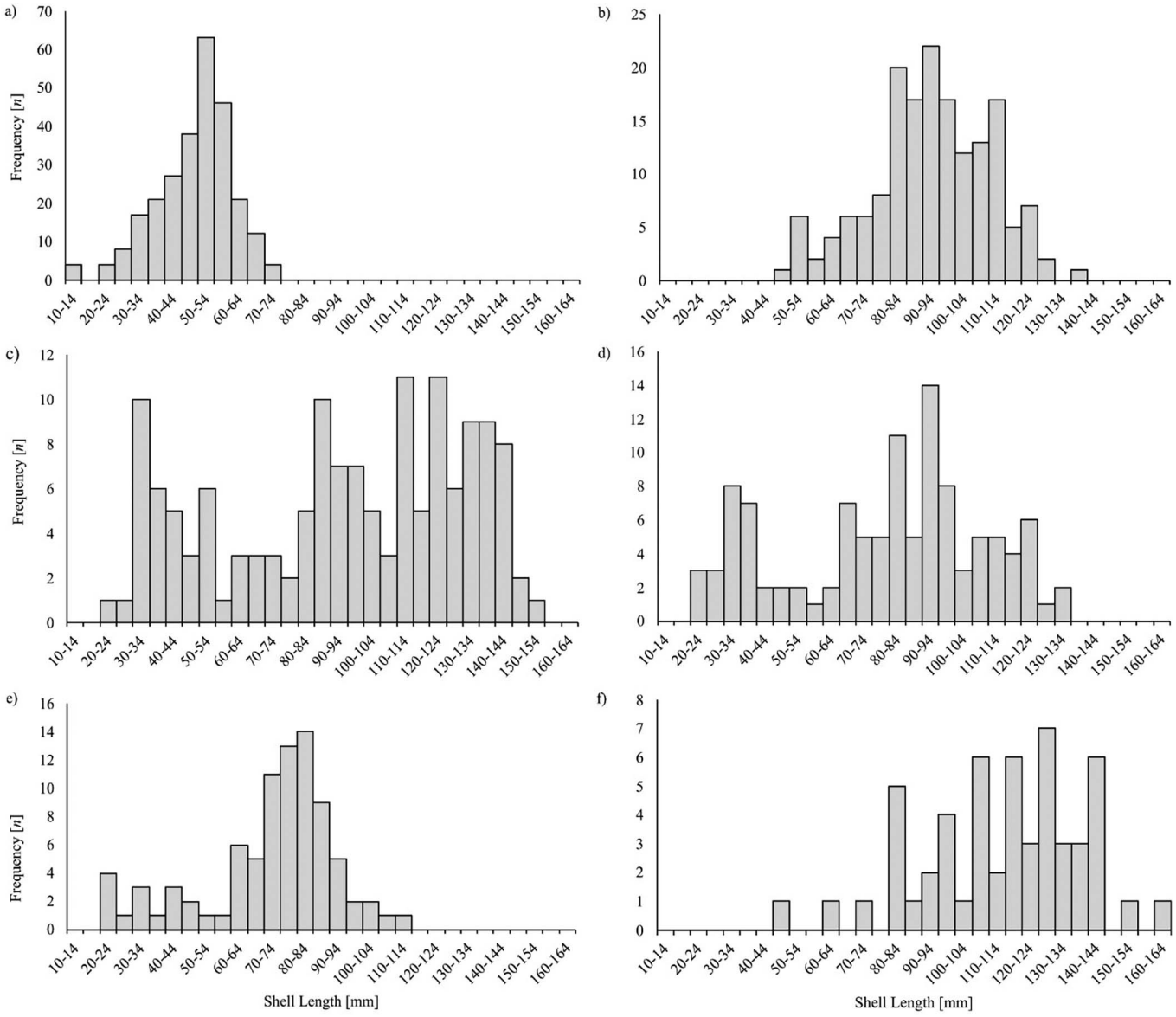

Figure 4. Shell length-frequency plots of the six most abundant species found at our 46 survey sites in the lower Wabash River. (a) *Obliquaria reflexa* ($n = 265$), (b) *Potamilus capax* ($n = 166$), (c) *Potamilus ohiensis* ($n = 143$), (d) *Potamilus fragilis* ($n = 111$), (e) *Quadrula quadrula* ($n = 85$), and (f) *Tritogonia verrucosa* ($n = 54$).

and teeth marks next to freshly dug holes, suggesting that terrestrial, opportunistic molluscivores may capitalize on these trails.

Our results generally support the greater efficiency of timed searches and transect surveys for estimating species richness but did not support the greater efficiency of quadrat surveys for detecting smaller individuals. Typically, timed searches and transects provide better estimates of species richness because a larger area can be searched, while excavated quadrat sampling provides better estimates of recruits or small species (Vaughn et al. 1997; Obermeyer 1998; Smith et al. 1999). However, in our study, average lengths in quadrat samples were longer than in timed searches or transect samples (Table 4) for two of three species. These differences may be explained by the sand/silt substrate and the wide spatial distribution of mussels. The absence of hard substrate facilitated locating mussels of all sizes (smallest individual = 12 mm). Additionally, mussels were so widely distributed that the probability of an individual being in a quadrat was low (Table 4), and quadrat sampling does not appear productive in this system. Nevertheless, our results show that the use of multiple sampling methods in conjunction can provide more robust assessments of abundance, species richness, and size distributions.

Potamilus capax

Our results indicate that the lower Wabash River continues to support *P. capax*, though it was widely spatially

Table 3. Summary of sampling data from the lower Wabash River, including survey method(s), mussel abundance, species richness and density estimates, survey effort in person-hours, diversity and evenness indices, and the relative abundances of mussel life-history strategies. Abbreviations: TS = timed search, TR = transect, QT = quadrat, *n* = total number of mussels, CPUE = catch-per-unit-effort (mussels/person-hour; data from timed searches), density = mussels/m^2 (data from quadrat samples), E = equilibrium, O = opportunistic, P = periodic, H' = Shannon Wiener species diversity index, and J' = Pielou's evenness index. A dash (–) indicates mussels were not detected.

Site	Survey Method	*n*	Species Richness	Survey Effort	CPUE	Density	Life-History Strategy			H'	J'
							% E	% O	% P		
1	TS	0	0	1	0.0	–	–	–	–	–	–
2	TS	0	0	1	0.0	–	–	–	–	–	–
3	TS	0	0	1	0.0	–	–	–	–	–	–
4	TS	5	3	6	0.8	–	–	40.0	60.0	1.05	0.96
5	TS	11	5	6	1.8	–	45.5	36.3	18.2	1.52	0.94
6	TS	113	12	6	18.8	–	19.5	16.8	63.7	1.63	0.65
7	TS	45	9	6	7.5	–	42.2	2.2	55.6	1.66	0.76
8	TS	118	13	6	19.7	–	61.0	6.8	32.2	1.95	0.76
9	TS, TR, QT	44	7	6	4.8	0.33	27.3	43.2	29.5	1.74	0.89
10	TS	43	8	6	7.2	–	11.6	41.9	46.5	1.58	0.76
11	TS	12	4	6	2.0	–	16.7	66.6	16.7	1.24	0.9
12	TS	11	6	6	1.8	–	18.2	54.5	27.3	1.72	0.96
13	TS	66	8	6	11.0	–	13.6	16.7	69.7	1.14	0.55
14	TS	23	10	6	3.8	–	69.6	13.0	17.4	1.83	0.8
15	TS, TR, QT	3	3	6	0.5	0.00	33.3	33.3	33.3	0.87	0.79
16	TS, TR, QT	9	5	6	1.3	0.00	22.2	77.8	–	1.39	0.86
17	TS	2	1	6	0.3	–	–	100.0	–	0.00	0.00
18	TS, TR, QT	28	8	6	4.2	0.00	7.1	85.8	7.1	1.58	0.76
19	TS	0	0	1	0.0	–	–	–	–	–	–
20	TS, TR, QT	64	9	6	7.3	0.00	3.2	93.6	3.2	1.33	0.61
21	TS	44	9	6	7.3	–	36.4	45.4	18.2	1.83	0.88
22	TS	15	6	6	2.5	–	26.7	33.3	40.0	1.53	0.85
23	TS, TR, QT	22	6	6	3.3	0.00	9.1	72.7	18.2	1.24	0.69
24	TS	12	4	6	2.0	–	–	33.3	66.7	0.98	0.71
25	TS, TR, QT	14	3	6	2.2	0.00	–	100.0	–	1.08	0.98
26	TS, TR, QT	25	5	6	3.2	0.33	8.0	92.0	–	1.02	0.63
27	TS	21	6	6	3.5	–	19.0	23.9	57.1	1.37	0.76
28	TS	12	3	6	2.0	–	–	100.0	–	0.82	0.75
29	TS	0	0	1	0.0	–	–	–	–	–	–
30	TS	0	0	1	0.0	–	–	–	–	–	–
31	TS, TR, QT	61	10	6	9.7	0.33	11.5	45.9	42.6	1.84	0.8
32	TS, TR, QT	77	7	6	11.5	0.00	2.6	94.8	2.6	1.14	0.58
33	TS, TR, QT	15	3	6	2.3	0.00	–	100.0	–	0.95	0.87
34	TS	2	1	6	0.3	–	–	100.0	–	0.00	0.00
35	TS	0	0	1	0.0	–	–	–	–	–	–
36	TS	16	6	6	2.7	–	31.3	25	43.7	1.49	0.83
37	TS, TR, QT	33	9	6	1.0	0.33	3.0	84.9	12.1	1.32	0.63
38	TS	13	7	6	2.2	–	15.4	69.2	15.4	1.63	0.84
39	TS	0	0	1	0.0	–	–	–	–	–	–
40	TS	0	0	1	0.0	–	–	–	–	–	–

Table 3, continued.

Site	Survey Method	*n*	Species Richness	Survey Effort	CPUE	Density	Life-History Strategy % E	% O	% P	H'	J'
41	TS	0	0	1	0.0	–	–	–	–	–	–
42	TS	0	0	1	0.0	–	–	–	–	–	–
43	ST	0	0	1	0.0	–	–	–	–	–	–
44	ST	0	0	1	0.0	–	–	–	–	–	–
45	TS, TR, QT	7	2	6	0.3	0.00	–	100.0	–	0.38	0.54
46	TS, TR, QT	10	5	6	1.0	0.33	10.0	90.0	–	1.36	0.85
Total		996	23	211	4.3	0.08	21.8	46.9	31.3		

distributed. It was located at 22 sites but was found only downstream of site 8 (Fig. 1). Abundances were low per site and only four sites had > 10 individuals. The species was usually found in water ≤ 1 m in depth within the first 10-m from the bank (> 70% of total *P. capax*), in sand and silt substrates, congruent with habitat observations made in other studies (Miller and Payne 2005; Peck et al. 2014). Interestingly, a single individual was located on the substrate surface in the midchannel of the river and may have been washed into the area (Fig. 5a).

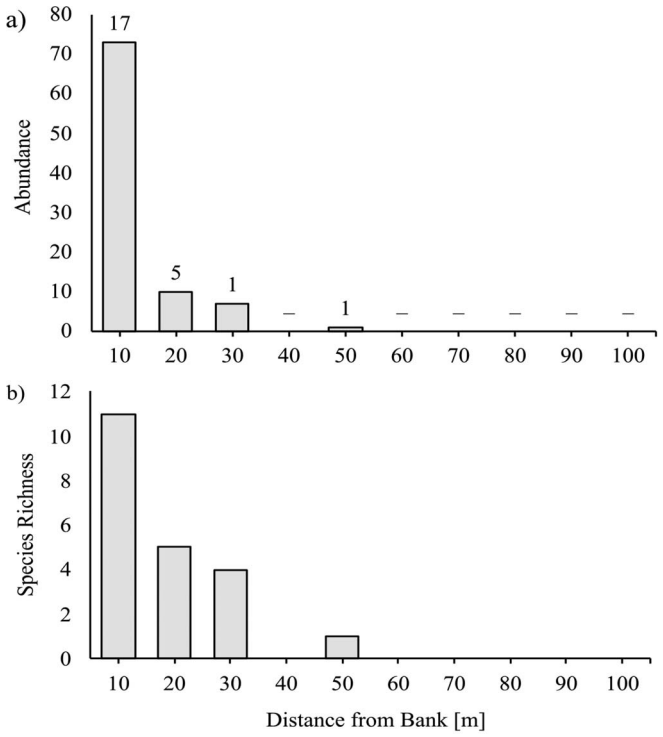

Figure 5. Mussel abundance (a) and species richness (b) per 10-m interval from transect samples. Data are combined from all transects ($n = 180$) by 10-m interval, and for all species. Values above the columns are *Potamilus capax* abundances found in each interval. A dash (–) indicates no *P. capax* were detected in the interval.

Potamilus capax mortality observed in our study was similar to that reported by Tiemann et al. (2012; 75.9%), though higher than those of other studies (Miller and Payne 2005; Peck et al. 2014). Fresh dead shells were abundant, and at some sites, they were so prevalent the entire search effort could have been spent recovering them. We found many adult live *P. capax* stranded, gaping and in distress, in shallow pools with elevated water temperatures. Though we also located fresh dead *P. ohiensis* and *P. fragilis*, neither were found in comparable numbers, despite similar behaviors, habitat preferences, and fish hosts (Haag 2012; Table 2). The high adult mortality may be a natural occurrence or an artifact of an environmental event. Future studies could explore if it was caused by a unique event, a natural generational process, or some other factor.

Despite high mortality, multiple size classes of *P. capax* were present, including several small individuals (Figs. 3 and 4b), and recruitment appears to be occurring at nearly half of sites. The rapidly declining abundance of individuals ≥ 120 mm probably indicates the size at which age-related mortality occurs. Although considered a low-density species (Miller and Payne 2005), *P. capax* appears stable and healthy in the lower Wabash River, especially compared to other federally listed mussels. For example, *Lampsilis higginsii* is thought to have historically comprised approximately 0.50% of an assemblage in the upper Mississippi River (Havlik and Marking 1981), and *Plethobasus cyphyus* made up 0.03% of the fauna in a survey of the Ohio River (Ford, unpublished data). In contrast, *P. capax* made up 16.70% of our assemblage. Unfortunately, we could not compare our data with historical assemblages, as those data are lacking. Regardless, the overall demography of the *P. capax* population mirrors other studies (Harris 2001; Miller and Payne 2005) and suggests moderate but relatively steady annual recruitment, high longevity, and moderately low annual mortality during the earliest and middle parts of the lifespan.

CONCLUSIONS

This study describes the current status and distribution of mussels in the lower Wabash River and builds on previous

Table 4. Total abundance (*n*), shell lengths (mean ± SE [range]), and proportion of recruits (Rec.) per species found using each sampling method at the 14 sites in the lower Wabash River where all sampling methods were used. Also presented are the results (*F* values [degrees freedom] and *P* values) of the ANOVA model comparing shell length among methods. A dash (–) indicates a species was not detected.

	Timed Searches			Transect Samples			Quadrat Samples			ANOVA (Shell Lengths)	
Species	*n*	Length (mm)	Rec.	*n*	Length (mm)	Rec.	*n*	Length (mm)	Rec.	*F* (df)	*P*
Equilibrium Species											
Quadrula quadrula	13	55.7 ± 6.4 (23–86)	38.5	6	73.3 ± 10.2 (26–93)	16.7	–	–	–	2.282 (1, 17)	0.149
Tritogonia verrucosa	5	125.0 ± 10.8 (96–154)	–	1	70.0 ± 0.0 (70)	–	–	–	–	4.302 (1, 4)	0.107
Opportunistic Species											
Potamilus alatus	2	123.5 ± 14.5 (109–138)	–	3	100.0 ± 20.1 (77–140)	–	–	–	–	0.700 (1, 3)	0.464
Potamilus capax	120	91.3 ± 1.6 (49–126)	0.8	24	91.0 ± 3.5 (60–126)	–	3	104.3 ± 1.9 (102–108)	–	0.871 (2, 144)	0.421
Potamilus fragilis	51	70.9 ± 3.2 (25–118)	19.6	24	70.9 ± 6.7 (24–121)	37.5	1	47.0 ± 0.0 (47)	–	0.408 (2, 73)	0.666
Potamilus ohiensis	63	82.1 ± 3.9 (30–142)	14.3	18	112.7 ± 7.0 (52–141)	–	–	–	–	13.697 (1, 79)	<0.001
Pyganodon grandis	–	–	–	1	156.0 ± 0.0 (156)	–	–	–	–	–	–
Truncilla donaciformis	2	25.0 ± 5.0 (20–30)	100.0	2	25.0 ± 8.0 (17–33)	50.0	–	–	–	0.000 (1, 2)	1.000
Truncilla truncata	4	38.0 ± 4.8 (25–48)	25.0	1	54.0 ± 0.0 (54)	–	–	–	–	2.210 (1,3)	0.234
Periodic Species											
Obliquaria reflexa	20	43.0 ± 2.4 (22–60)	45.0	9	43.8 ± 3.7 (30–57)	44.4	1	52.0 ± 0.0 (52)	–	0.324 (2, 27)	0.726
Obovaria olivaria	23	58.3 ± 3.2 (23–78)	8.7	2	56.0 ± 23.0 (33–79)	50.0	–	–	–	0.036 (1, 23)	0.851
Total	303			91			5				

research (Fisher 2006; Stodola et al. 2014). Additionally, we provide evidence that the use of multiple sampling methods in conjunction can provide more robust assessments of mussel abundance, species richness, diversity, and population demographics. Though much depleted from historical norms, the lower Wabash River continues to support about a third of its original mussel community and does not appear altered since the most recent surveys more than a decade ago. Unfortunately, suitable habitat for many species was largely lacking and will likely remain so. Regardless, the lower Wabash River will likely continue to retain reproducing populations of *P. capax* and other smooth-shelled species unless large-scale alterations of the river occur.

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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).

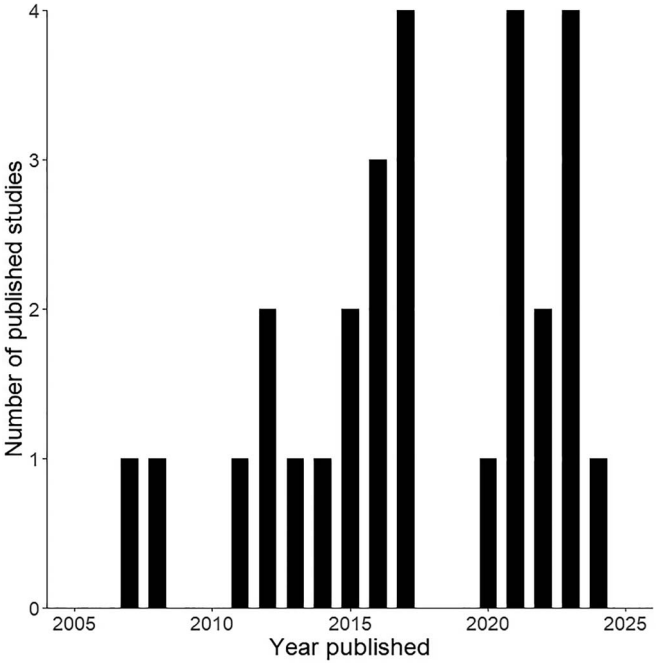

Figure 1. Cumulative number of studies published in the peer-review literature based on a search of the terms "freshwater mussels" and "PIT tags" in Web of Science and Google Scholar searches. Twenty-eight papers were published between 2007 and 2024 (as of May 8, 2024).

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 ~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).

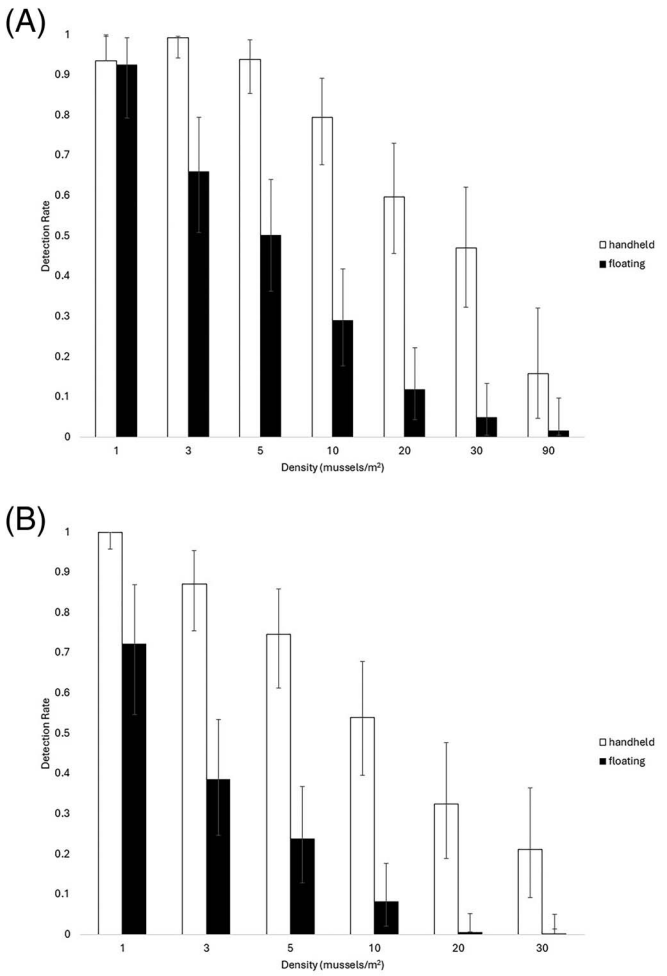

Figure 4. Linear model predictions of detection rate of tags and 95% confidence intervals using handheld (white) and floating (black) antennas as a function of tag density in a (A) 1-m × 1-m cell and (B) 3-m × 1-m cell ($P < 0.001$).

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 non-invasive 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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