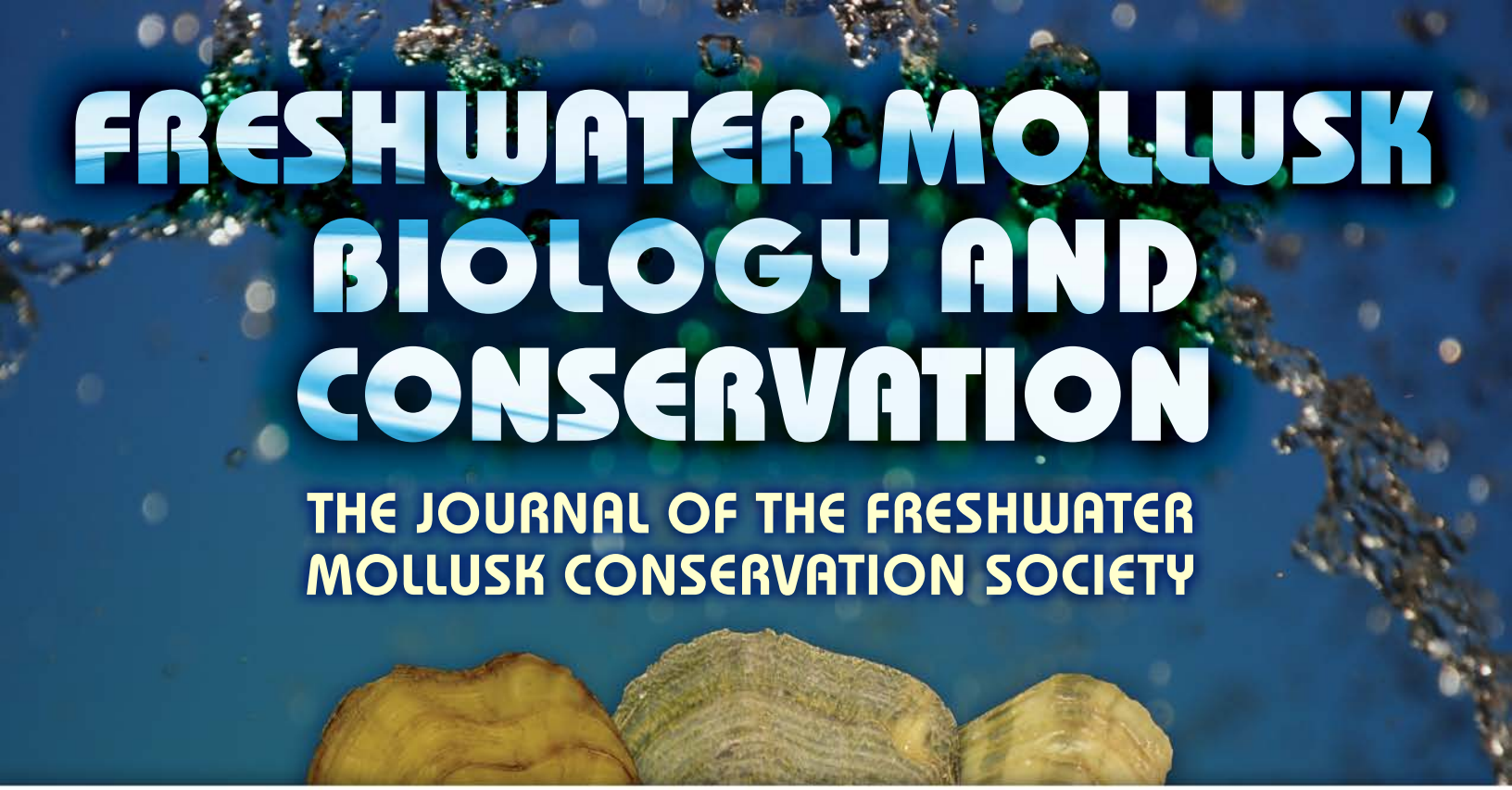

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REGULAR ARTICLE

RELOCATION OF WESTERN PEARLSHELL BEFORE AND AFTER STREAM RESTORATION IN TINCUP CREEK, IDAHO

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ABSTRACT

Freshwater mussels can be negatively affected by heavy machinery during stream restoration projects, requiring mussels to be relocated from the project area to unaffected areas. We assessed recapture and survival of Western Pearlshell (*Margaritifera falcata*) relocated in Tincup Creek, Idaho before and after a stream restoration project. From 2018 to 2020, we searched 4,350 m of Tincup Creek before restoration and salvaged 1,213 Western Pearlshell. Mussels were measured, marked with shellfish tags, and relocated among 10 sites in previously restored reaches elsewhere in Tincup Creek. At the time of salvage, mussels ranged from 19 to 84 mm with 83% of the mussels ≥50 mm, and most mussels were found in run habitats (63%). We surveyed all sites for tagged mussels 1 to 3 yr after relocation. We recaptured tagged mussels at seven of the 10 sites, and the recapture rate was positively related to the number of relocated mussels and mussel size. Tag retention was high but varied among relocation years. Estimated survival after 3 yr was 69.9–87.4% at two sites, and detection probability was 60.3–62.9%. Estimated survival after 1 yr was 55.8–91.3% at four other sites. Survival was low at three sites, likely due to low numbers of relocated mussels or scarcity of suitable habitat, and survival decreased dramatically at one site (from 91.3% to 28.6%) in 2 consecutive years, likely due to beaver activity. Our results suggest that stream restoration created habitat suitable for Western Pearlshell, and relocation was a successful strategy for avoiding direct mortality associated with restoration activities.

KEY WORDS: *Margaritifera falcata,* freshwater mussels, shellfish tags, conservation planning, unionids, North America, translocation

INTRODUCTION

In recent decades, much effort has been dedicated to stream restoration to offset negative impacts of anthropogenic degradation on aquatic habitats (Bernhardt et al. 2005). These projects are usually focused on fishes and aim to enhance habitat availability and complexity. Benefits of stream restoration can include increased macroinvertebrate abundance, increased periphyton production, and enhanced reproductive success for target fishes (Mueller et al. 2014). Stream restoration projects also can improve habitat quality for freshwater mussels, which are among the most imperiled animals in North America (Ricciardi and Rasmussen 1999; Lydeard et al. 2004; Haag and Williams 2014). However, initial restoration activities can result in direct mussel mortality from heavy equipment, burial in sediments, or stranding in dewatered channels. Managers should consider negative impacts on mussels before initiating restoration projects and make efforts to minimize those effects (Blevins et al. 2017a).

Relocation is a common strategy for temporarily or permanently removing mussels from areas that will be affected by construction or other human activities (Cope and Waller 1995; Eveleens and Febria 2022). Mussel survival after relocation varies widely among projects (Cope and Waller 1995; Tiemann et al. 2016), but proper collecting and handling practices, as well as prior evaluation of habitat suitability and mussel density at the relocation site, can increase the chances of success (Bolden and Brown 2002; Luzier and Miller 2009). Careful documentation and monitoring of relocation projects

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can provide additional case studies for improving relocation methods and success (Cope and Waller 1995, Hamilton et al. 1997).

We assessed the success of relocating Western Pearlshell (*Margaritifera falcata*) in Tincup Creek, Idaho before and after a stream restoration project. Western Pearlshell is considered near threatened globally and imperiled and a species of greatest conservation need in Idaho (Blevins et al. 2017b; Idaho Department of Fish and Game 2017). The project required use of heavy machinery, which likely would have resulted in direct mussel mortality in the project area. We salvaged mussels from the project area, relocated them to 10 previously restored sites elsewhere in Tincup Creek, and assessed recovery, tag retention, and survival after relocation.

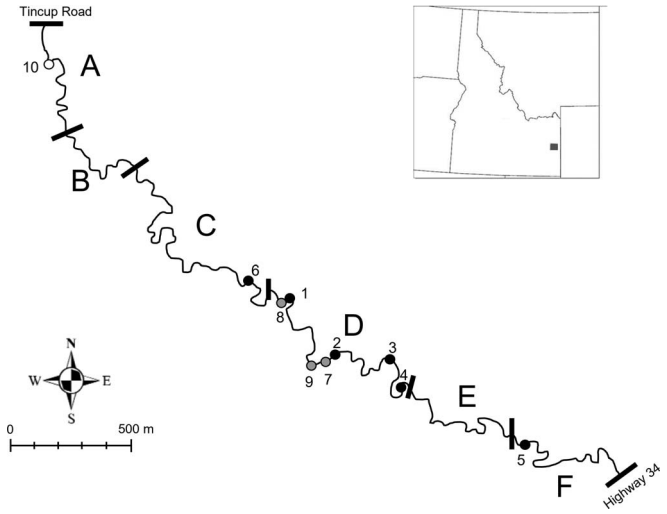

Figure 1. Map of the study sites in Tincup Creek, Idaho. Reaches (A-F) are delineated with thick black lines. Relocation sites are indicated by numbered dots; black dots represent sites to which mussels were relocated in 2018, gray dots represent relocation in 2019, and the white dot represents relocation in 2020.

METHODS

Study Area and Restoration Project

Tincup Creek is a 60-km-long tributary of the Salt River in the upper Snake River drainage in Bonneville and Caribou counties, Idaho. The stream flows east off the Caribou Range in the Caribou–Targhee National Forest and drops from 2,766 to 1,445 m in elevation from source to mouth. The hydrograph is typical of snowmelt-driven systems, having high spring flows followed by base flows for the remainder of the year.

Restoration of Tincup Creek was a collaborative project by Trout Unlimited, the U.S. Forest Service, and other groups; it was designed to improve habitat for Cuttthroat Trout (*Oncorhynchus clarkii*) and other aquatic species by addressing channel destabilization caused by prior removal of riparian vegetation. Specific actions included reconnecting historical meanders, planting willows in riparian areas, elevating riffles, and adding large woody debris. The project took place within a 6.5-km section of upper Tincup Creek from the Tincup Road bridge (U.S. Forest Service Road 117) downstream to the Highway 34 bridge (Fig. 1). Heavy equipment was used in the restoration and portions of the existing channel were dewatered, which prompted concern about the negative effects on Western Pearlshell (Blevins et al. 2017a). Restoration took place in phases from 2017 to 2020 in different reaches (Fig. 1). Restoration was completed in reaches D and F in 2017, Reach E in 2018, reaches A and B in 2019, and Reach C in 2020. Restoration of Reach A was originally scheduled to be completed in 2018 but was delayed until 2019.

Mussel Salvage

We did not salvage mussels from reaches D and F before restoration; however, mussels observed within these reaches during restoration prompted concerns about the impact of restoration on mussels in other reaches. Consequently, we salvaged mussels from reaches A and E in 2018, reaches A and B in 2019, and Reach C in 2020 before each reach was restored. Reach lengths were as follows: A, 843 m; B, 754 m; C, 1,849 m; and E, 928 m. Salvage occurred in all reaches in June and July at or near base flow; water temperatures were 8–18°C. We salvaged mussels by having two people search the entire reach in an upstream direction using plexiglass-bottomed view buckets. We carefully removed mussels from the substrate and placed them in mesh bags that remained in the stream during salvage. In each reach, we recorded the macrohabitat type (i.e., pool, riffle, or run) in which each mussel was found. Salvage in each reach required 45 to 96 person-hours.

We measured the length of each mussel from anterior end to posterior end and affixed two 8- × 4-mm polyethylene shellfish tags (model FPN8X4; Hallprint, Hindmarsh Valley, Australia) to the shell of each mussel with Loctite 60 Second Universal Glue (Henkel, Düsseldorf, Germany), which was allowed to dry for at least 30 s. The maximum time mussels were out of the water for measuring and tagging was 3 min.

Characterization of Mussel Habitat

In addition to recording the macrohabitat type where each mussel was found in each reach, we characterized Western Pearlshell habitat use during mussel salvage in reaches A and E in 2018 to guide subsequent relocation efforts in Tincup Creek. We divided each habitat unit where mussels were found into five equally spaced transects (perpendicular to flow) and measured the wetted width, three depths (at 25%, 50%, and 75% of channel width), and thalweg water velocity at each transect. We quantified substrate size in each transect using a modified pebble count (Wolman 1954), in which we measured the size of four substrate particles at equally spaced points across the transect (total = 20 particles/habitat unit) with a gravelometer (Wildco, Yulee, FL. USA).

Table 1. Number of Western Pearlshell (*Margaritifera falcata*) relocated from 2018 to 2020 and recaptured in 2019 to 2021 at 10 sites in Tincup Creek, Idaho. Not applicable (NA) indicates that the site was not surveyed for mussels that year.

Year Relocated	Reach	Site	Number Relocated	Recaptures in 2019	Recaptures in 2020	Recaptures in 2021
2018	D	1	28	0	0	NA
	D	2	22	0	0	NA
	D	3	117	64	68	61
	D	4	83	37	36	32
	F	5	62	0	0	NA
	C	6	96	NA	33	NA
2019	D	3	17	—	9	13
	D	7	64	—	NA	36
	D	8	51	—	28	9
	D	9	52	—	28	NA
2020	A	10	621	—	—	284

Mussel Relocation

After salvage and tagging, we relocated mussels to previously restored reaches of Tincup Creek. We relocated mussels to reaches C, D, and F in 2018, D in 2019, and A in 2020 (Table 1). We relocated mussels to one to seven sites within each reach (Reach A, one site; Reach C, one site; Reach D, seven sites; Reach F, one site). We chose relocation sites that had habitat types similar to those identified during salvage in reaches A and E (see Results), and we avoided livestock crossings. Sites were 20 to 30 m long; we placed mussels in these smaller areas to facilitate relocation and monitoring. However, site 10 was approximately 145 m long because of the high number of mussels relocated to this site. Relocation sites had a mean depth of 0.32 m (0.08 SD) and a mean thalweg velocity of 0.45 m/s (0.24 SD). Median substrate size generally was large gravel (32–64 mm), except for site 5, which had a median substrate size of small gravel (2–32 mm). We relocated mussels to each site in only 1 yr, except for site 3, to which we relocated mussels in 2018 and 2019.

We transported mussels to relocation sites in mesh bags placed in buckets with water; transit time was 10–30 min. Before placing mussels in the stream, we searched the site for resident mussels for about 30 min with a view bucket; we did not find resident mussels at any relocation site. We placed mussels in runs or riffles and avoided deep pools or low-flow areas. We placed relocated mussels on their side on the substrate surface and allowed them to burrow into the substrate; we did not attempt to bury the mussels to avoid damaging them (Blevins et al. 2017a). In areas with strong current, we placed mussels in pockets near large rocks or boulders to lessen the chances of dislodgement.

Postrelocation Surveys

We conducted mussel surveys at all relocation sites from 2019 to 2021 to evaluate relocation success. We surveyed each site one to three times (Table 1); surveys occurred 1 to 3 yr after relocation. We surveyed for mussels using plexiglass-bottomed view buckets throughout and within 100 m upstream and downstream of each site; search time at each site averaged 4 person-hours. We measured each mussel encountered and inspected it for the presence of tags. After the survey was completed, we released mussels where they were found within the relocation site. Mussels that were recaptured at site 6 in 2020 were moved to site 10 because restoration was scheduled for 2020 at that site.

Data Analysis

To determine the effect of mussel size on the probability of recapture after 1 yr, we used a generalized linear model with a binomial response in R (R Development Core Team 2018). We used recapture data collected in 2019, 2020, and 2021 that represented recaptures 1 yr after mussels had been relocated. Mussels that were not recaptured were given a value of 0, whereas mussels that were recaptured were given a value of 1. We determined the significance of mussel size on the probability of recapture using a drop-in-deviance test assuming a chi-squared distribution of deviances (Rasmussen and Belk 2012).

We estimated survival of relocated mussels using recapture data. We first estimated survival and detection probabilities for mussels at sites 3 and 4 because we surveyed those sites in 3 consecutive years (2019–2021). Our initial surveys found no resident mussels at any site before relocation (see previous), and all recaptured mussels were tagged. Therefore, we were unable to use simple mark–capture estimators that compare the proportions of marked and unmarked individuals. For sites 3 and 4, we estimated the abundance of surviving mussels at each site using the Schnabel estimator with 95% confidence intervals (CIs) estimated using the normal approximation (Krebs 1998). For the Schnabel estimator we used three sample occasions (2019, 2020, and 2021). The survey for recaptures in 2019 was considered the first sample occasion, and mussels captured in 2019 were considered captured for the first time for the Schnabel estimator. For 2020 and 2021, we considered mussels that had not been recaptured in previous sample occasions as "unmarked," whereas mussels that had been recaptured in previous sample occasions were considered "marked." We estimated survival of mussels at sites 3 and 4 by dividing estimated abundance and 95% CIs by the number of mussels originally relocated at each site. We estimated detection probability for sites 3 and 4 by dividing the number of mussels recaptured by the estimated abundance of surviving mussels. On the basis of the similarity of detection probabilities at sites 3 and 4 (see Results), we assumed that detection was similar at the other sites. We used the mean detection probability for sites 3 and 4 to estimate the number of surviving mussels at the other sites that only had one or two recapture occasions (sites 6–10).

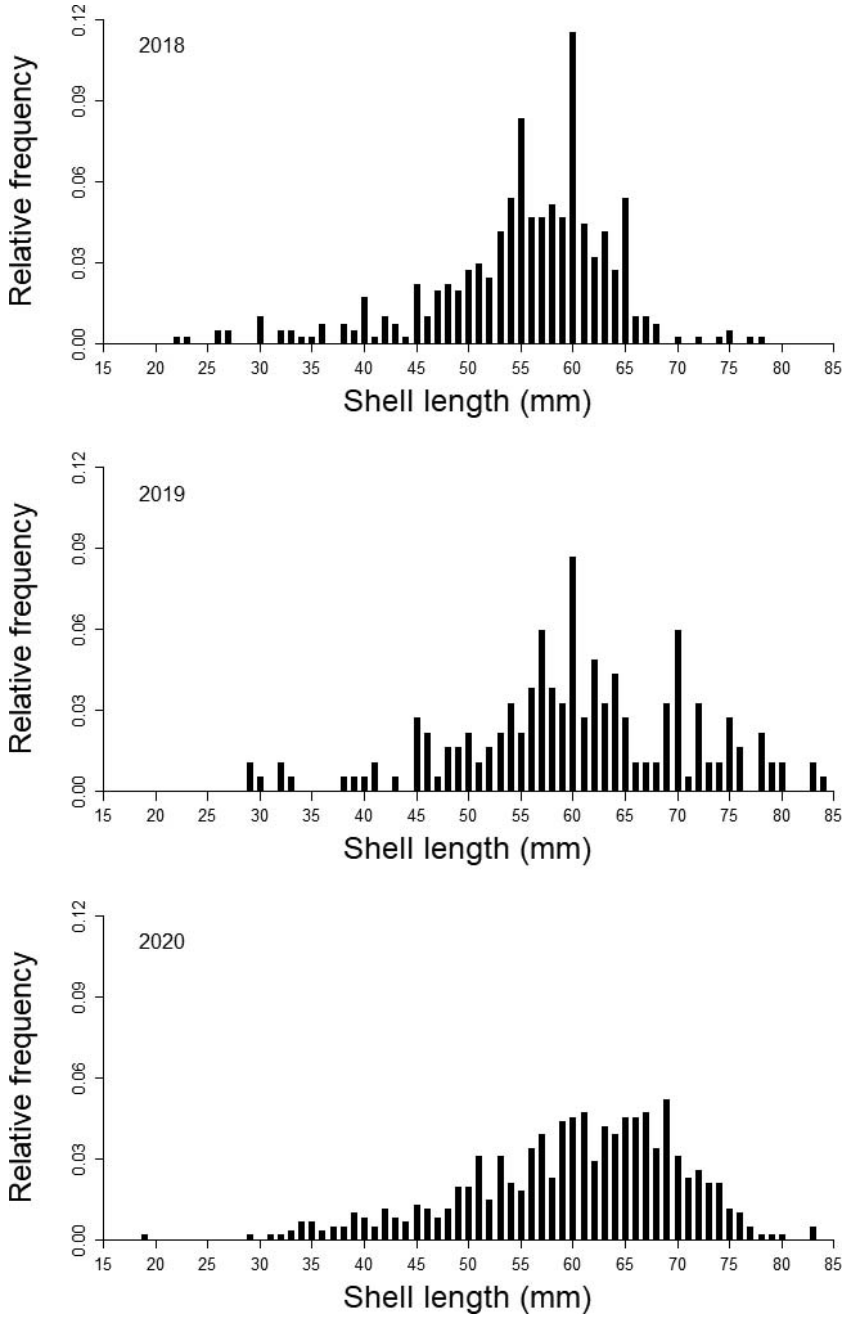

Figure 2. Size frequency distribution of Western Pearlshell (*Margaritifera falcata*) relocated in 2018 ($N = 408$ mussels), 2019 ($N = 184$ mussels), and 2020 ($N = 621$ mussels) in Tincup Creek, Idaho.

RESULTS

We salvaged and relocated a total of 1,213 Western Pearlshell from reaches A–C and E in Tincup Creek from 2018 to 2020. The size distribution of mussels at the time of salvage and relocation was similar among years; mussels were between 19 and 84 mm, and 80% of individuals were ≥50 mm (Fig. 2). Most Western Pearlshell (63%) were found in runs; 16% occurred in riffles and 21% in pools. Mussels in pools and riffles were often found in the short, runlike transition between riffles and pools where the channel was deeper than that found in the riffles, but the water velocity had not slowed completely to mean pool water velocity.

Mean channel depth in habitats where mussels were salvaged in 2018 was 40 cm ± 6.7 cm (SE) in Reach A and 29 cm ± 5.1 cm (SE) in Reach E. Mean thalweg water velocity in habitats where mussels were salvaged was 1.30 m/s ± 0.309 m/s (SE) in Reach A and 0.44 m/s ± 0.073 m/s (SE) in Reach E. Habitats where mussels were salvaged in both reaches had a similar mean substrate size (Reach A = 44 mm; Reach E = 48 mm) and the same median substrate size (32 mm).

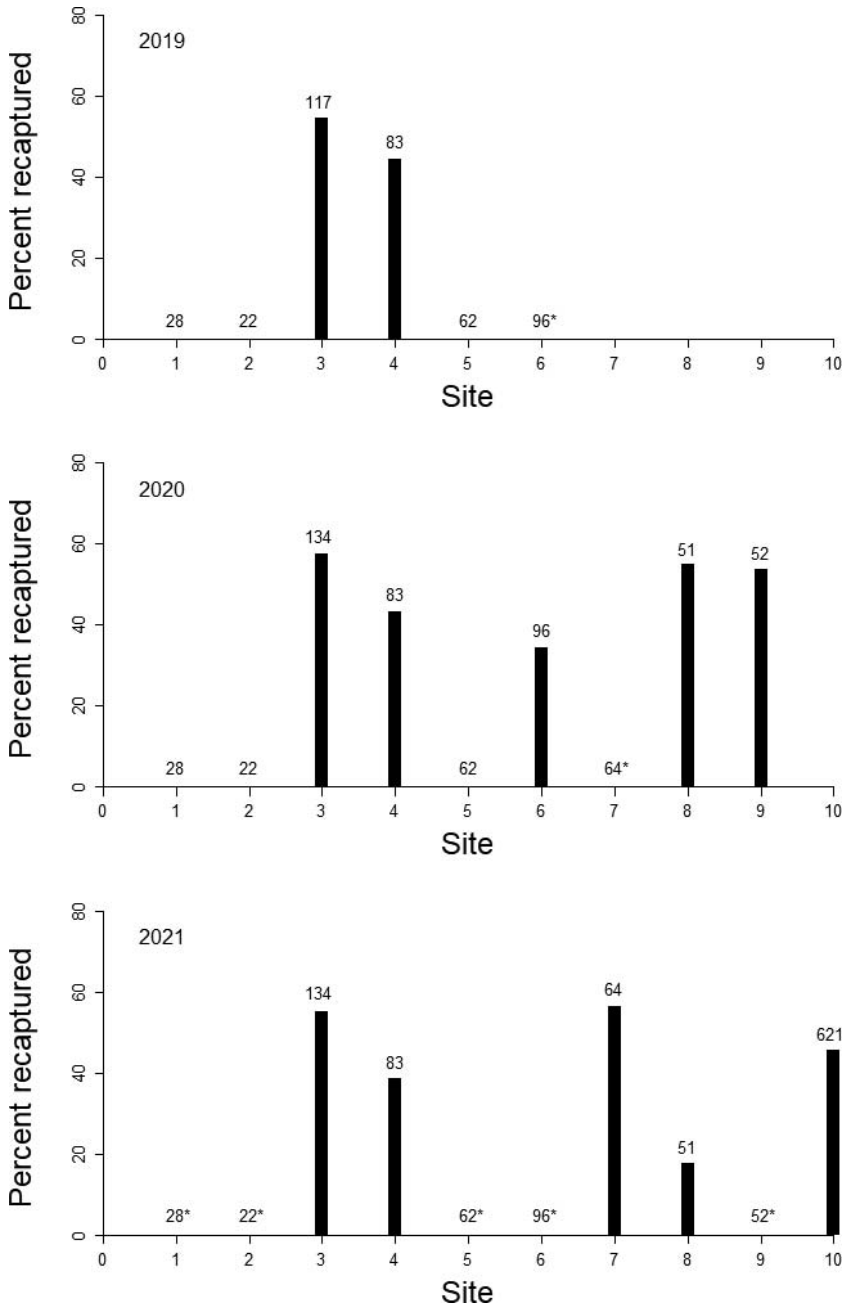

Figure 3. Percentage of Western Pearlshell (*Margaritifera falcata*) recaptured at relocation sites in Tincup Creek, Idaho in 2019, 2020, and 2021. Numbers above bars indicate the number of mussels relocated at each site. Note: the increase of mussels relocated at site 3 in 2020 reflects additional mussels relocated to the site in 2019 (see Table 1). An asterisk (*) indicates that the site was not surveyed for mussels during that year.

We recaptured Western Pearlshell at seven of the 10 sites to which mussels were relocated (Table 1; Fig. 3). At the sites that we sampled 1 yr after relocation (sites 1–5, 8–10), the number of recaptures was predicted remarkably well by the number of relocated mussels ($y = 0.468x - 4.695$, $R^2 = 0.983$, $P < 0.0001$), and the recapture rate after 1 yr was similar among sites at which mussels were recaptured (44.6–54.9%). Some mussels were recaptured in multiple years; we recaptured 71 mussels in 2 different years and 44 mussels in 3 different years. The recapture rate was similar between the first year and after 3 yr at sites 3 and 4, but it declined markedly at site 8 after 2 yr (17.7%). The probability of recapturing Western Pearlshell 1 yr after being relocated was positively related to mussel size ($\chi^2 = 51.32$; $P < 0.001$; Fig. 4). The few dead and tagged mussels we found were discovered only in 2021: two mussels from site 3 and three mussels from site 8.

Tag retention varied among years in which mussels were relocated (Table 2). For mussels relocated in 2018 and 2019, an average of 83.8% ± 5.9% (SE) retained both tags in each

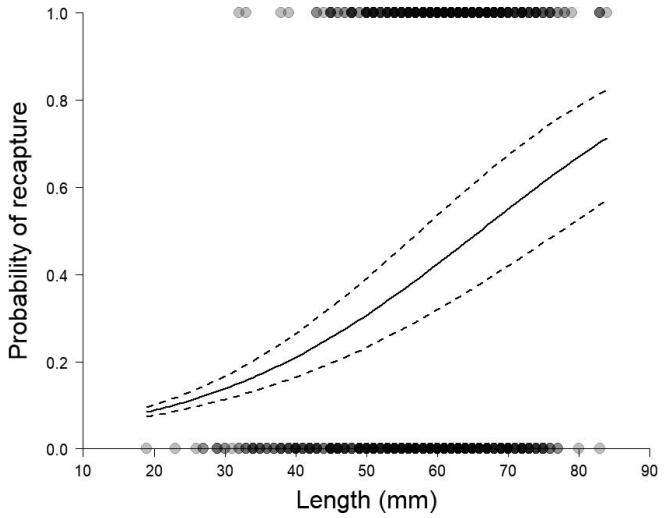

Figure 4. Probability of recapture of Western Pearlshell (*Margaritifera falcata*) as a function of mussel size 1 yr after relocation in Tincup Creek, Idaho. Recaptured mussels ($N = 157$) have a value of 1, whereas undetected mussels ($N = 146$) have a value of 0. Points are shaded on the basis of the number of individuals per point, with darker points representing a greater number of individuals. Equation of regression line is logit (recapture) $= -3.35 + 0.051 \times$ length. Error bars represent ± 1 SE.

recapture event, 15.5 % ± 4.9% (SE) retained one tag, and only three mussels were found with no tags (<1%). However, only 52.2% of mussels relocated in 2020 retained both tags after 1 yr, 30.0% retained one tag, and 17.7% lost both tags. Mussels that had lost both tags retained glue on the shell; we did not find mussels without glue.

Estimated survival after 3 yr was 87.4% (95% CI = 73.0–98.3) at site 3 and 69.9% (54.9–96.1) at site 4. Detection probability was 62.9% ± 3.43% (SE) at site 3 and 60.3% ± 4.56% (SE) at site 4. On the basis of the mean recapture probability for sites 3 and 4 (61.6%), estimated survival of mussels after 1 yr was 55.8% at site 6, 74.2% at site 10, and 87.4% at site 9. Estimated survival after 2 yr was 91.3% at site 7. At site 8, estimated survival was 89.1% after 1 yr, but it decreased to 28.6% after 2 yr. The lack of recaptures in 2 yr of sampling at sites 1, 2, and 5 suggested that mussels did not survive after relocation to these sites.

Table 2. Retention of shellfish tags on Western Pearlshell (*Margaritifera falcata*) in Tincup Creek, Idaho 1 to 3 yr after relocation.

Year Relocated	Year Recaptured	Number of Mussels Recaptured	Number with Two Tags	Number with One Tag	Number with No Tags
2018	2019	101	90	11	0
	2020	136	120	16	0
	2021	105	82	21	2
2019	2020	65	50	14	1
	2021	61	53	8	0
2020	2021	276	144	83	49

DISCUSSION

Survival of relocated Western Pearlshell at most of our sites was high and comparable with survival rates reported in previous studies (71–93%, Tiemann et al. 2016). Earlier mussel relocations reported generally poorer survival (~50%; Cope and Waller 1995). We followed recent improvements in relocation protocols, such as avoiding extreme temperatures and overcrowding and keeping mussels moist (e.g., Blevins et al 2017a), which may have been responsible for high survival at most sites. Mussel mortality after relocation can be caused by handling stress during relocation or environmental factors at recipient sites. Mortality caused by handling stress is most likely to occur within the first year after relocation (Cope and Waller 1995). It is unlikely that low survival after 1 yr at sites 1, 2, and 5 was caused by handling stress because we used consistent relocation methods for all sites. The strong relationship we found between initial relocation number and recaptures suggests that the low recapture and survival rates at sites 1 and 2 were due simply to the low number of relocated mussels at those sites. Low survival at other sites may have been due to environmental factors. The low survival we observed at site 5 may have been due to the scarcity of suitable run habitat at that site. The abrupt decline in survival at site 8 between 2020 and 2021 may have been caused by construction of a beaver dam 100 m upstream, which rerouted the stream into an old channel and lowered current velocity at the relocation site by 2021.

Selection of suitable relocation sites is the most important consideration to be made before relocation (Dunn et al. 1999). Characteristics of sites that support healthy mussel assemblages, such as substrate composition and stability, stream size, surface geology, hydrological variability, and riparian vegetation, can be used to guide relocation site selection (e.g., Stober 1972; Vannote and Minshall 1982; Lewis and Riebel 1984; DiMaio and Corkum 1995; Morris and Corkum 1996). Our characterization of Western Pearlshell habitat use and subsequent selection of relocation sites on the basis of those criteria resulted in generally high mussel survival. Notably, we observed low survival at the site (5) that deviated most widely from our characterization of suitable habitat, which is similar to, and augments, previous characterizations for Western Pearlshell (Stober 1972; Vannote and Minshall 1982; Stone et al. 2004). We were unable to statistically test the relationship between relocation success and specific habitat variables, but our results demonstrate that careful consideration of habitat characteristics at relocation sites can lead to successful mussel relocation.

It is more difficult to anticipate other environmental factors during relocation site selection. Beavers are a natural and formerly abundant part of the ecosystem in streams that supported large Western Pearlshell populations (Humphries and Winemiller 2009), and beavers can have positive influences on mussel populations (Bylak et al. 2020). Future relocation efforts should weigh potential positive effects of beavers on overall stream health against localized negative effects, such as those we observed in our study. Other

environmental factors such as drought and floods are difficult to predict, but selection of optimal habitats for relocation can maximize the chances that sites are resilient to those factors.

Our use of two shellfish tags/mussel was effective for short-term monitoring of relocation success, and >98% of mussels tagged in 2018 and 2019 retained at least one tag for as long as 3 yr. The lower tag retention we observed in 2020 may have been due to a combination of insufficient time for the glue to dry and placing a higher number of mussels in mesh bags after tagging, which may have dislodged tags. In addition to allowing sufficient time for the glue to dry, tag retention may be improved by placing mussels by themselves in water to provide additional time for the glue to cure before placing mussels in mesh bags or back in the stream (Lemarie et al. 2000). Nevertheless, 82% of mussels marked in 2020 retained at least one tag. Passive integrated transponder (PIT) tags can improve mussel detection, especially for small mussels, against which our sampling was biased (Kurth et al. 2007; Hua et al. 2015; Tiemann et al. 2016). However, PIT tags are also subject to loss, and these tags and associated equipment are more costly than shellfish tags.

To our knowledge, no previous studies have evaluated the effectiveness of stream restoration involving major channel reconfiguration in creating habitat suitable for mussels. Because all the potential relocation sites available to us were in previously restored reaches, we were not able to evaluate success of relocation into control reaches that were not restored. However, the high survival we observed at most sites indicates that newly restored habitats in Tincup Creek were suitable for Western Pearlshell. Continued monitoring is needed to determine the long-term success of Western Pearlshell relocation in Tincup Creek, but our initial results demonstrated that relocation was an effective conservation tool for avoiding direct mussel mortality associated with stream restoration.

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REGULAR ARTICLE

DISTRIBUTION AND STATUS OF FRESHWATER MUSSELS IN THE BEAR CREEK WATERSHED, MISSISSIPPI

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ABSTRACT

Bear Creek is a tributary of the Tennessee River in northwestern Alabama and northeastern Mississippi. The watershed supports a diverse freshwater mussel assemblage including several species of conservation concern. We conducted a mussel survey at 55 sites in the Mississippi portions of Bear Creek and its largest tributary, Cedar Creek, during September and October 2020. We found a total of 30 species, of which 25 were represented by live individuals. The invasive Asian Clam, *Corbicula fluminea*, was widespread in the watershed, but we found no evidence of Zebra Mussel, *Dreissena polymorpha*. Notable species found live included two federally endangered species, Cumberlandian Combshell, *Epioblasma brevidens* and Slabside Pearlymussel, *Pleuronaia dolabelloides*; one federally threatened species, Rabbitsfoot, *Theliderma cylindrica*; and two state endangered species. In addition, we report the first documented occurrence of the Mountain Creekshell, *Villosa vanuxemensis*, in Mississippi. Mussel abundance and species richness were low at most sites in the watershed, but the upper portion of Bear Creek had the highest mussel abundance and species richness. We compare our results with previous surveys in the watershed and discuss conservation issues pertinent to the Bear Creek mussel fauna.

KEY WORDS: Unionidae, threatened, survey, Tennessee River system

INTRODUCTION

Bear Creek is a major tributary within the Tennessee River system that supports one of the most diverse freshwater mussel faunas on Earth (Haag 2012). The Bear Creek watershed covers approximately 2,450 km^2 in northwestern Alabama and northeastern Mississippi. Bear Creek flows 219 km from its headwaters to its confluence with the Tennessee River. In Mississippi, Bear Creek flows approximately 44 km through Itawamba and Tishomingo counties and converges with Cedar Creek, a major tributary, at the Alabama–Mississippi border (Fig. 1). Although it retains a diverse mussel fauna, Bear Creek historically contained several species that now appear to be extirpated, and the fauna in general may have declined (McGregor and Garner 2004). The causes of mussel declines and species loss in Bear Creek are unknown, but the watershed has experienced a wide range of anthropogenic modifications.

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The upper portion of the Bear Creek watershed in Alabama is impounded by four Tennessee Valley Authority (TVA) dams constructed between 1969 and 1979 for flood control and recreation. These include two dams on Bear Creek, one on Little Bear Creek, and one on Cedar Creek. As Bear Creek enters Mississippi, two separate elevated channelized sections run alongside the sinuous original channel. These channelized sections were constructed in 1973 by TVA as overflow channels to alleviate flooding and reduce bank erosion during high-flow events. A grade-control structure is present at the head of both sections and consists of about 50 m of large riprap that slowly drops in elevation until it reaches the channelized streambed. Both sections hold water during low flow but are stagnant and do not provide suitable mussel habitat. After leaving Mississippi, Bear Creek flows back into Alabama where the lower 30 km of Bear Creek are inundated by the backwaters of Pickwick Reservoir, which was constructed in 1938 (McGregor and Garner 2004).

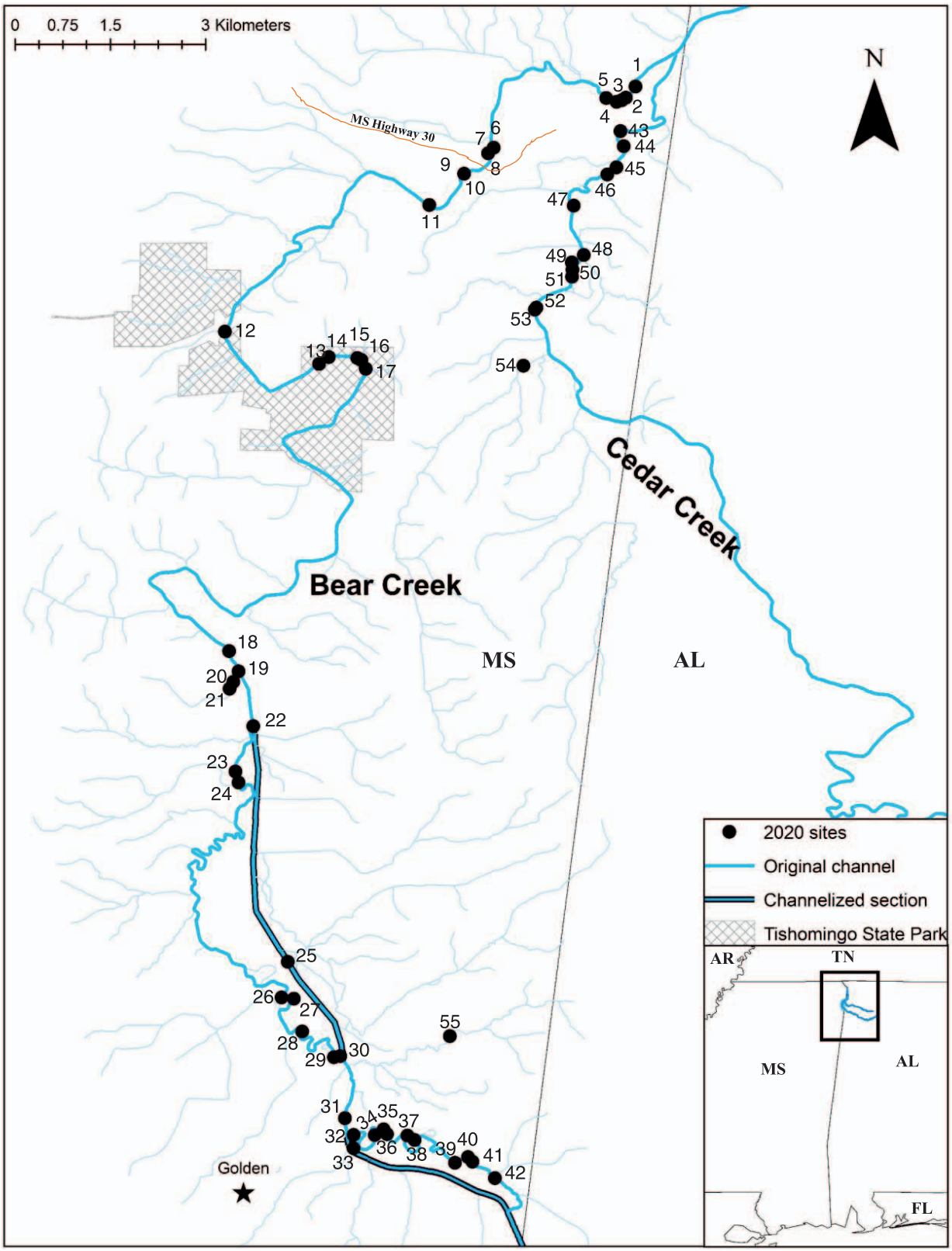

Figure 1. Map of the Bear Creek watershed in Mississippi showing sites sampled for mussels in 2020.

Table 1. Mussel species documented in the Bear Creek watershed, Alabama and Mississippi. MMNS = Mississippi Museum of Natural Science, Jackson, MS. L = live individuals reported; FD = freshly dead shells only; L/FD = live individuals or freshly dead shells reported, but not differentiated; R = relic shells only; X = species present but condition not reported; — = species not reported. All records reported by MMNS and this study are from Mississippi. Records reported by other studies from Mississippi are indicated by an asterisk (*), and their condition (if reported) is given in parentheses.

Species	Ortmann (1925)	Isom and Yokely (1968)	McGregor and Garner 1995-2000 (2004)	MMNS 1999-2008	MMNS 2009-2018	This Study (2020)
Unionids						
Actinonaias pectorosa (Conrad, 1834)	X	—	—	—	—	—
Alasmidonta marginata (Lea, 1858)	X	—	—	—	—	—
Amblema plicata (Say, 1817)	X	X	L/FD(R*)	L	—	L
Arcidens confragosus (Say, 1829)	—	—	L/FD	FD	—	L
Cyclonaias pustulosa (Lea, 1831)	X	X	L/FD(L/FD*)	L	R	L
Cyclonaias tuberculata (Rafinesque, 1820), SE[a]	—	—	L/FD(R*)	L	—	L
Ellipsaria lineolata (Rafinesque, 1820)	—	X	L/FD(R*)	L	—	R
Elliptio crassidens (Lamarck, 1819)	—	X	L/FD(R*)	L	R	L
Epioblasma brevidens (Lea, 1831), SE, FE	—	X	L/FD	FD	L	L
Epioblasma capsaeformis (Lea, 1834), FE	X	—	—	—	—	—
Epioblasma triquetra (Rafinesque, 1820), SE, FE	X*	—	—	—	—	—
Epioblasma turgidula (Lea, 1858), FE	X	—	—	—	—	—
Fusconaia cuneolus (Lea, 1840), FE	X	—	—	—	—	—
Lampsilis abrupta (Say, 1831), FE	—	—	L/FD	—	—	—
Lampsilis fasciola Rafinesque, 1820	X	X	L/FD	FD	FD	L
Lampsilis ovata (Say, 1817)	X	X	L/FD(R*)	L	L	L
Lampsilis teres (Rafinesque, 1820)	—	—	—	—	FD	L
Lampsilis virescens (Lea, 1858), FE	X	—	—	—	—	—
Lasmigona complanata (Barnes, 1823)	—	—	L/FD	L	—	L
Lasmigona costata (Rafinesque, 1820), SE	X	—	L/FD	R	—	L
Ligumia recta (Lamarck, 1819), SE	—	X	L/FD(R*)	L	—	L
Megalonaias nervosa (Rafinesque, 1820)	—	X	L/FD(L/FD*)	L	R	L
Obliquaria reflexa Rafinesque, 1820	—	X	L/FD(L/FD*)	FD	—	L
Obovaria subrotunda (Rafinesque, 1820), SE, FC	X	—	—	—	—	—
Pleurobema oviforme (Conrad, 1834)	X	—	R	—	—	—
Pleuronaia barnesiana (Lea, 1838), FC	X	X*	—	—	—	—
Pleuronaia dolabelloides (Lea, 1840), SE, FE	—	—	L/FD	L	L	L
Potamilus alatus (Say, 1817)	—	X	L/FD(L/FD*)	L	L	L
Potamilus ohiensis (Rafinesque, 1820)	—	—	FD	—	—	—
Potamilus fragilis (Rafinesque, 1820)	—	X	L/FD(R*)	L	—	L
Ptychobranchus fasciolaris (Rafinesque, 1820), SE	X	—	L/FD(R*)	L	—	FD
Pyganodon grandis (Say, 1829)	—	—	L/FD(R*)	—	—	R
Quadrula apiculata (Say, 1829)	X	—	L/FD	FD	R	—
Quadrula quadrula (Rafinesque, 1820)	—	—	L/FD(R*)	L	R	L
Reginaia ebenus (Lea, 1831)	—	—	L/FD	FD	—	L
Strophitus undulatus (Say, 1817)	—	—	(R*)	FD	—	L
Theliderma cylindrica (Say, 1817), SE, FT	X	X	L/FD	L	R	L
Toxolasma lividum Rafinesque, 1831	X	—	—	—	—	—
Toxolasma parvum (Barnes, 1823)	—	—	FD	—	—	—
Tritogonia verrucosa (Rafinesque, 1820)	X	X	L/FD(R*)	L	—	L
Truncilla donaciformis (Lea, 1828)	—	X	L/FD	FD	—	L
Truncilla truncata Rafinesque, 1820	X	X	L/FD	FD	FD	L
Utterbackia imbecillis (Say, 1829)	—	—	L/FD	—	—	FD
Utterbackiana suborbiculata (Say, 1831)	—	—	L/FD	—	—	—
Villosa iris (Lea, 1829)	X	—	—	—	—	—
Villosa vanuxemensis (Lea, 1838)	X	—	L/FD	—	—	FD
Nonnative Bivalves						
Corbicula fluminea (Müller, 1774)	—	—	L	L	L	L
Dreissena polymorpha (Pallas, 1771)	—	—	R	—	—	—

[a]SE = state endangered; FT = federally threatened; FE = federally endangered; FC = candidate for federal listing.

Table 2. Locations of sites surveyed in the Bear Creek watershed, Mississippi in 2020.

Site	Locality	Date	Latitude	Longitude	Time (min)
1*	Bear Creek upstream of Indian Mound	September 8, 2020	34.64549	−88.13305	87
2	Bear Creek upstream of Indian Mound	September 8, 2020	34.64393	−88.13441	138
3	Bear Creek upstream of Indian Mound	September 8, 2020	34.64357	−88.13498	189
4	Bear Creek upstream of Indian Mound	September 8, 2020	34.64333	−88.13574	78
5*	Bear Creek upstream of Indian Mound	September 8, 2020	34.64388	−88.13719	75
6	Bear Creek downstream Highway 30	Septembe 10, 2020	34.63684	−88.15314	200
7	Bear Creek downstream Highway 30	September 10, 2020	34.63605	−88.15393	120
8	Bear Creek downstream Highway 30	September 10, 2020	34.63605	−88.15393	124
9	Bear Creek upstream Highway 30	September 29, 2020	34.63315	−88.15733	69
10	Bear Creek upstream Highway 30	September 29, 2020	34.63315	−88.15733	24
11	Bear Creek upstream Highway 30	September 29, 2020	34.62872	−88.16227	333
12	Bear Creek by Natchez Trace overpass in Tishomingo State Park	September 9, 2020	34.61079	−88.19122	114
13	Bear Creek upstream of Swinging Bridge	September 9, 2020	34.60622	−88.17788	360
14	Bear Creek upstream of Swinging Bridge	September 9, 2020	34.60719	−88.1765	198
15	Bear Creek upstream of Swinging Bridge	September 9, 2020	34.60707	−88.17247	148
16	Bear Creek upstream of Swinging Bridge	September 9, 2020	34.60678	−88.17188	112
17	Bear Creek upstream of Swinging Bridge	September 9, 2020	34.60552	−88.17126	104
18	Bear Creek downstream of Dennis Bridge	September 21, 2020	34.56554	−88.19061	57
19	Bear Creek upstream of Dennis Bridge	September 21, 2020	34.56268	−88.18928	108
20	Bear Creek upstream of Dennis Bridge	September 21, 2020	34.56118	−88.19002	18
21	Bear Creek upstream of Dennis Bridge	September 21, 2020	34.56022	−88.19056	30
22	Bear Creek upstream of Dennis Bridge at mouth of channelized section	September 21, 2020	34.5549	−88.1872	29
23	Bear Creek upstream of Dennis Bridge at powerlines in sinuous section	September 21, 2020	34.54848	−88.18973	33
24	Bear Creek upstream of Dennis Bridge in sinuous section	September 21, 2020	34.54692	−88.18928	87
25	Bear Creek auxiliary channel upstream County Road (CR) 993	September 30, 2020	34.52154	−88.1823	20
26*	Bear Creek upstream CR 993	September 30, 2020	34.51649	−88.18319	16
27	Bear Creek upstream CR 993	September 30, 2020	34.51632	−88.18145	16
28	Bear Creek upstream CR 993	September 30, 2020	34.51167	−88.18027	36
29	Bear Creek downstream of Golden, below grade-control structure in sinuous section	September 17, 2020	34.50803	−88.17579	93
30	Bear Creek downstream of Golden, below grade-control channelized section	September 17, 2020	34.50818	−88.17488	90
31*	Bear Creek downstream of Golden, channelized section	September 17, 2020	34.4994	−88.17423	27
32	Bear Creek upstream of Golden, sinuous section	September 17, 2020	34.49702	−88.17299	17
33	Bear Creek upstream of Golden, channelized section	September 17, 2020	34.49513	−88.17299	51
34	Bear Creek upstream of Golden, sinuous section	September 16, 2020	34.497	−88.16999	210
35	Bear Creek upstream of Golden, sinuous section	September 16, 2020	34.49782	−88.16874	26
36	Bear Creek upstream of Golden, sinuous section	September 16, 2020	34.49713	−88.16825	128
37	Bear Creek upstream of Golden, sinuous section	September 16, 2020	34.49692	−88.16536	134
38	Bear Creek upstream of Golden, sinuous section	October 8, 2020	34.49629	−88.16436	144
39	Bear Creek upstream of Golden, sinuous section	October 8, 2020	34.49307	−88.15862	240
40	Bear Creek upstream of Golden, sinuous section	October 14, 2020	34.49388	−88.15678	270
41	Bear Creek upstream of Golden, sinuous section	October 14, 2020	34.49322	−88.15615	15
42	Bear Creek upstream of Gee Branch, sinuous section	October 14, 2020	34.49089	−88.15297	123
43	Cedar Creek downstream Maudeal Road/CR 98	September 22, 2020	34.63919	−88.13519	72
44	Cedar Creek downstream Maudeal Road/CR 98	September 22, 2020	34.63705	−88.13472	21
45	Cedar Creek downstream Maudeal Road/CR 98	September 22, 2020	34.63405	−88.13575	5
46	Cedar Creek downstream Maudeal Road/CR 98 (beach walk)	September 22, 2020	34.63305	−88.13706	10
47	Cedar Creek at Maudeal Road/CR 98	September 21, 2020	34.62864	−88.14181	48
48*	Cedar Creek upstream Maudeal Road/CR 98	September 22, 2020	34.62165	−88.14039	15
49	Cedar Creek upstream Maudeal Road/CR 98	September 22, 2020	34.62059	−88.14208	27

Table 2, continued.

Site	Locality	Date	Latitude	Longitude	Time (min)
50	Cedar Creek upstream Maudeal Road/CR 98 (beach walk)	September 22, 2020	34.61955	−88.14198	10
51	Cedar Creek upstream Maudeal Road/CR 98	September 22, 2020	34.61857	−88.14206	30
52	Cedar Creek upstream Maudeal Road/CR 98	September 22, 2020	34.61418	−88.14709	27
53	Cedar Creek upstream Maudeal Road/CR 98	September 22, 2020	34.61391	−88.14731	21
54*	Holly Branch on CR 85	September 29, 2020	34.60595	−88.14893	90
55*	Brumley Branch on CR 68	September 29, 2020	34.51102	−88.15936	65

*Previously unsurveyed sites.

Previous surveys documented a total of 46 native mussel species and two invasive bivalves (Zebra Mussel, *Dreissena polymorpha*; Asian Clam, *Corbicula fluminea*) in the entire Bear Creek watershed (Table 1). Thirty-one mussel species and one invasive bivalve (Asian Clam) are reported previously in the Mississippi portion of the watershed (Table 1). These include three federally endangered species (Cumberlandian Combshell, *Epioblasma brevidens*; Snuffbox, *Epioblasma triquetra*; Slabside Pearlymussel, *Pleuronaia dolabelloides*), one candidate for federal listing (Tennessee Pigtoe, *Pleuronaia barnesiana*), and two state endangered species (Purple Wartyback, *Cyclonaias tuberculata*; Kidneyshell, *Ptychobranchus fasciolaris*), all of which are reported from Mississippi only in the Bear Creek watershed (Jones et al. 2021). In addition, one federally threatened species (Rabbitsfoot, *Theliderma cylindrica*) is reported from Bear Creek but is also found elsewhere in Mississippi. Its high diversity, including nine species of conservation concern, demonstrates the regional and global importance of the Bear Creek watershed for mussel conservation.

Previous mussel surveys devoted comparatively little effort to the Mississippi portion of the Bear Creek watershed. For example, McGregor and Garner (2004) surveyed 40 sites in the watershed but only four of those sites were in Mississippi. On the basis of records in a statewide mussel distribution database maintained by the Mississippi Museum of Natural Science (MMNS, Jackson, MS; MMNS Freshwater Invertebrate Collection, https://www.mdwfp.com/museum/seek-study/biological-collections/freshwater-invert/), 58 shell collections were made in Bear Creek in Mississippi between 1966 and 2018. However, most of these collections were made incidentally during fish surveys and were not the result of targeted mussel surveys. The few targeted surveys sampled only one to three sites each year and did not comprehensively cover the system. Excluding incidental collections, no mussel surveys have been conducted in the Mississippi portion of Bear Creek in over 10 yr, and a single, comprehensive survey of this section has never been undertaken. We conducted the first intensive mussel survey of the Mississippi portion of the Bear Creek watershed, including surveys at 55 sites. We report species richness, mussel abundance (as catch per unit effort [CPUE]), and size structure at these sites, and we discuss the conservation applications of our findings.

METHODS

We surveyed 55 sites throughout the Bear Creek watershed in Mississippi (Fig. 1, Table 2). We chose both previously surveyed and unsurveyed sites on the basis of site accessibility and the presence of apparently suitable mussel habitat (riffles or runs with stable, sand/gravel substrate), as well as the presence of shell material. One site was on a small tributary to Cedar Creek, 11 sites were on main-stem Cedar Creek, one site was on a small tributary to Bear Creek, and 42 sites were on main-stem Bear Creek; most main-stem Bear Creek sites were on the original channel, but we surveyed four sites on the channelized sections. Surveys were conducted in September and October 2020.

We searched for live mussels at most sites using a combination of snorkeling and tactile search (grubbing). This was done by lightly disturbing the substrate with our hands to detect partially buried mussels either by touch or by sight. We also searched gravel bars and shorelines for freshly dead and relic shells. We defined freshly dead shells as those having lustrous nacre, and relic shells as those with chalky shells or badly eroded nacre and periostracum, indicating that they had been dead for an extended time. At two sites, 46 and 50, we searched for shells but did not search for live mussels because the habitat did not appear suitable. We established a sampling area at each site on the basis of the extent of suitable mussel habitat. We conducted timed searches for live mussels at each site within the designated sampling area. We determined search time on the basis of amount of available habitat as well as mussel species richness at the site. If initial sampling revealed a high number of species, we searched the site for a longer time. Time began when all searchers entered the water and ended when searching ceased; shell searches were not included in the search time. We counted and measured all live native mussels (length, greatest anterior–posterior dimension, nearest 1 mm). We counted Asian Clams, but we did not measure them. We expressed native mussel abundance and Asian Clam abundance at each site as CPUE (number of live individuals/person-hours search time). We generated length–frequency histograms on the basis of live individuals for species that were represented by 10 or more individuals across all sites. We included freshly dead and relic shells for calculating species richness, but we used live individuals only when calculating CPUE and length–frequency distributions.

Table 3. Results of mussel surveys at 55 sites in the Bear Creek watershed, Mississippi in 2020. Cell entries are catch per unit effort (CPUE, number of live mussels/h), followed by numbers of live individuals encountered (in parentheses). Species that were present but not represented by live individuals are indicated as FD (freshly dead) or R (relic); "—" indicates that a species was not found at the site.

	Site													
	Bear Creek													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Unionids														
Amblema plicata	—	0.4 (1)	R	R	—	—	—	1 (2)	—	—	0.2 (1)	—	—	—
Arcidens confragosus	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cyclonaias pustulosa	—	0.9 (2)	0.6 (2)	—	—	R	R	R	R	—	3.6 (20)	R	0.2 (1)	0.9 (3)
Cyclonaias tuberculata	—	—	—	0.8 (1)	—	R	—	—	—	—	R	—	—	—
Ellipsaria lineolata	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Elliptio crassidens	—	1.7 (4)	1.6 (5)	1.5 (2)	—	R	—	R	—	—	—	R	R	—
Epioblasma brevidens	—	—	0.3 (1)	—	—	—	—	—	—	—	—	—	—	—
Lampsilis fasciola	—	—	—	—	—	—	—	—	R	—	0.2 (1)	R	R	0.3 (1)
Lampsilis ovata	—	0.4 (1)	1 (3)	R	—	R	0.5 (1)	0.5 (1)	R	—	1.6 (9)	0.5 (1)	—	0.9 (3)
Lampsilis teres	—	—	—	—	—	R	—	—	—	—	0.4 (2)	—	—	—
Lasmigona complanata	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lasmigona costata	—	—	—	—	—	—	—	—	—	—	0.2 (1)	—	—	—
Ligumia recta	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Megalonaias nervosa	—	R	3.2 (10)	2.3 (3)	—	—	—	—	—	—	—	0.5 (1)	R	—
Obliquaria reflexa	—	—	—	—	—	R	—	R	—	—	0.2 (1)	—	—	—
Pleuronaia dolabelloides	—	0.4 (1)	0.3 (1)	0.8 (1)	—	—	R	—	—	—	R	—	—	0.3 (1)
Potamilus alatus	—	R	—	R	—	—	—	R	R	—	0.4 (2)	0.5 (1)	—	0.3 (1)
Potamilus fragilis	—	R	—	—	—	R	0.5 (1)	R	FD	—	0.4 (2)	R	—	—
Ptychobranchus fasciolaris	—	—	—	—	—	—	—	—	—	—	R	FD	—	—
Pyganodon grandis	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Quadrula quadrula	—	—	—	—	—	0.6 (2)	2 (4)	1.5 (3)	0.9 (1)	—	2.7 (15)	—	0.5 (3)	0.6 (2)
Reginaia ebenus	—	—	0.6 (2)	—	—	—	R	—	—	—	—	—	—	—
Strophitus undulatus	—	—	—	—	—	—	—	1 (2)	—	—	0.2 (1)	—	—	—
Theliderma cylindrica	—	—	—	0.8 (1)	—	—	0.5 (1)	—	—	—	—	—	—	—
Tritogonia verrucosa	—	—	—	—	—	—	—	—	—	—	0.2 (1)	—	—	—
Truncilla donaciformis	—	—	—	—	—	0.3 (1)	—	R	—	—	—	—	—	—
Truncilla truncata	—	R	—	—	—	R	R	—	—	—	R	—	—	—
Villosa vanuxemensis	—	—	—	—	—	FD	—	—	—	—	—	—	—	—
Utterbackia imbecillis	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nonnative bivalves														
Corbicula fluminea	1.4 (2)	217 (499)	1 (3)	—	—	0.6 (2)	—	—	—	—	0.9 (5)	—	6.7 (40)	R
Number of unionid species	0	4	8	5	0	2	4	4	1	0	12	3	2	6
Total CPUE (all species)[a]	0	3.4	7.6	6.2	0	0.9	3.5	4	0.9	0	10.3	1.5	0.7	3.3

[a]Total CPUE excludes *Corbicula fluminea*.

We collected representative live, freshly dead, or relic shells of each species encountered at each site and deposited them in the MMNS Freshwater Invertebrate Collection.

RESULTS

We documented a total of 30 native mussel species and one invasive bivalve, the Asian Clam (Table 1). We found no live individuals or shells of *D. polymorpha*, which has been found in upper Bear Creek in Alabama and Pickwick Reservoir (McGregor and Garner 2004). We found live individuals of 25 native mussel species and the Asian Clam. The Kidneyshell, Paper Pondshell (*Utterbackia imbecilis*), and Mountain Creekshell (*Villosa vanuxemensis*) were represented only by freshly dead shells, and no live individuals were found. The Butterfly (*Ellipsaria lineolata*) and Giant Floater (*Pyganodon grandis*) each were represented only by a single relic shell.

Average mussel abundance and species richness across all sites were low (mean CPUE = 4.5 live mussels/h; 5.4 native species/site; Table 3). However, mussel abundance and species

Table 3, extended.

Site																			
Bear Creek																			
15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
—	—	R	—	—	—	—	—	—	—	—	—	—	—	—	R	—	R	—	7.7 (27)
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.3 (1)
0.4 (1)	0.5 (1)	R	—	0.6 (1)	—	R	—	—	—	—	—	R	1.7 (1)	3.2 (5)	R	—	R	—	12.9 (45)
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	R	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	R	—	—	—	—	—	—	—	—	—	—	—
0.4 (1)	1.1 (2)	0.6 (1)	-	0.6 (1)	R	R	FD	—	0.7 (1)	—	—	—	—	1.3 (2)	R	—	—	—	—
—	—	—	R	—	—	R	—	—	—	—	—	—	—	R	R	—	—	R	0.3 (1)
—	—	—	—	—	—	—	R	—	—	—	—	—	—	—	R	—	—	—	—
—	—	—	—	—	—	R	—	—	—	—	—	—	—	—	0.7 (1)	—	—	—	0.6 (2)
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	0.5 (1)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.6 (16)
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.3 (8)
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	R
R	0.5 (1)	R	—	—	—	R	FD	—	2.1 (3)	R	—	3.8 (1)	5 (3)	5.2 (8)	R	—	3.5 (1)	—	2.3 (8)
—	—	—	—	—	—	—	R	—	0.7 (1)	—	—	—	—	R	FD	—	—	—	0.9 (3)
R	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
R	-	1.7 (3)	—	—	—	—	—	—	0.7 (1)	R	—	—	1.7 (1)	0.6 (1)	—	—	—	—	0.6 (2)
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	R	—	—	—	—	—	—	0.7 (1)	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	R
—	R	—	—	—	—	—	—	—	0.7 (1)	—	—	—	1.7 (1)	0.6 (1)	—	—	—	—	4.3 (15)
R	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	1.7 (3)	—	0.6 (1)	—	4 (2)	—	5.5 (3)	—	—	48.8 (13)	22.5 (6)	33.3 (20)	0.6 (1)	—	—	—	—	12.6 (44)
2	4	2	0	2	0	0	0	0	6	0	0	1	4	5	1	0	1	0	11
0.8	2.6	2.3	0	1.2	0	0	0	0	5.6	0	0	3.8	10.1	10.9	0.7	0	3.5	0	36.8

richness were distributed unevenly in the watershed. Mussel abundance and species richness were highest in Bear Creek (mean CPUE = 5.6 mussels/h; mean richness = 6.3 species/site). Within Bear Creek, mussel abundance was consistently high only in the section from site 34 to site 42 (mean CPUE = 17.0 mussels/h), which included the four highest CPUE values observed (site 34, 36.8; site 39, 31.9; site 42, 13.4; site 37, 12.5). Species richness also was highest in this section (mean = 10.4 species/site), with the highest values at sites 39 and 40 (each having 17 species). Beyond that section, mussel abundance and species richness were relatively high only at sites 3 and 4 (mean CPUE = 6.9, mean richness = 6.5), site 11 (CPUE = 10.3, richness = 12), site 24 (CPUE = 5.6, richness = 6), and sites 28 and 29 (mean CPUE = 10.5, mean richness = 4.5). CPUE was <4.0/h at all other Bear Creek sites, and few other sites had more than four native species.

Mussel abundance and species richness were low in Cedar Creek (mean CPUE = 0.8/h; mean richness = 3.0 species/site). The highest abundance and species richness in Cedar Creek were observed at sites 43 (CPUE = 4.1) and 44 (8 species), respectively. There was little recent evidence of mussels in the channelized sections of Bear Creek. We found only one live

Table 3, extended.

Site																				
Bear Creek								Cedar Creek											Holly Branch	Brumley Branch
35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
R	1.4 (3)	0.9 (2)	0.8 (2)	0.3 (1)	0.9 (4)	—	1.5 (3)	—	—	R	—	R	—	—	—	—	—	—	—	—
—	—	—	0.4 (1)	2 (8)	0.4 (2)	—	1 (2)	—	—	—	—	—	—	—	—	—	—	—	—	—
R	0.9 (2)	2.7 (6)	1.7 (4)	6.3 (25)	4.2 (19)	—	1.5 (3)	0.8 (1)	R	FD	—	R	—	—	FD	—	—	—	—	—
—	—	—	—	0.3 (1)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	R	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	R	—	0.3 (1)	0.2 (1)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	R	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	0.4 (1)	0.4 (1)	1 (4)	1.6 (7)	4 (1)	1.5 (3)	R	2.9 (1)	—	—	R	—	—	—	2 (1)	R	—	—	—
R	R	0.4 (1)	R	R	R	—	1 (2)	—	R	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	0.3 (1)	0.2 (1)	—	1 (2)	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	1.3 (3)	—	0.7 (3)	—	—	—	R	—	—	R	—	—	R	—	—	—	—	—
—	—	—	0.4 (1)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4.6 (2)	0.9 (2)	7.2 (16)	10.8 (26)	10.3 (41)	7.8 (35)	—	—	—	R	—	—	—	—	—	—	R	—	—	—	—
—	—	—	R	0.5 (2)	—	—	FD	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	R	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2.3 (1)	—	0.9 (2)	0.4 (1)	2 (8)	1.3 (6)	4 (1)	4.9 (10)	2.5 (3)	R	FD	—	—	—	—	R	FD	—	—	—	—
R	R	—	R	0.8 (3)	R	—	FD	—	—	FD	—	R	—	—	—	—	—	—	—	—
—	—	—	—	—	R	—	—	—	R	—	R	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	R	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	R	R	1 (4)	1.6 (7)	—	1 (2)	0.8 (1)	—	—	—	—	—	—	—	—	R	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	FD	0.8 (3)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	0.8 (2)	1.5 (6)	1.1 (5)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	FD	—	—	—	0.2 (1)	—	R	—	—	—	—	—	—	—	—	—	—	—	—	—
R	0.9 (2)	FD	R	4.5 (18)	1.8 (8)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	FD	—	—	—	—	—	—	—	—	—	—
FD	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6.9 (3)	—	4 (9)	R	3.8 (15)	—	—	0.5 (1)	—	—	—	—	—	—	—	—	—	—	—	—	—
2	4	6	9	15	13	2	8	3	1	0	0	0	0	0	0	1	0	0	0	0
6.9	4.1	12.5	17	31.9	22	8	13.4	4.1	2.9	0	0	0	0	0	0	2	0	0	0	0

mussel in these sections (Flutedshell, *Lasmigona costata*, site 30), and only one freshly dead shell (Mapleleaf, *Quadrula quadrula*, site 30). We found relic shells of seven other species in the channelized sections. No live mussels or shells were found at either of the two tributary sites (sites 54 and 55). We found Asian Clams at about half of the Bear Creek sites, but we did not find them in Cedar Creek or the other two tributary sites.

The most abundant and widely distributed native species were Washboard (*Megalonaias nervosa*, mean CPUE = 4.8/h, 153 live individuals, live individuals or shells found at 15 sites), Pimpleback (*Cyclonaias pustulosa*, 2.4/h, 142 individuals, 33 sites), Pink Heelsplitter (*Potamilus alatus*, 2.3/h, 61 individuals, 32 sites), Deertoe (*Truncilla truncata*, 2.1/h, 47 individuals, 15 sites), Mapleleaf (1.2/h, 52 individuals, 21 sites), and Pocketbook (*Lampsilis ovata*, 1.1/h, 46 individuals, 31 sites). All other species occurred at a mean abundance of $\leq$2.0 mussels/h and were found live at $\leq$10 sites.

Federally listed species were uncommon throughout the watershed (Table 3). We found only one live Cumberlandian Combshell (adult male, 36.0 mm length) in the lower section of Bear Creek (site 3), one relic shell at site 21 in Bear Creek,

and one relic juvenile or subadult (23.4 mm length) in Cedar Creek (site 50). We found a single live Slabside Pearlymussel at each of four sites in Bear Creek (sites 2, 3, 4, and 14), and we found single relic shells at sites 7, 11, 34, and 40; all live individuals appeared to be adults (lengths = 52.0–68.0 mm). We found six live Rabbitsfoot among four sites in Bear Creek (sites 4, 7, 24, and 39; lengths = 50.0–96.0 mm), two freshly dead shells (site 38), and a single relic shell (site 17).

State-listed species were similarly uncommon. We found two live Purple Wartyback in Bear Creek (sites 4 and 39, lengths = 85.0 mm and 137.0 mm) and three relic shells (sites 6 and 11). We found no live Kidneyshell, but we found one freshly dead juvenile or subadult in Bear Creek (site 12; 34.5 mm length) and relic shells in Bear Creek (sites 11, 15, and 40) and Cedar Creek (sites 44 and 46).

The Flutedshell, Black Sandshell (*Ligumia recta*), and Mountain Creekshell are proposed for state listing in Mississippi. We found 10 live Flutedshell in Bear Creek (sites 1, 30, 34, 38, and 40) and five relic shells (Bear Creek, site 21; Cedar Creek, sites 44, 47, and 50). We found one live Black Sandshell in Bear Creek (site 38). We found one freshly dead Mountain Creekshell in Bear Creek (site 6), one freshly dead shell in Cedar Creek (site 45), and one relic shell in Bear Creek (site 15).

Most of the 11 species for which we constructed length–frequency histograms were represented by a wide range of sizes, and several species were represented by individuals <50 mm length (Fig. 2). A conspicuous exception was the Elephantear (*Elliptio crassidens*), for which all 13 live individuals were ≥ 100 mm.

DISCUSSION

We found all species previously reported from the Mississippi section of Bear Creek except Snuffbox, Tennessee Pigtoe, and Southern Mapleleaf (*Quadrula apiculata*). Snuffbox and Tennessee Pigtoe have not been reported from anywhere in the Bear Creek watershed for over 50 yr and likely are extirpated from the system. Southern Mapleleaf recently colonized the lower Tennessee River system, including Bear Creek (Garner and McGregor 2001; McGregor and Garner 2004), and it likely still occurs in the Mississippi section. Notably, we found living individuals of all previously reported species except Butterfly and Giant Floater. Butterfly is predominantly a large-river species, and a large population exists in Pickwick Reservoir (Garner and McGregor 2001); it is likely that a small population exists in the Mississippi portion of Bear Creek. Giant Floater is a stream-size generalist, but it typically occurs in pools or depositional areas (Haag 2012), which we did not sample extensively; it probably occurs at least sparingly in those habitats in Bear Creek.

Our finding of the Mountain Creekshell in Bear Creek is the first report of this species anywhere in Mississippi, but the species was reported previously in the Alabama portion of the watershed (Ortmann 1925; McGregor and Garner 2004). We did not find live Mountain Creekshell, but our finding of two freshly dead shells suggests that a small population exists in the Mississippi portion of the watershed. The Flutedshell previously was reported from the Mississippi portion of Bear Creek only as relic shells (MMNS). Our finding of 10 live individuals confirms the continued existence of this species in the state. Our findings of Mountain Creekshell and Flutedshell prompted consideration of both species for listing as state endangered in Mississippi because of their apparently small population size and restricted range in the state.

Paper Pondshell was the only other species we found that had not been reported previously in the Mississippi portion of Bear Creek. We found only freshly dead shells of this species, but like the Giant Floater, it typically occurs in depositional areas and a population probably occurs in the Mississippi portion of Bear Creek. The Yellow Sandshell (*Lampsilis teres*) was reported previously from the Mississippi portion of Bear Creek only as freshly dead or relic shells (MMNS), and it was not reported previously from the Alabama portion; our collections represent the first findings of live individuals in the watershed. The Ebonyshell (*Reginaia ebenus*) was previously known from Bear Creek in Mississippi by a single freshly dead shell (MMNS), but our records of two live individuals confirm the species' presence and suggest that it is moving upstream in the system (see McGregor and Garner 2004).

Length–frequency distributions of most of the more common species showed individuals of a wide range of sizes, which suggests that at least some recruitment is occurring for these species. The only exception was the Elephantear, which was represented only by large individuals. Elephantear populations in other areas are similarly dominated by large individuals and show no evidence of recent recruitment, potentially due to restriction of movement of their host fishes (herrings, *Alosa* spp.) by dams (Haag 2012). We were unable to assess recent recruitment for federally endangered or threatened species because of our low sample sizes for these species. However, Rabbitsfoot was represented by a wide range of sizes (lengths = 50.0–96.0 mm), suggesting the presence of several age classes.

The continued survival of most previously reported species and the presence of recent recruitment suggests that mussel populations in the Mississippi portion of the Bear Creek watershed have been relatively stable since the 1995–2000 survey of McGregor and Garner (2004). However, our study is the first to provide quantitative estimates of mussel abundance, so it is impossible to make inferences about changes in mussel abundance during the last 25 yr. It seems clear that major changes occurred in the Bear Creek fauna before the McGregor and Garner (2004) study. In addition to Snuffbox and Tennessee Pigtoe, nine other species had disappeared from the stream by that time. Although we have no information about historical mussel abundance, the overall low abundance we observed at most sites suggests that the stream continues to be negatively affected by some factor or has not recovered from previous anthropogenic insults.

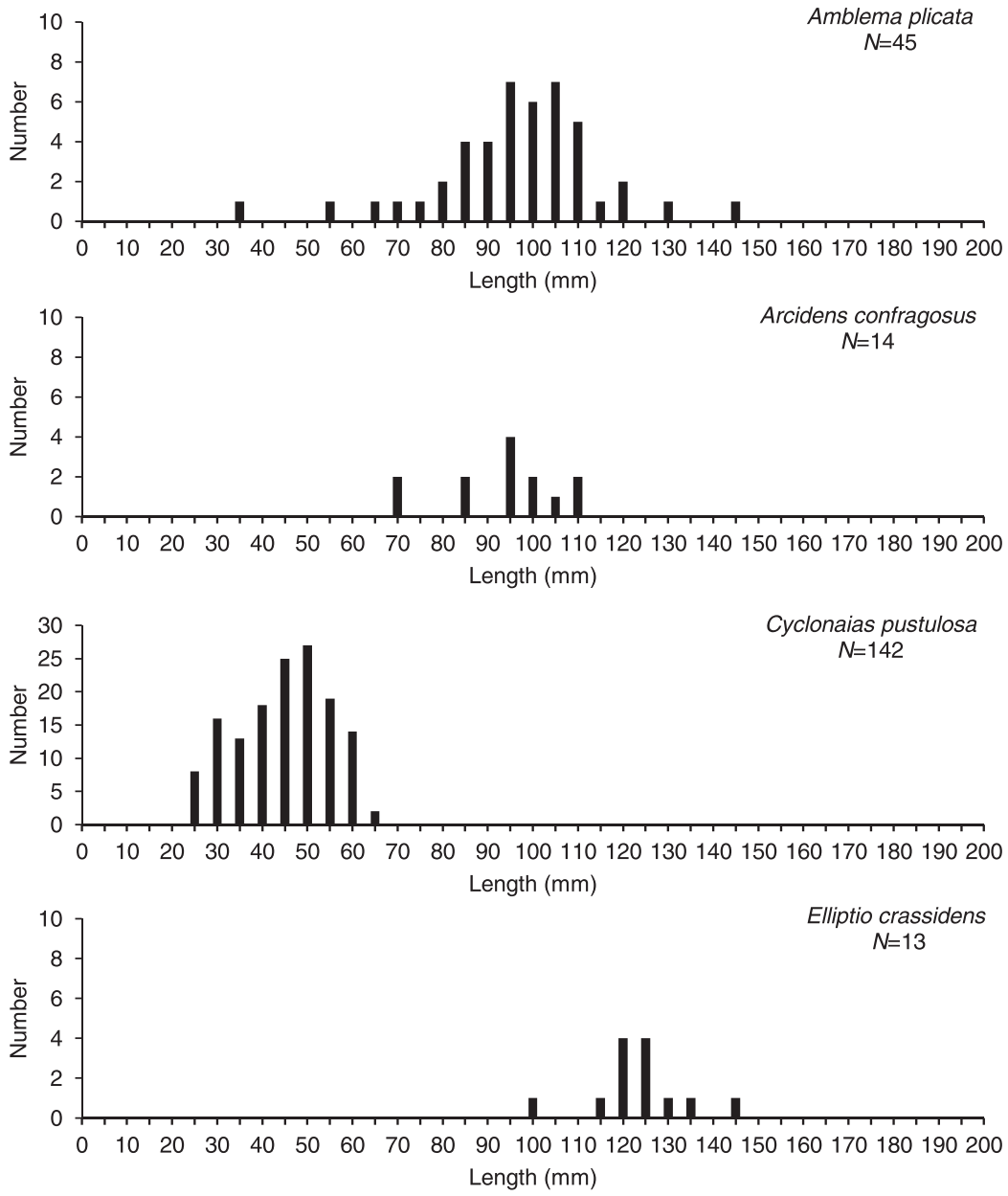

Figure 2. Length frequency histograms for 11 mussel species in the Bear Creek watershed, Mississippi in 2020. Sample sizes (*N*) represent all live individuals collected throughout the watershed.

The reasons for species loss and the currently low mussel abundance in Bear Creek are unknown. McGregor and Garner (2004) proposed that altered flow regimes caused by TVA reservoirs in the upper watershed have negatively affected the mussel fauna. TVA initiated minimum flows from these reservoirs in 2007 to improve aquatic habitats in the system (USFWS 2006), but we are unable to assess potential effects of this action because of the absence of previous estimates of mussel abundance. Stream habitats in the Bear Creek watershed have been degraded in other ways, including channelization and channel alteration, loss of riparian vegetation, and bank erosion, but the effect of these factors on the mussel fauna is unknown.

Bear Creek continues to support a diverse and important mussel fauna. Bear Creek represents the approximate downstream extent of the endemic mussel fauna of the Tennessee River system (Haag 2012), and it is distant from other populations of endemic species in the system. For example, the Bear Creek population of Cumberlandian Combshell is separated from the nearest surviving population by 748 river km and numerous dams (Gladstone et al. 2022). This isolation illustrates the biogeographic importance of Bear Creek, as well as its vulnerability to stochastic effects. The results from our comprehensive survey of Bear Creek, including the first estimates of mussel abundance in the system, will be important

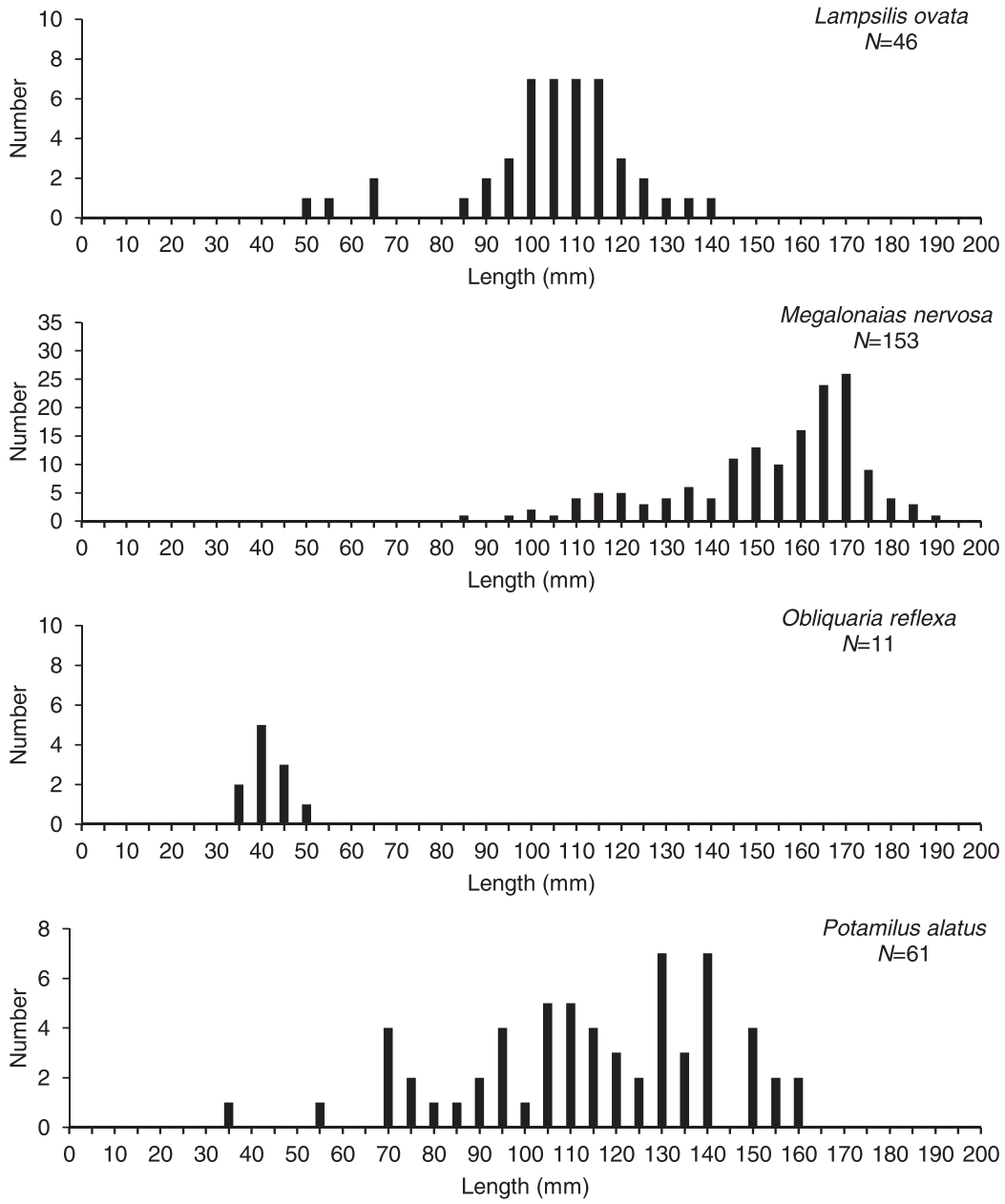

Figure 2, continued.

for monitoring the fauna and assessing the effects of future conservation actions.

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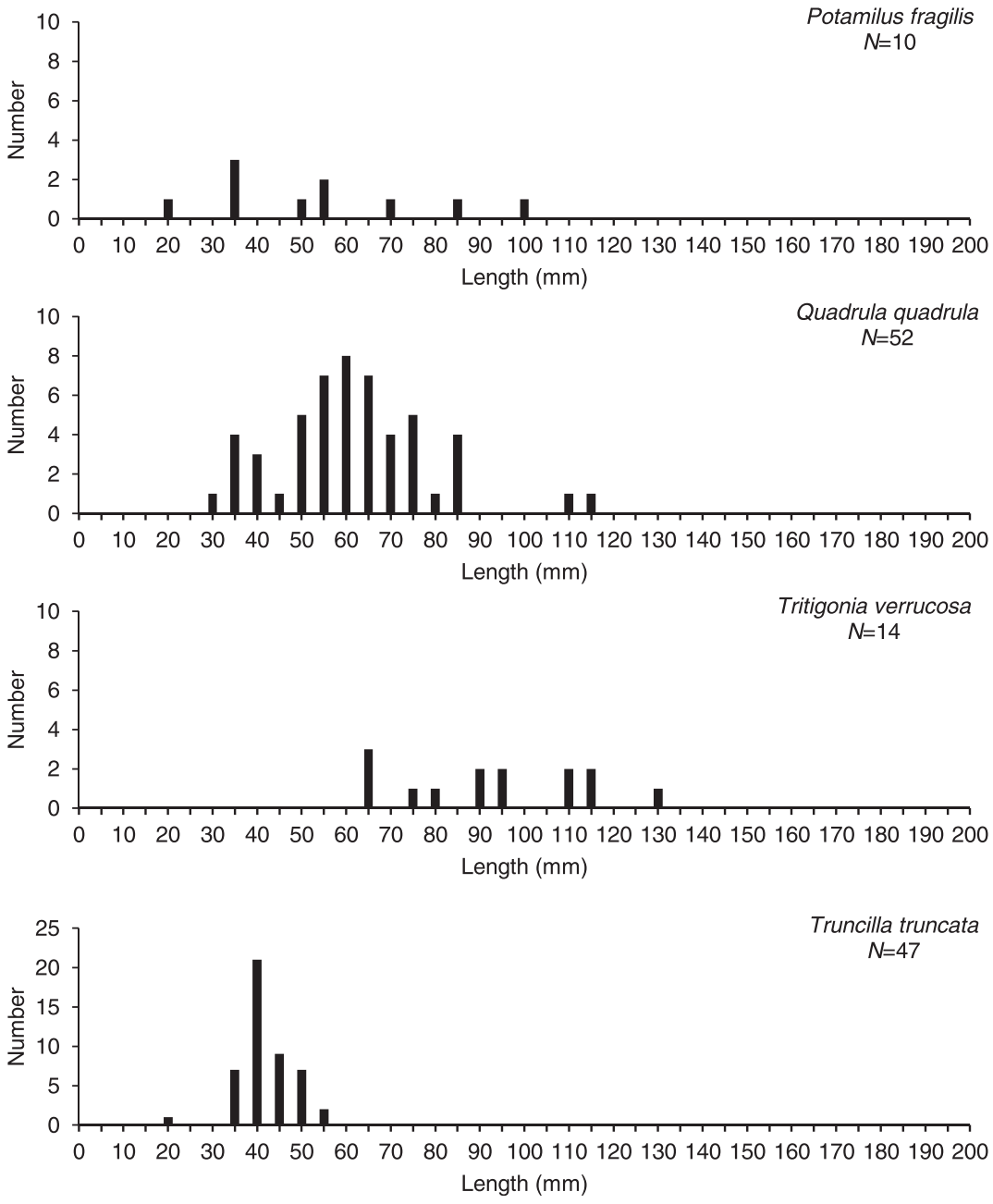

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REGULAR ARTICLE

DENSITY, APPARENT SURVIVAL, AND LOCAL POPULATION SIZE OF LOUISIANA PIGTOE (*PLEUROBEMA RIDDELLII*) IN THE NECHES RIVER, TEXAS

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ABSTRACT

Most North American unionids are imperiled to some degree, including the Louisiana Pigtoe, *Pleurobema riddellii*, which is currently under review for listing under the U.S. Endangered Species Act. Understanding a species' population dynamics, including spatial and temporal variation in survival, density, recruitment, and population size, is vital for conservation, but this information is lacking for *P. riddellii*. We conducted a mark–recapture study to estimate apparent survival, density, recruitment, and population size of *P. riddellii* within a 25-m^2 area at three sites (75 m^2 total) in the Neches River, Texas from 2014 to 2019. We used the program MARK to evaluate POPAN models for estimating population parameters. We collected a total of 392 unique individuals of *P. riddellii* over the 5-yr period and the observed recapture rate averaged 55.6%. The most parsimonious POPAN model indicated that apparent survival varied temporally, the recapture rate varied temporally and spatially, and both the entry probability (recruitment) and population size varied spatially. Apparent survival averaged 83.3% ± 3.4% (SE)/yr, overall population size across the three sites was 429 individuals (overall density = 5.7/m^2), and recruitment averaged 6.3%/yr. High survival, relatively high density, the presence of recruitment, and the lack of temporal changes in population size suggest that these populations are stable. The presence of *P. riddellii* throughout a long section of the river, including localized patches of higher abundance, suggests that the total population size in the Neches River is relatively large and the river is a global stronghold for the species.

KEY WORDS: recapture rates, mark–recapture, MARK, population dynamics, vital rates, long term

INTRODUCTION

Estimates of population vital rates and population size are important for effective species conservation (Matter et al. 2013). Vital rates, such as survival and recruitment, are the main determinants of a population's growth rate and ultimately, its viability (Akçakaya et al. 2004; Bonnot et al. 2011; Connette and Semlitsch 2015; Newton et al. 2020). Population size can influence viability primarily because small populations can be more vulnerable to Allee effects or biotic and abiotic factors (Kramer et al. 2009; Nystrand et al. 2010). Population models incorporating vital rates and population size can inform conservation efforts by making predictions about the resilience of a species to environmental impacts (Fonnesbeck and Dodd 2003; Connette and Semlitsch 2015).

North America's freshwater mussels (Unionoidae) are one of the most highly imperiled faunal groups on the continent (Williams et al. 1993; Bogan 2008; Haag 2012). Information about mussel population dynamics is especially important for evaluating population viability and responses to various environmental and anthropogenic factors. Annual survival and recruitment differ widely among mussel species, and these patterns can have a large influence on population growth and stability (e.g., Payne and Miller 2000; Villella et al. 2004; Haag 2012). However, vital rates remain unknown for numerous mussel species, and the long life span of many species requires multiyear sampling to estimate those factors (Villella et al. 2004; Newton et al. 2011, 2020). Mark–recapture studies can provide relatively unbiased estimates of

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population size and survival rates, which can be difficult to estimate directly (Daura-Jorge and Simões-Lopes 2014; Pace et al. 2017; Schachat et al. 2019).

We used a mark–recapture study to estimate apparent survival, recruitment, and population size for the Louisiana Pigtoe, *Pleurobema riddellii*, at three sites in the Neches River of eastern Texas from 2014 to 2019. This species is currently under review by the U.S. Fish and Wildlife Service (2009) for listing under the U.S. Endangered Species Act. Little life-history and population information is available for this species, and these data will be valuable to future conservation efforts.

METHODS

Study Species

Pleurobema riddellii was known historically from portions of western Louisiana, eastern Texas, and Red River tributaries in Arkansas (Vidrine 1993; Howells et al. 1996; Howells 2010, 2014). The species has experienced a large range constriction over the past decades, and sizable populations in Texas are currently known only from the upper Neches River basin (Burlakova et al. 2011; Ford et al. 2014; D. F. Ford et al. 2016). In the Neches River, *P. riddellii* occurs in riffles and shallow to moderately deep runs in stable gravel-and cobble-substrates (N. B. Ford et al. 2016; Glen 2017) and is a host specialist on drift-feeding minnows (*Pimephales vigilax*, *Cyprinella venusta*, and *Cyprinella lutrensis*; Hinkle 2018; Marshall et al. 2018). Estimates of individual growth are available for the species and maximum life span is likely over 40 yr (Ford et al. 2020). However, estimates of population vital rates and population size are lacking.

Study Area

The Neches River is a sixth-order stream and drains approximately 26,000 km^2 (Texas Parks and Wildlife Department 1974; Horizon System Corporation 2015). Seasonal stream flow patterns were similar among all years of our study (2014–2019; U.S. Geological Survey gauge 0803200 Neches River near Neches, Texas, https://waterdata.usgs.gov/tx/nwis/nwis, accessed February 10, 2021), except for the winter of 2015 and most of 2016 during which flow was consistently high (>30 m^3/s, maximum = 134 m^3/s).

We selected three study sites that supported the highest abundance of *P. riddellii* observed over multiple years of mussel surveys in the Neches River basin (Walters et al. 2017; Ford et al. 2020). The most upstream site (HWY 79) was 8.6 km downstream of the Highway 79 bridge (Anderson County), the next site (CHC) was 22.2 km downstream of the HWY 79 site near Cherokee Hunting Club Road (Cherokee County), and the most downstream site (HWY 294) was 11.3 km downstream of CHC, upstream from the Highway 294 bridge (Cherokee County). We established a 150-m study reach at each site.

In 2014, we conducted initial site sampling by dividing each 150-m study reach into three 50-m segments and excavating 27 0.25-m^2 quadrats in each segment (total of 81 quadrats in each 150-m reach). In each 50-m segment we distributed the 27 quadrats across the stream by placing nine quadrats at randomly chosen locations in the center of the stream and nine quadrats at randomly chosen locations along each bank. We calculated an estimate of mean density of *P. riddellii* in each of the three 150-m reaches as the mean density among the 81 total quadrats (three sets of 27 quadrats per reach). In each 150-m reach, we identified the quadrat with the highest number of *P. riddellii* and established a 5 m × 5 m grid (25 m^2) centered on that quadrat for the mark–recapture study. No *P. riddellii* were collected in initial site sampling at HWY 79; we conducted a qualitative search at this site and located the 5 m × 5 m grid where the first specimen was found.

Sampling Methods

We sampled the 25-m^2 grids at each site once/yr in late summer or early fall during low-water conditions from 2014 to 2019, but we did not sample in 2018. We sampled each grid by placing a 1-m^2 quadrat at one corner of the grid, searching it for mussels by excavating the substrate, and then flipping the quadrat over to the adjacent 1-m^2 location until the entire 25-m^2 area was searched. We affixed a passive integrated transponder (PIT) tag (Biomark, Boise, ID, USA) to the shell and a numbered bee tag (Betterbee, Greenwich, NY, USA) to the opposite valve. We measured shell length of each *P. riddellii* encountered and then returned all individuals to the substrate in the grid. After 2014, we made an initial pass over the grid with a PIT tag receiver to locate previously tagged individuals and then excavated the grid as described above to ensure that all individuals were collected. On each sampling occasion, we recorded the tag numbers and measured all recaptured *P. riddellii* and tagged and measured newly encountered individuals. We also recorded dead individuals encountered in the grid. Loss of tags was rare, and no individuals lost both tags, which allowed us to identify all recaptured mussels.

Mark–Recapture Analysis

We calculated recapture rates of *P. riddellii* for each sampling event as:

$$R^c = T^r/T^m,$$

where R_c is the recapture rate for the sampling event, T_r is the number of marked *P. riddellii* recovered during the sampling event, and T_m is the total number of *P. riddellii* marked before the sampling event.

We used the POPAN model in the program MARK (White and Burnham 1999) for our mark–recapture analysis. This model has the following assumptions: (1) marks are not lost and can be read correctly, (2) sampling is instantaneous and animals are released immediately after sampling, (3) the study area remains constant and its size does not change, (4) all animals (marked and unmarked) have an equal probability of survival between each sampling event, and (5) all animals

Table 1. Densities (number/m^2) of *Pleurobema riddellii* estimated from initial site sampling and later sampling of the 25-m^2 grids at three sites in the Neches River, Texas from 2014 to 2019. Numbers in parentheses are the number of unique individuals located during each sampling event. The column "Mean" represents mean values across all 5 yr. The column "Totals" represents density estimates based on the total number of unique individuals encountered across all 5 yr and the sample area (25 m^2, or 75 m^2 for "Overall").

Site	Initial Site Sampling (2014)	25-m^2 Mark–Recapture Grid						
		2014	2015	2016	2017	2019	Mean	Totals
HWY 79	0.0	1.3 (32)	2.2 (54)	2.2 (54)	2.2 (56)	1.6 (40)	1.9 (47)	3.2 (79)
CHC	2.8	1.2 (29)	1.9 (48)	2.6 (65)	1.5 (37)	1.7 (42)	1.8 (44)	3.5 (87)
HWY 294	0.2	2.6 (64)	4.2 (104)	4.9 (123)	3.4 (86)	4.7 (118)	4.0 (99)	9.0 (226)
Overall	1.0	1.7 (125)	2.8 (206)	3.2 (242)	2.3 (172)	2.7 (200)	2.5 (189)	5.2 (392)

(marked and unmarked) have an equal probability of being captured between each pair of sampling events. Generally, these assumptions were met by our study. Passive integrated transponder tags may have allowed somewhat greater capture rates of previously marked individuals, but our thorough excavation of the grids likely effectively detected untagged individuals (see Discussion).

The POPAN model calculates four statistics, apparent survival (ϕ), the recapture probability during the sampling event (p), the probability of a new individual entering or being located within the sample area from the total population (entry probability, p_{ent}), and superpopulation size (N). Apparent survival is the probability of an individual surviving between sampling events, given that the organism is still present within the site, whereas the recapture probability is the probability of an individual being captured during a sampling event assuming it is alive. Entry probability (p_{ent}) is the probability of entry from the population (the population in the 25-m^2 grid) into the study area as a result of immigration or birth (i.e., recruitment). We interpreted estimates of p_{ent} derived from the POPAN model to represent annual recruitment. Adult mussels are relatively sedentary, but it is possible that some individuals moved into or out of a sampling grid. However, given the large size of the grid, this is unlikely except along the edges (Schwalb and Pusch 2007), and the number of immigrating or emigrating adults is expected to be low (Newton et al. 2015, 2020). Juveniles that recruited to a grid by dropping off host fishes initially are too small to be detected by our sampling but are detectable after about 3 yr, at which time they average >20 mm in length (Ford et al. 2020). The superpopulation size (hereafter referred to as population) is considered the number of individuals ever present in the sampling area. We calculated N for each of the three 25-m^2 grids. Both N and recruitment were rounded to the nearest whole individual. We calculated all parameters using a 1-yr time interval between successive samples, except for 2017 to 2019, where we used a 2-yr time interval to account for the lack of sampling in 2018.

We included a group effect (sampling site) and a time effect (year) in the POPAN models to evaluate spatial and temporal variation in model parameters. We used Akaike's information criterion corrected for small sample size (AIC_c) to rank candidate models. We used quasi-AIC_c ($QAIC_c$) values to select the most parsimonious model from the list of candidate models, and we used a goodness-of-fit test in the program RELEASE in MARK to determine the fit of a chosen model. The most parsimonious model is the one with the smallest $QAIC_c$ value, which explains most of the variation in the data, while using the least number of model parameters.

RESULTS

Between 2014 and 2019 we captured a total of 392 *P. riddellii* individuals from all three sites and found eight (2.0%) dead individuals (Table 1). All dead individuals were recovered from HWY 79 in 2017 (three individuals) and 2019 (five individuals). Of the 392 *P. riddellii* individuals, we had a total of 944 captures, including 138 individuals (35.2%) that were captured once and not recaptured, 69 (17.6%) that were recaptured once (initial capture + one recapture), 94 (24.0%) that were recaptured twice, 69 (17.6%) that were recaptured three times, and 22 (5.7%) that were recaptured in all sampling events after 2014. Recapture rate averaged 55.6% across all sites and years. Recapture rates did not differ between sites (analysis of variance, $F_{9,11} = 4.26$, $P = 0.480$) but were significantly different between sampling years ($F_{12,15} = 3.49$, $P = 0.001$). Recapture rates differed only between 2016 and 2019 (Tukey honestly significant difference, $P < 0.001$). At all sites, initial captures of untagged individuals declined from 2014 to 2017, but initial captures increased in 2019 (Fig. 1). Conversely, recapture rates generally increased during the first 3 yr, then remained relatively steady after 2016, except in 2019, when recapture rates appeared to decrease substantially, particularly at HWY 294. Mean *P. riddellii* density across all three 25-m^2 grids was 1.7/m^2 in 2014 and 2.7/m^2 in 2019 (mean = 2.5/m^2; Table 1).

The most parsimonious POPAN model included apparent survival (ϕ), which varied temporally; recapture probability (p), which varied spatially and temporally; and entry probability (p_{ent}) and population size (N), which both varied spatially ($\chi^2_{(21)}$, $P = 0.002$; Table 2). Mean survival across sites was 83.3% ± 3.4% (SE). Apparent survival was >80% in all years, except between 2017 to 2019 when it was 73.8% (Table 3). Recapture probability (p) averaged 67.9% (range = 38.5–95.3%) across all sites and years. Both the lowest (2015)

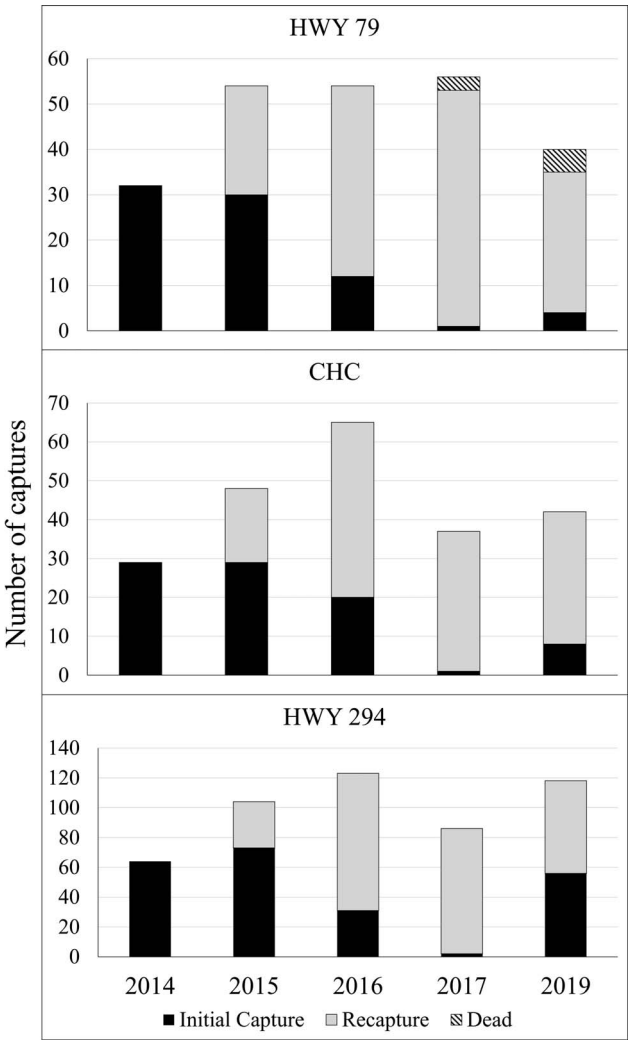

Figure 1. Captures of *Pleurobema riddellii* at three sites in the Neches River, Texas from 2014 to 2019.

and highest (2017) recapture probabilities were found at CHC. Recapture probability was lowest for all sites in 2015 and highest in 2017, except at HWY 79, where recapture probability was highest in 2019. Entry probability (p_{ent}) across all three sites ranged from 2.8% ± 1.4% (SE) at HWY 79 to 10.8% ± 1.3% at HWY 294 (mean across sites = 6.3% ± 1.5%). Assuming all individuals entering the populations originated from recruitment, values of p_{ent} represent the addition of 2 ± 2 (SE) to 27 ± 3 individuals/yr. The estimated total population size across all three sites was 429 ± 8 (SE) individuals (Table 3).

Of the total 79 *P. riddellii* captures at HWY 79, 14 (17.7%) individuals were captured once, 10 (12.7%) were recaptured once, 32 (40.5%) were recaptured twice, 17 (21.5%) were recaptured three times, and six (7.9%) were recaptured in all sampling events after 2014. The recapture rate at HWY 79 averaged 63.6%. We found no *P. riddelli* at HWY 79 during the initial site sampling in 2014. Within the 25-m^2 grid, densities were 1.3/m^2 and 1.6/m^2 in 2014 and 2019, respectively (Table 1). Entry probability was the lowest of the three sites, and an estimated 2 ± 2 (SE) new individuals immigrated to the site each year. The estimated population size at HWY 79 (87 ± 4 [SE] individuals; Table 3) was the lowest of any site.

Table 2. Quasi-Akaike information criterion corrected for small sample size ($QAIC_c$) ranking of POPAN models for estimating mark–recapture parameters for *Pleurobema riddellii* at three sites in the Neches River, Texas from 2014 to 2019. Model parameters are apparent survival (ϕ), recapture probability (p), probability of entry (p_{ent}), and population size (N). Parameters denoted with (t) indicate variance by survey year, (g) indicates variance by sampling site, and (.) indicates no variance by sampling time or site. Parameters that are a function of year and site simultaneously are denoted by the interaction term (g*t). NP is the number of parameters used in the model.

Model	$QAIC_c$	$QAIC_c$ Weight	NP
Φ(t) p(g*t) p_{ent}(g) N(g)	1403.565	0.506	25
Φ(g*t) p(t) p_{ent}(.) N(g)	1405.074	0.238	21
Φ(g*t) p(t) p_{ent}(g) N(t)	1405.452	0.197	21
Φ(.) p(g*t) p_{ent}(g) N(g*t)	1409.072	0.032	22
Φ(g*t) p(g*t) p_{ent}(.) N(g*t)	1410.979	0.012	31
Φ(g) p(t) p_{ent}(g) N(g)	1411.691	0.009	14
Φ(g*t) p(g*t) p_{ent}(g) N(t)	1413.145	0.004	31
Φ(t) p(t) p_{ent}(.) N(g*t)	1415.697	0.001	13
Φ(g) p(t) p_{ent}(g) N(.)	1418.395	0.000	12
Φ(g*t) p(t) p_{ent}(.) N(.)	1418.772	0.000	19
Φ(t) p(t) p_{ent}(.) N(.)	1418.909	0.000	11
Φ(.) p(g*t) p_{ent}(.) N(.)	1418.931	0.000	20

Of the total 87 *P. riddellii* captures at CHC, 27 (31.0%) were captured once, 18 (20.7%) were recaptured once, 16 (18.4%) were recaptured twice, 20 (23.0%) were recaptured three times, and six (6.9%) were recaptured in all sampling events after 2014. The recapture rate at CHC averaged 58.1%. Density of *P. riddellii* was 2.8/m^2 during the initial site sampling in 2014 (Table 1). Within the 25-m^2 grid, densities were 1.2/m^2 in 2014 and 1.7/m^2 in 2019 (Table 1). Entry probability indicated that an estimated 5 ± 2 (SE) new individuals immigrated to the site each year. The estimated population size at CHC (96 ± 4 [SE] individuals) was similar to that of HWY 79 but much lower than that of HWY 294 (Table 3).

Of the total 226 *P. riddellii* captures at HWY 294, 97 (42.9%) were captured once, 41 (18.1%) were recaptured once, 46 (20.4%) were recaptured twice, 32 (14.2%) were recaptured three times, and 10 (4.4%) were recaptured during all sampling events after 2014. Recapture rate at HWY 294 averaged 50.5%. Density of *P. riddellii* was 0.2/m^2 during the initial site sampling. Within the 25-m^2 grid, densities were 2.6/m^2 in 2014 and 4.7/m^2 in 2019 (Table 1). Entry probability was highest at this site, and an estimated 27 ± 3 (SE) new individuals immigrated to the site each year. The estimated population size at HWY 294 (246 ± 6 [SE] individuals) was the highest observed at any site (Table 3).

Table 3. POPAN model mark–recapture parameter estimates for *Pleurobema riddellii* at three sites in the Neches River, Texas from 2014 to 2019. Parameters are apparent survival (ϕ), recapture probability (p), probability of entry into the sampling area from the overall population in the area (p_{ent}), population size (N), and recruitment (r) from the most parsimonious model (see Table 2). Error for each estimate is SE. Values of apparent survival, p_{ent}, and N are the same across sites or years, respectively, following the most parsimonious POPAN model, which indicated only temporal variation for apparent survival and only spatial variation for p_{ent} and N; the POPAN model indicated both temporal and spatial variation for p. Recruitment is the estimated number of recruits in each year and was estimated as $(p_{ent}/100) \times N$. Population size and recruitment are rounded to the nearest whole individual.

Year	Φ	p	p_{ent}	N	r
HWY 79					
2015	85.0 ± 3.7	41.3 ± 6.3	2.8 ± 1.4	87 ± 4	2 ± 2
2016	89.1 ± 2.6	74.3 ± 5.9	2.8 ± 1.4	87 ± 4	2 ± 2
2017	85.3 ± 4.3	80.3 ± 5.4	2.8 ± 1.4	87 ± 4	2 ± 2
2019	73.8 ± 3.1	88.2 ± 5.3	2.8 ± 1.4	87 ± 4	2 ± 2
CHC					
2015	85.0 ± 3.7	38.5 ± 6.7	5.4 ± 1.8	96 ± 4	5 ± 2
2016	89.1 ± 2.6	68.8 ± 6.8	5.4 ± 1.8	96 ± 4	5 ± 2
2017	85.3 ± 4.3	95.3 ± 3.2	5.4 ± 1.8	96 ± 4	5 ± 2
2019	73.8 ± 3.1	62.4 ± 7.9	5.4 ± 1.8	96 ± 4	5 ± 2
HWY 294					
2015	85.0 ± 3.7	45.9 ± 5.6	10.8 ± 1.3	246 ± 6	27 ± 3
2016	89.1 ± 2.6	74.0 ± 4.8	10.8 ± 1.3	246 ± 6	27 ± 3
2017	85.3 ± 4.3	84.6 ± 3.8	10.8 ± 1.3	246 ± 6	27 ± 3
2019	73.8 ± 3.1	61.6 ± 5.5	10.8 ± 1.3	246 ± 6	27 ± 3

Density estimates differed substantially among sampling approaches and analytical methods. On the basis of the area of the 25-m^2 grid and estimates of population size from the POPAN model, estimated densities were 3.5/m^2 at HWY 79, 3.8/m^2 at CHC, and 9.8/m^2 at HWY 294 (overall = 5.7/m^2; see Table 3). These estimates were very similar to estimates based on area sampled and the total number of unique individuals captured across all 5 yr of sampling in the 25-m^2 grid (3.2/m^2 at HWY 79, 3.5/m^2 at CHC, 9.0.m^2 at HWY 294, 5.2/m^2 overall; Table 1). However, density estimates from quadrat sampling in individual years were about 50% lower than estimates made by the previous two methods (Table 1).

DISCUSSION

Although density and population size varied among sites, all three of our study sites in the Neches River appear to support relatively large populations of *P. riddellii*, with densities of about three to nine individuals/m^2. Other parameters suggest that these populations are stable, particularly the lack of temporal variation in population size. Survival varied across time but not by location, suggesting that annual riverwide variation in environmental factors was a more important determinant of survival than local variation among sites. However, apparent survival was generally high (usually >80%), similar to values reported for several other mussel species from stable populations (e.g., 87–>97%, Hart et al. 2001; Villela et al. 2004; Meador et al. 2011; Reátegui-Zirena et al. 2013; Wisniewski et al. 2014; Hyde et al. 2017). Values of p_{ent} were relatively low, but they represent recruitment strength similar to that seen in other stable mussel populations (e.g., 1–45%, Villella et al. 2004; Haag and Warren 2010; Matter et al. 2013); in contrast, declining populations are often characterized by a near absence of recruitment (Haag 2012).

It is important to note that our density and population size estimates for these local populations do not reflect the overall abundance of *P. riddellii* throughout the Neches River. Our estimates were obtained from small areas of high *P. riddellii* density at sites identified by previous surveys as having the highest density of the species in the river. Indeed, previous surveys at other sites in the Neches River basin found lower densities of *P. riddellii* at most sites (0.39–0.79/m^2; Andrew Glen, personal communication; Ford et al. 2014; D. F. Ford 2016). Nevertheless, the presence of *P. riddellii* throughout a long section of the river, including localized patches of higher abundance, suggests that the total population size in the Neches River is relatively large.

Variation in habitat characteristics among sites (see Ford et al. 2020) may partially explain the higher density and population size observed at HWY 294. The HWY 294 site had an extensive shallow riffle with gravel-and-cobble substrate. The CHC site had a deeper riffle with more mud and silt, and HWY 79 did not have a riffle but instead consisted of deeper, pooled habitat. *Pleurobema riddellii* is thought to prefer gravel-and-cobble substrates (Glen 2017; Ford et al. 2020), and the known hosts for *P. riddellii* are riffle-dwelling minnows (Hinkle 2018; Marshall et al. 2018). The greater abundance of riffle habitat at HWY 294 may have provided more habitat for *P. riddellii* and its host fishes.

Actual survival is difficult to estimate but is often higher than apparent survival because permanent emigration from the study area results in biased estimates of apparent survival

(Gilroy et al. 2012; Hyde et al. 2017). Permanent emigration is considered less of an issue for estimating survival of mussels because of their sedentary nature (Balfour and Smock 1995; Villela et al. 2004; Newton et al. 2020), but mussels can move substantial distances in some cases (Haag 2012; Daniel and Brown 2014; Newton et al. 2015). Because we did not sample for missing *P. riddellii* outside of the mark–recapture area, we could have missed individuals that emigrated out of the 25-m^2 grid or that were displaced by the 2016 flood. Temporary emigration, such as burrowing deeper into the substrate during colder months or higher flows, also could have biased our survival estimates, but we sampled in late summer and early fall when the water was warm and the flow was low.

The use of PIT tags may have introduced some bias into our parameter estimates by increasing the likelihood of recapturing tagged individuals compared with previously uncaptured individuals (see Kurth et al. 2007). However, even by using a PIT tag reader we missed a substantial proportion of tagged individuals in any given year. This fact, combined with our extensive excavation of the substrate to find unmarked individuals, probably minimized any bias associated with the use of PIT tags.

Our estimates of density varied markedly between the initial site sampling of the 150-m reach in 2014 and later sampling of the 25-m^2 grids, even in 2014. Higher density in the 25-m^2 grids, as observed at two of the sampling sites, was expected because the areas with the highest density in the 150-m reach were selected for the sampling grids. However, our density estimates were substantially lower in the 25-m^2 grids than over the 150-m reach at CHC. The variation in density estimates between our 150-m reach and the 25-m^2 grids illustrates the characteristically patchy nature of mussel distribution (Strayer 1999; Strayer et al. 2004) and the effects of scale on sample estimates. On the basis of the results of our broader-scale initial site sampling at HWY 79 and HWY 294, *P. riddellii* might have been considered absent or rare, respectively, at those sites, but our more focused sampling of the 25-m^2 grids revealed that both sites supported substantial populations. Conversely, our initial site sampling at CHC indicated a higher density than revealed by our sampling of the 25-m^2 grids.

The large difference in density estimates between our annual samples and longer-term sampling illustrates other sample design issues. Our estimates from quadrat sampling in individual years were about 50% lower than estimates from the mark–recapture model or from the combined 5-yr quadrat sampling data set. This discrepancy is probably explained by our overall observed annual recapture rate (55.6%) and our estimate of overall recapture probability from the POPAN model (67.9%). Detectability is rarely 100%, but we appear to have missed a substantial proportion of the population in any given year despite our focused sampling in a small area and extensive excavation of the substrate. Multiyear sampling is often impractical to implement on a large scale. Our sampling methods were broadly similar to adaptive sampling, in which additional sampling effort is allocated in areas where the target species is found (Strayer and Smith 2003). A more formalized application of adaptive sampling may be appropriate when the goal of a study is to provide accurate density estimates in a single sampling effort for a patchily distributed species. In addition, accounting for detectability may help provide more accurate density estimates in single sampling events (e.g., Smith et al. 2000; Bailey et al. 2004; Wisniewski et al. 2014).

Our multiyear sampling of *P. riddellii* populations provided estimates of density, survival, population size, and recruitment that are important for conservation efforts. These estimates provide baseline data for monitoring of the species' status over time. The lack of temporal variation in population size, high survival, and apparent levels of recruitment we document suggest that these local populations are stable. Our population parameter estimates can be coupled with other demographic information to construct population models, which can provide a quantitative assessment of the current trajectory and viability of *P. riddellii* populations (e.g., increasing, stable, decreasing). The occurrence of *P. riddellii* at relatively high density throughout a long, interconnected reach of the Neches River indicates that the river is a global stronghold for this species. The decline of *P. riddellii* and other mussel species across Texas (Howells et al. 1997; Randklev et al. 2010) highlights the imperative for protection of the Neches River basin.

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REGULAR ARTICLE

POPULATION DENSITY AND REPRODUCTIVE SEASONALITY OF *TRYONIA CHEATUMI* (GASTROPODA: COCHLIOPIDAE), THE PHANTOM TRYONIA

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ABSTRACT

We studied population density, population size, and reproductive seasonality of the Phantom Tryonia, *Tryonia cheatumi* (Pilsbry, 1935). This endangered freshwater snail is found only in the San Solomon Spring system, a cienega, or karst-based, arid-land freshwater spring system, in western Texas, USA. We sampled populations at seven locations in the system seasonally over a 2-yr period. San Solomon Spring, the system's largest spring and modified into a swimming pool, had the largest population of *T. cheatumi*, with an estimated 49 million individuals and a mean density as high as 23,626 ± 39,030 (individuals/m^2 ± SD). There were seasonal differences in mean density (up to 25-fold) and median snail size at all sites, but consistent seasonal patterns of mean density or size were not observed. Median snail size among samples was not related to water temperature, and juveniles were present in most samples in all seasons. These results support continuous, aseasonal reproduction, as expected in thermally stable habitats, but differences in median size and mean density among seasons and sites suggest that other factors affect reproduction and seasonal variation in population size of *T. cheatumi*.

KEY WORDS: spring snails, desert springs, life history, snail reproduction, conservation

INTRODUCTION

Many aquatic snails in arid regions are narrowly endemic, usually restricted to one or a few nearby springs, and these species often are of conservation concern (Lydeard et al. 2004; Hershler et al. 2014). Spring snails in the genus *Tryonia* are characteristic of mineral and thermal (hot or warm) springs in the southwestern United States and Mexico, and most are restricted to a single spring or spring system (Hershler 2001; Hershler et al. 2011, 2014). *Tryonia* are small (< 7 mm), obligately aquatic, ovoviviparous snails with separate sexes that graze on periphyton (Brown et al. 2008). *Tryonia* are most abundant at spring heads, where they are locally dominant members of the invertebrate community (Meffe and Marsh 1983; Hershler 2001). However, *Tryonia* typically occupy narrower microhabitat niches than other spring-dwelling snails (Sada 2008), thereby limiting their spatial distribution and total population size. Furthermore, population size of *Tryonia* and related genera can vary annually, seasonally, or spatially (Taylor 1983; Lang 2001, 2011; Brown et al. 2008; Johnson et al. 2019).

Fishes inhabiting thermally stable spring systems are expected to have largely aseasonal population dynamics, such as continuous or aseasonal reproduction (Winemiller 1989), but freshwater snails may deviate from this pattern (Whelan

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and Strong 2014). For example, spring snails in the genus *Pyrgulopsis* have well-defined, population-specific reproductive seasons that correspond to small, seasonal differences in water temperature (e.g., Mladenka and Minshall 2001; Lysne et al. 2007). Aseasonal or continuous reproduction is proposed for *Tryonia* in warm, thermally stable springs, but seasonal reproduction is proposed in cooler, more thermally variable spring systems (Taylor 1983; Brown et al. 2008). However, these conclusions were based on limited evidence, and detailed life history information is available for only a few species in this group (e.g., Sada 2008). Critically, seasonal temperature variability has not been assessed in springs that support *Tryonia*, and temperature is potentially a primary driver of reproduction (Brown et al. 2008).

The endemic spring snails of Texas, USA, include five species of *Tryonia* (Hershler 2001; Hershler and Liu 2017), all of which are critically imperiled (G1) under NatureServe criteria (Faber-Langendoen et al. 2012; Johnson et al. 2013). *Tryonia cheatumi*, the Phantom Tryonia, is listed as endangered under the U.S. Endangered Species Act (USFWS 2013). *Tryonia cheatumi* occurs only in the San Solomon Springs system in arid western Texas within the Chihuahuan Desert ecoregion (Allan 2011; Lang 2011). All known populations are restricted to a 13-km-long series of spring outflows near the town of Balmorhea, including Phantom Lake, Giffin, San Solomon, and East and West Sandia springs, and associated aquatic habitats. Flows from the San Solomon Springs system are declining over the long term due to groundwater extraction for agriculture, and concern exists regarding potential effects from nonconventional oil and gas development in the region (Texas Water Development Board 2005). Limited sampling of *T. cheatumi* from 2001 to 2009 found high densities (thousands per square meter) at Phantom Lake, San Solomon Springs, and East Sandia Springs (Lang 2011). However, effective conservation of the species requires more recent density estimates and life history data to understand population trends over time.

We examined seasonal patterns of population density, population size, and size structure in *T. cheatumi* throughout the San Solomon Spring system over 2 yr. We examined size structure to make inferences about when reproduction occurred and whether reproduction was seasonal or continuous. We also examined temporal patterns of water temperature in different parts of the spring system and how thermal stability was related to size structure.

METHODS

Study Area

San Solomon Springs is an artesian spring system consisting of three main areas, all of which appear to arise from the same groundwater sources (Chowdhury et al. 2004). The main spring, San Solomon Spring (water source in San Solomon Pool), within Balmorhea State Park, has been modified into a partially concrete-lined swimming pool that discharges into the concrete-lined San Solomon Canal that, in turn, feeds two restored wetland areas, San Solomon Cienega and Hubbs Cienega. Giffin Spring is on private land approximately 0.2 km northwest of San Solomon Spring (Lang 2011). The system includes two additional spring areas that support *T. cheatumi*. Phantom Lake Spring, approximately 2.4 km southwest of the state park, is upgradient from the main spring and discharges groundwater from a large cavern system (Brune 2002). The spring discharge from the cavern ceased to flow in 1999, but water is now provided to a constructed canal and wetland by a pump located inside the cave. East and West Sandia springs, near Balmorhea, are downgradient of the main spring system and located on property owned by The Nature Conservancy. East Sandia Spring currently consists of a series of isolated pools and marshy areas. In 2000, West Sandia Spring was reduced to a moist soil area, and no aquatic snails were found (J.J. Landye, Arizona Game and Fish Department, retired, personal communication). However, West Sandia Spring was flowing during our study. All springs in the system have been connected intermittently via a series of irrigation canals since the 1940s, but it is unknown whether *T. cheatumi* occurs in the canals.

Field Sampling

We sampled *T. cheatumi* and water temperature at seven sites within the San Solomon Spring system (Fig. 1): (1) East Sandia Spring (30.990978, –103.729036; 603-m^2 surface area), (2) West Sandia Spring (30.986838, –103.73635; 339 m^2), (3) San Solomon Pool (30.944279, –103.788395; 5,556 m^2), (4) San Solomon Canal (30.944538, –103.785917; 2,859 m^2), (5) Hubbs Cienega (30.945479, –103.786001; 1,050 m^2), (6) San Solomon Cienega (30.945138, –103.784405; 4,340 m^2), and (7) Phantom Lake Spring (30.935005, –103.849613; 120 m^2). These sites encompass all known populations of *T. cheatumi* except Giffin Spring, which we were unable to sample. We sampled most sites in spring (March), summer (June), fall (October), and winter (December) in 2017 and 2018 (Table 1), except for the fall 2018 sample at San Solomon Pool, which was taken in August. Because of limited access to the site, we did not sample Phantom Lake Spring in the fall.

At each site on each sampling event, we estimated *T. cheatumi* density at 10–25 randomly generated points distributed among mesohabitat types found at the sites. Before sampling, we delineated broad mesohabitat types based on benthic substrate composition and macrophyte cover, and we estimated the area of each mesohabitat with a hand-held Trimble GPS unit (Trimble, Inc., Sunnyvale, CA, USA). Based on our delineation, there were one to three mesohabitat types at each site. The number of sampling points at each site was dependent on the size of the site and the number of mesohabitat types present, and we allocated the number of samples in each mesohabitat proportional to the area of the mesohabitat at the site on the date of sampling.

On each sampling date and before sampling for *T.*

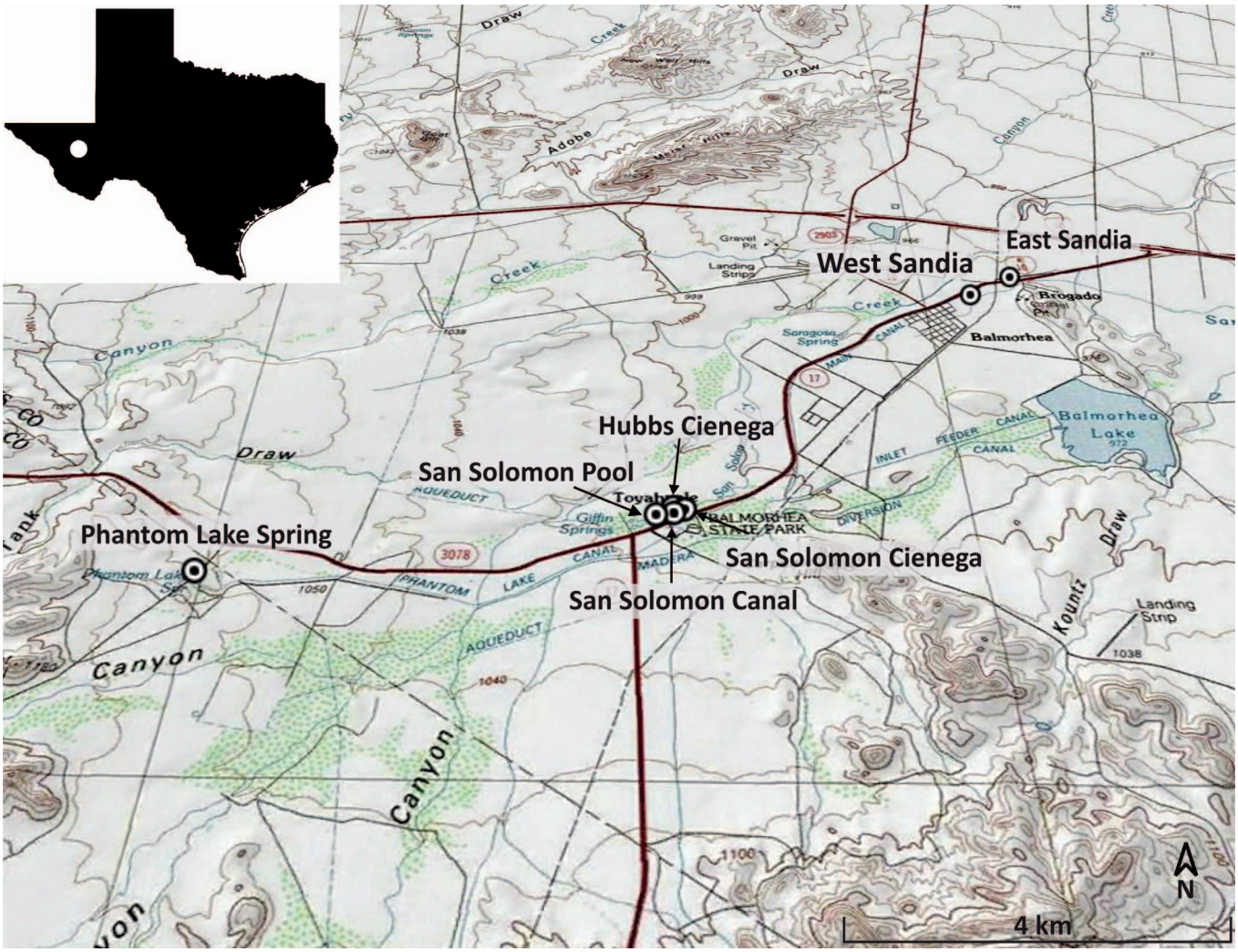

Figure 1. Map showing the location of sampling sites in the San Solomon Spring system (imagery from USGS 2013). Inset shows the location of the study area in Texas, USA.

Table 1. Parameter estimates for the generalized linear model examining the effect of site and season on mean density of *Tryonia cheatumi* at seven sites in the San Solomon Spring system. b is the slope and W_T is the Wald chi-squared test statistic. Degrees of freedom for all parameters = 1. Redundant parameters were set to zero and are not included. Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC = San Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring.

Parameter	b	SE	95% Confidence Interval	W_T	P
Intercept	0.04	1.00	−1.91 to 1.99	0.002	0.966
CAN	6.65	1.02	4.65 to 8.66	42.34	< 0.001
ES	5.78	1.13	3.56 to 8.00	26.04	< 0.001
HC	6.23	1.09	4.10 to 8.37	32.71	< 0.001
PHA	8.67	1.08	6.56 to 10.78	64.75	< 0.001
SSC	2.40	1.10	0.20 to 4.57	4.74	0.03
SSP	8.80	1.06	6.72 to 10.87	68.90	< 0.001
Fall	0.76	0.64	−0.48 to 2.00	1.45	0.23
Spring	0.23	0.45	−0.65 to 1.12	0.27	0.60
Summer	0.42	0.44	−0.45 to 1.29	0.88	0.35

cheatumi, we measured water temperature immediately above the bottom at each sampling point by using a pre- and postcalibrated Manta multiprobe (Eureka Water Probes, Austin, TX, USA). We then collected benthic material (containing *T. cheatumi*) at each point with a 100-cm^2 benthic basket sampler designed for quantitative sampling of spring snail populations (Lang sampler, 10 cm × 10 cm × 3 cm, 500-μm mesh; Lang 1999, 2001; Johnson et al. 2019). The number of samples taken on each date at each site was as follows: East Sandia Spring, $N = 10$–16; West Sandia Spring, $N = 10$–11; San Solomon Pool, $N = 18$–20; San Solomon Canal, $N = 15$–25; Hubbs Cienega, $N = 5$–6; San Solomon Cienega, $N = 14$–16; and Phantom Lake Spring, $N = 5$–20. We preserved samples in the field in 95% ethanol and later sorted, counted, and identified the contents under a dissecting microscope at the San Marcos Aquatic Resource Center, U.S. Fish and Wildlife Service and the Aquatic Ecology Laboratory in the Freeman Aquatic Station at Texas State University, San Marcos. Samples contained two invasive snail species, *Melanoides tuberculata* and *Thiara granifera*, that we distinguished from *T. cheatumi* by shell coloration and sculpture.

We obtained samples of *T. cheatumi* large enough for size structure analysis (> 50 individuals) from three sites: San Solomon Pool ($N = 1{,}260$), San Solomon Canal ($N = 645$), and Phantom Lake Spring ($N = 524$). We measured shell height (maximum shell height parallel to the axis of coiling) with a stereoscopic microscope and attached Infinity-1 camera (Teledyne Lumenera, Ottawa, Ontario, Canada). Infinity Analyze (Teledyne Lumenera, Ottawa, Ontario, Canada) was used to calibrate measurement by using a stage micrometer (Meiji Techno America, San Jose, CA, USA; 1 mm with 0.01-mm divisions). Each shell was placed on clay in the same orientation for measurement, conforming to a standardized shell photography guide (Callomon 2019).

Data Analysis

We tested for differences in median water temperature across seasons at each site by using a Kruskal–Wallis test for independent samples and pairwise comparisons (two-sided tests), due to heterogenous variance and nonnormal error structure. Analyses were conducted in SPSS 27 (IBM, Armonk, New York, United States). We tested for differences in the variance around median water temperature among sites with Levene's test (Zar 1999).

We examined whether *T. cheatumi* density differed across sites or seasons by using a generalized linear model (GzLM). We used GzLM because our data contained many zero observations, exhibited substantial nonnormal error structure, and had heteroscedastic variance (Maindonald and Braun 2007; O'Hara and Kotze 2010). We modeled density by using a negative binomial distribution and a log link function. Site (San Solomon Canal, East Sandia Spring, Hubbs Cienega, Phantom Lake Spring, San Solomon Cienega, San Solomon Pool, and West Sandia Spring) and season (fall, winter, summer, and spring) were categorical variables, and we applied the Huber–Sandwich estimator procedure to account for heterogeneous variance. We assessed overall model significance by comparing the fitted model to an intercept-only model, and pairwise post hoc comparisons among sites and seasons were performed with a sequential Bonferroni procedure. These analyses were performed in R 3.6.3.

To assess the relationship between reproduction of snails and water temperature, we examined two-tailed Pearson correlation coefficients (R 3.6.3; R Core Team 2022) between median shell height of snails at each site on each sampling date and mean water temperature measured at that site. Shell height was not normally distributed in most samples (Shapiro–Wilk test: $W = 0.860856$–0.979127, $P = 0.4063$ to < 0.001). We tested for differences in shell size among sites and seasons using Kruskal–Wallis tests followed by comparison of each pair with the Wilcoxon method including a Bonferroni correction for multiple comparisons when needed. These analyses were conducted in JMP Pro 15.0.0 (SAS Institute, Cary, NC, USA).

RESULTS

Density and Population Size

Water temperatures differed among sites (Fig. 2a). Water temperatures differed among sites across the entire study period (Kruskal–Wallis test: $H_6 = 350.06$, $P < 0.001$). Temperature at East and West Sandia springs did not differ from each other ($P = 0.703$), but both had consistently lower temperatures (~20.5°C) throughout the study period than the other sites ($P < 0.001$ for all comparisons). San Solomon Cienega had lower water temperatures than San Solomon Pool and San Solomon Canal, and Phantom Lake Spring had slightly lower temperatures than San Solomon Canal ($P < 0.001$ for all comparisons).

Water temperatures varied seasonally across sites (Kruskal–Wallis test: $H_3 = 184.94$, $P < 0.001$) following expected seasonal patterns (Fig. 2b). Winter temperatures were lower than those in the spring, summer, and fall ($P < 0.006$ for all comparisons); spring temperatures were lower than those in the summer and fall ($P < 0.001$ for both comparisons); and fall temperatures were lower than those in the summer ($P < 0.001$). Seasonal variation in water temperatures differed among sites (Levene's statistic = 9.32; df = 6, 46; $P < 0.001$), with San Solomon Cienega having the greatest seasonal variation in water temperature, differing > 9°C between the winter minimum and the summer maximum (Fig. 2a). All other sites showed limited variation in water temperature across seasons, with a range of variation of < 4°C annually.

Mean density of *T. cheatumi* differed significantly among sites (Table 1). The highest mean density of *T. cheatumi* was observed in San Solomon Pool (mean density over all sampling events = 8,976 individuals/m^2 ± 19,900 [SD]; Table 2 and Fig. 3). Phantom Lake Spring also had high mean density (7,438/m^2 ± 12,963 SD), but mean density at this site

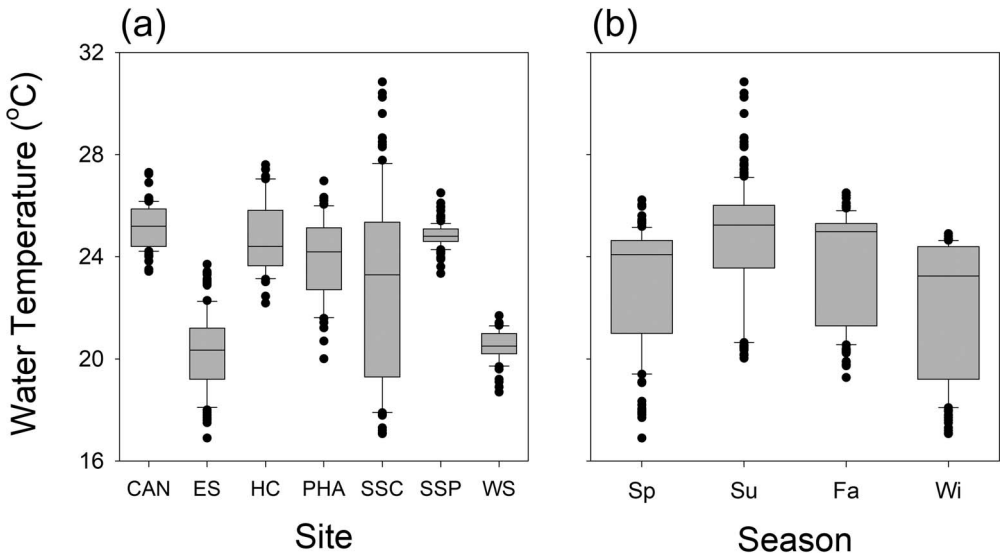

Figure 2. Box-and-whisker plots showing variation in water temperature among (a) sites and (b) seasons in the San Solomon Spring system. Boxes represent the 25% and 75% quartiles, lines within boxes are the median, whiskers are 1.5 × the interquartile range, and dots are outliers. Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC = San Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring. Season abbreviations are as follows: Sp = spring, Su = summer, Fa = fall, and Wi = winter.

did not differ significantly from the other sites except San Solomon Pool due to extremely high seasonal variation. We observed *T. cheatumi* at West Sandia Spring in only one sample, where it was present at low density (spring 2018, 9/m^2 ± 29 SD; mean density across sampling events = 1/m^2 ± 12 SD). Mean density at the other four sites ranged from 7 to 2,099/m^2. Mean estimates of density appeared to vary widely among seasons at all sites, particularly San Solomon Pool and Phantom Lake Spring, but confidence intervals (CIs) around these estimates overlapped widely, and season was not a significant factor in the GzLM model (Table 2). Population size appeared to vary annually at some sites, but CIs overlapped between years at all sites except San Solomon Canal, where estimated population size in 2017 was nearly 20 times higher than in 2018 (Table 3).

The two invasive snail species were present at all study sites except East and West Sandia springs. At Phantom Lake Spring, *Terebia* mean density was 175/m^2 ± 228 SD and *Melanoides* mean density was 4,793/m^2 ± 6,397 SD. At San Solomon Pool, *Terebia* mean density was 3,846/m^2 ± 766 SD and *Melanoides* mean density was 1,593/m^2 ± 1,262 SD. At San Solomon Canal, *Terebia* mean density was 15,445/m^2 ± 6,482 SD and *Melanoides* mean density was 1,245/m^2 ± 834 SD. At San Solomon Cienega, *Terebia* mean density was 19,504/m^2 ± 25,203 SD and *Melanoides* mean density was 8,495/m^2 ± 6,989 SD. At Hubbs Cienega, *Terebia* mean density was 9,535/m^2 ± 12,102 SD and *Melanoides* mean density was 12,060/m^2 ± 5,809 SD.

San Solomon Pool had the highest estimated population size of *T. cheatumi*, with 49,477,642 individuals (95% CI = 25,520,917–73,434,367) (Table 3), followed by San Solomon Canal, Phantom Lake Spring, Hubbs Cienega, San Solomon Cienega, and East Sandia Spring. Estimated population size at

Table 2. Mean density (individuals/m^2 ± SD) of *Tryonia cheatumi* at seven sites in the San Solomon Spring system. Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC = San Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring.

Year	2017				2018			
Site	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter
ES	318 ± 737	283 ± 1,050	29 ± 70	156 ± 641	156 ± 641	67 ± 249	0 ± 0	9 ± 29
WS	—[a]	0 ± 0	0 ± 0	0 ± 0	9 ± 29	0 ± 0	0 ± 0	0 ± 0
SSP	23,626 ± 39,030	15,425 ± 26,782	789 ± 976	1,505 ± 2,367	15,512 ± 47,354	4,530 ± 8,854	1,200 ± 2,781	9,221 ± 31,056
CAN	305 ± 714	2,116 ± 7,228	3,735 ± 13,763	3,243 ± 8,415	242 ± 721	55 ± 172	55 ± 218	300 ± 980
HC	0 ± 0	167 ± 197	83 ± 186	400 ± 563	1,000 ± 1,501	1,020 ± 1,235	200 ± 352	2,240 ± 4,232
SSC	0 ± 0	21 ± 77	0 ± 0	7 ± 26	0 ± 0	77 ± 266	54 ± 187	0 ± 0
PHA	3,050 ± 3,825	12,989 ± 21,828	—	1,917 ± 2,623	325 ± 507	2,980 ± 2,213	—	23,367 ± 46,780

[a]Dashes indicate that no sample was taken.

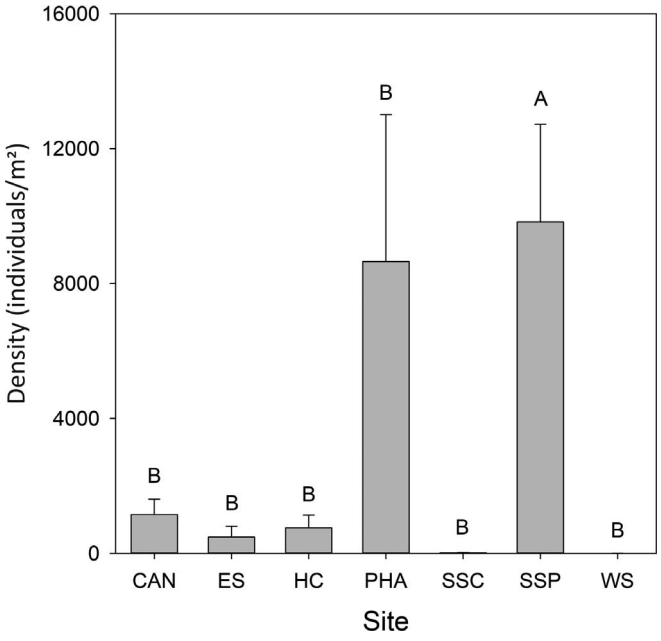

Figure 3. Estimated marginal mean density of *Tryonia cheatumi* across seasons at seven sites in the San Solomon Spring system. Error bars are ±1 SE. Means with different letters are significantly different (post hoc pairwise comparisons, $\alpha = 0.05$). Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC = San Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring.

West Sandia Spring was small (478 individuals, 95% CI = 0–1,413).

Size Structure

Snail size differed among seasons at all three sites where we examined size structure (Fig. 4). There was a significant difference in shell height among seasons at San Solomon Pool (Kruskal–Wallis test: $\chi^2 = 183.2108$, df = 3, $P < 0.0001$; Wilcoxon paired comparisons: $P < 0.0001$); median shell height was highest in the fall (2.76 mm) and lowest in the spring (1.77 mm). Four of six pairwise comparisons were significantly different (Fig. 4). There was a significant difference in shell height among seasons at San Solomon Canal (Kruskal–Wallis test: $\chi^2 = 16.7944$, df = 3, $P < 0.0001$; Wilcoxon paired comparisons: $P < 0.0001$–0.0005); median shell height was highest in the spring (1.90 mm) and lowest in the fall (1.49 mm). Two of six pairwise comparisons were significantly different. There was a significant difference in shell height between summer and winter at Phantom Lake (Kruskal–Wallis test: $\chi^2 = 84.6952$, df = 3, $P < 0.0001$); median shell height was higher in the winter (2.27 mm) than in summer (1.41 mm).

Shell height differed among sites in all seasons except spring (Fig. 5). Median shell height did not differ between San Solomon Pool and San Solomon Canal in the spring (Kruskal–Wallis test: $\chi^2 = 0.6710$, df = 1, $P = 0.4127$), but size appeared to be more variable in San Solomon Pool. There was a significant difference in shell height among all three sites in the summer (Kruskal–Wallis test: $\chi^2 = 236.9808$, df = 2, $P < 0.0001$; all pairwise comparisons significantly different); median shell height was highest in San Solomon Pool (2.57 mm) and lowest at Phantom Lake (1.41 mm). There was a significant difference in shell height between San Solomon Pool and San Solomon Canal in the fall (Kruskal–Wallis test: $\chi^2 = 160.8383$, df = 1, $P < 0.0001$); median shell height was higher in San Solomon Pool (2.76 mm) than in San Solomon Canal (1.49 mm). There was a significant difference in shell height among all three sites in the winter (Kruskal–Wallis test: $\chi^2 = 57.0120$, df = 2, $P < 0.0001$; all pairwise comparisons significantly different); median shell height was highest at Phantom Lake (2.20 mm) and lowest at San Solomon Canal (1.57 mm). There was no correlation between median shell height and water temperature across all sample events that yielded > 50 individuals ($r = -0.10$, $P = 0.654$; $N = 19$).

DISCUSSION

We found substantial populations of *T. cheatumi* at San Solomon Springs and Phantom Lake Spring and smaller populations at the other sites. We found large changes in density (up to 25-fold) of *T. cheatumi* across years and sampling periods. San Solomon Pool had high densities in both spring seasons, summer 2017, and winter 2018 and lower densities in winter 2017, summer 2018, and both fall seasons. At Phantom Lake Spring, peak densities occurred in summer 2017 and winter 2018. San Solomon Spring supports the largest population of *T. cheatumi* in the spring system despite

Table 3. Population size estimates (95% confidence interval) for *Tryonia cheatumi* at seven sites in the San Solomon Spring system in 2017 and 2018. Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC = San Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring.

Site	2017	2018	Mean across Both Years
ES	129,533 (1,155–257,912)	639,611 (0–1,867,644)	389,209 (0–1,016,600)
WS	0 (—)	827 (0–2,447)	478 (0–1,413)
SSP	57,162,692 (25,559,251–88,766,133)	41,377,184 (5,027,369–77,726,998)	49,477,642 (25,520,917–73,434,367)
CAN	6,154,181 (712,138–11,596,224)	320,672 (51,342–590,001)	3,333,041 (559,177–6,106,905)
HC	186,136 (25,389–346,884)	1,170,750 (15,867–2,325,633)	655,000 (85,865–1,224,135)
SSC	31,000 (0–78,772)	131,750 (0–315,577)	81,375 (0–176,376)
PHA	686,448 (69,342–1,303,554)	925,020 (0–1,303,554)	792,480 (115,830–1,469,130)

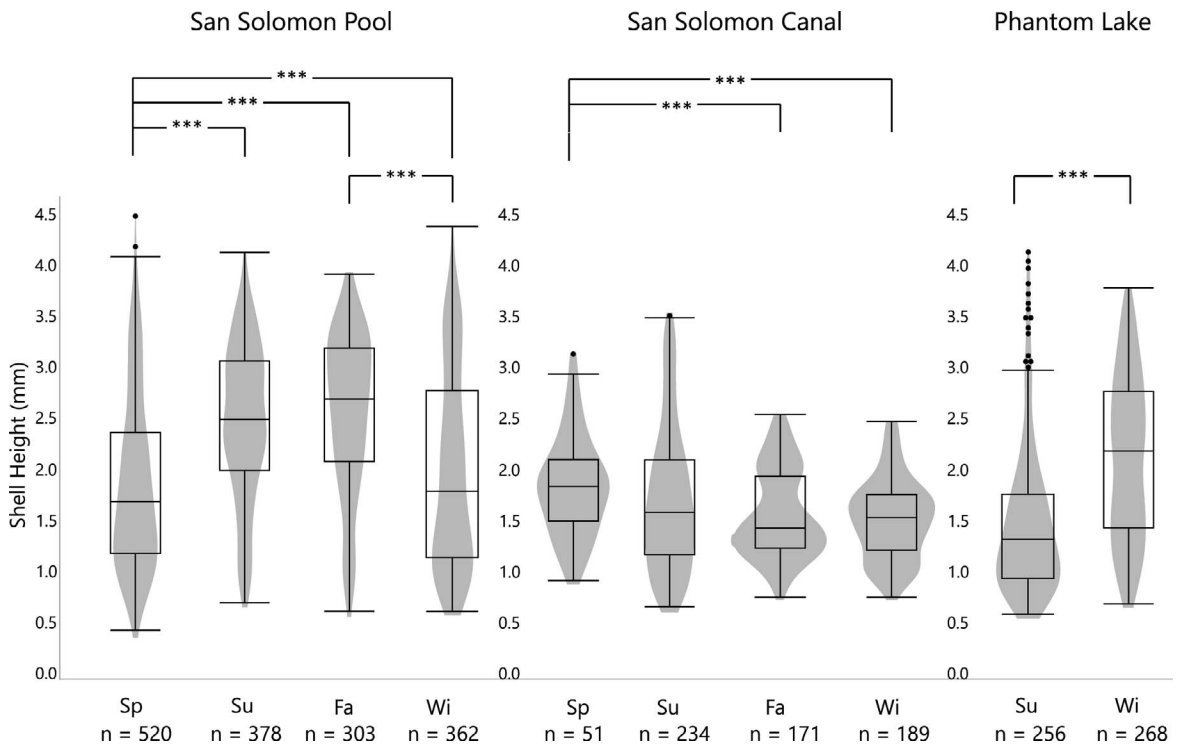

Figure 4. Comparison of size distributions of *Tyronia cheatumi* populations among seasons at three sites in the San Solomon Spring system. Data for 2017 and 2018 are combined for each season. Boxes represent the 25% and 75% quartiles, lines within boxes are the median, whiskers are 1.5 × the interquartile range, and dots are outliers. Season abbreviations are as follows: Sp = spring, Su = summer, Fa = fall, and Wi = winter. All comparisons denoted by *** were significant at $P < 0.001$; comparisons without a bracket were not significant.

the site's heavy recreational use and modification as a swimming pool (~2,000 people/d during the summer). The potential decline in density between spring and summer in both years may be related to annual drawdown and cleaning of the pool in late spring. However, we were unable to detect statistical differences in density between spring and summer, and we do not know the extent of snail mortality that occurs coincident with pool cleaning.

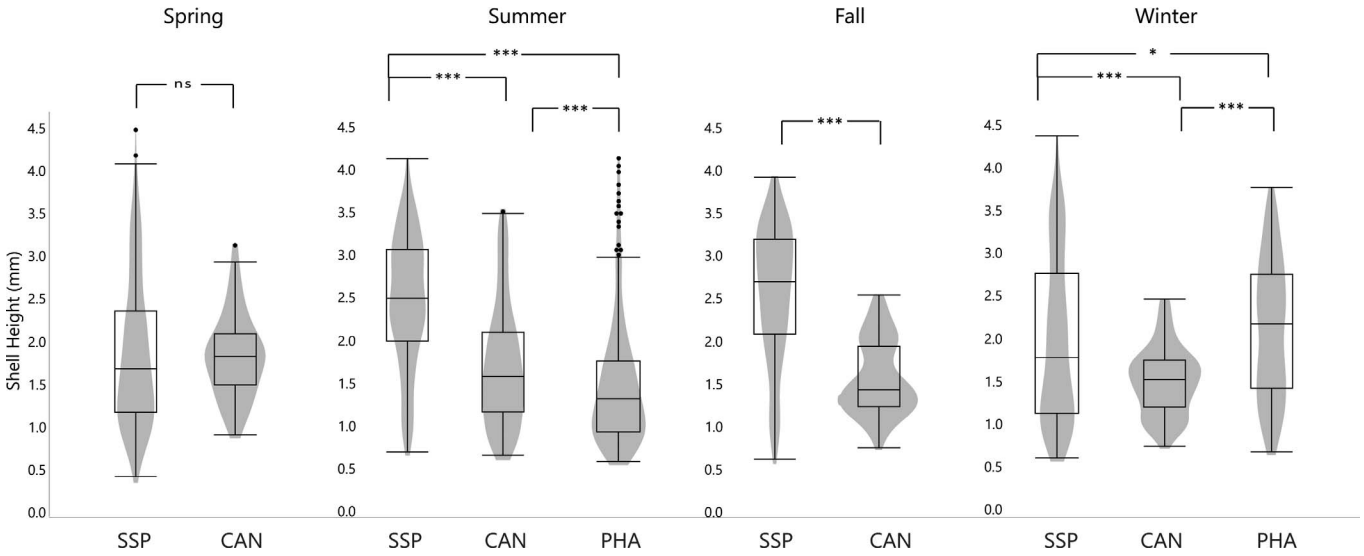

Figure 5. Comparison of size distributions of *Tyronia cheatumi* populations among three sites in the San Solomon Spring system within seasons. Boxes represent the 25% and 75% quartiles, lines within boxes are the median, whiskers are 1.5 × the interquartile range, and dots are outliers. Data for 2017 and 2018 are combined for each season. Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, HC = Hubbs Cienega, PHA = Phantom Lake Spring, SSC = San Solomon Cienega, SSP = San Solomon Pool, and WS = West Sandia Spring. Significance codes: *$P < 0.05$; ***$P < 0.001$; ns, not significant.

Table 4. Mean density (individuals/m^2 ± SD) of *Tryonia cheatumi* at four sites in the San Solomon Spring system in summer samples from 2001 to 2018. Values from 2001, 2003, and 2009 are from Lang (2011); values from 2017 and 2018 are from this study. Site abbreviations are as follows: CAN = San Solomon Canal, ES = East Sandia Spring, PHA = Phantom Lake Spring, and SSP = San Solomon Pool.

Site	May 2001	May 2003	June 2009	June 2017	June 2018
ES	42,206.6 ± 36,846.1	65,844.5 ± 60,961.9	4,187.6 ± 5,859.0	283 ± 1,050	67 ± 249
SSP	3,195.5 ± 910.1	—[a]	—	15,425 ± 26,782	4,530 ± 8,854
CAN	14,215.8 ± 7,434.8	348,617.7 ± 24,624.7	11,681.2 ± 11,924.9	2,116 ± 7,228	55 ± 172
PHA	47,661.5 ± 24,537.1	46,284.0 ± 51,526.7	31,462.1 ± 20,251.3	12,989 ± 21,828	2,980 ± 2,213

[a]Dashes indicate that no sample was taken.

There have been several prior efforts to document snail density at San Solomon Springs. Bradstreet (2012) found an average density of *T. cheatumi* of 168/m^2 in May 2010 and 0/m^2 throughout the rest of the year, values that are far lower than our estimates. However, their study sampled quadrats visually, instead of collecting and sorting benthic material. Juvenile *T. cheatumi* are < 1 mm in height and are difficult to find in underwater visual surveys, particularly in areas with vegetation. Juveniles comprised most of our samples in some seasons, and they are similar in appearance when unmagnified to juveniles of the invasive snails *Melanoides* and *Thiara*. Because of these issues, it is difficult to compare our results with those of Bradstreet (2012).

Lang (2011) sampled San Solomon Pool, San Solomon Canal, Phantom Lake Spring, and East Sandia Spring in summers 2001, 2003, and 2009 (Table 4) by using the same benthic sampling device that we used. That study found much higher *T. cheatumi* density at East Sandia Spring, San Solomon Canal, and Phantom Lake Spring than we found in our summer samples, but Lang (2011) found much lower density at San Solomon Pool. However, Lang (2011) took only three samples per site at targeted locations with known snail presence, compared with the 10–25 randomly selected points per site that we sampled. Consequently, the values reported by Lang (2011) represent estimates only from high snail-density areas, whereas our values represent estimates of site-wide mean density. Snail density showed high spatial variation in our study, and many samples had no snails. This resulted in very high error around our estimates of mean density; in contrast, the targeted sampling of Lang (2011) produced lower (but still high) error. The much lower densities of *T. cheatumi* that we observed at East Sandia Spring and Phantom Lake Spring in 2017 and 2018 are potentially concerning. However, because of the fundamental differences in sampling design between our study and that of Lang (2011), it is impossible to conclude whether *T. cheatumi* density has changed at any sites since 2001–2009. Sampling approaches such as stratified sampling are needed to account for the high spatial variability of *T. cheatumi* and to provide more precise density estimates needed to detect temporal changes in abundance. Furthermore, the high and unpredictable seasonal variation we observed means that single, annual samples may be insufficient for monitoring long-term changes in density.

Discharge from San Solomon Spring has not changed appreciably between 2009 and the present (U.S. Geological Survey Gage 08427500; https://waterdata.usgs.gov/tx/nwis/rt, accessed March 9, 2022). Although all habitats that support *T. cheatumi* are influenced by the same groundwater source, we do not know whether flows have changed at East Sandia and Phantom Lake springs and whether flow may be related to the potential decline of the species at those sites. Recent changes in the area have the potential to affect *T. cheatumi* populations. Oil and gas extraction has boomed in the past 10 yr, but the effects of this activity on groundwater flow in the San Solomon Spring system are unknown. Two sites that support large populations of *T. cheatumi*—San Solomon Pool and Phantom Lake Spring—have undergone major reconstruction in the past few years. Repair of the pool at San Solomon Springs (2018 and 2020) and construction of a cienega at Phantom Lake Springs (2010) have increased available habitat for *T. cheatumi*, but we do not know whether these changes are associated with changes in snail population size.

Our study partially supported the hypothesis of continuous, aseasonal reproduction, which is expected in thermally stable habitats (Winemiller 1989). As expected by this hypothesis, we found no relationship between snail size and water temperature, size structure showed no obvious seasonal pattern, and juvenile individuals were present in most samples in all seasons. However, size differed significantly among most seasons and sites, suggesting that other, unknown factors have some influence on reproductive cycles. A more detailed analysis of population dynamics, including estimation of seasonal patterns of individual growth and survival, could help explain the seasonal and spatial variation that we observed in *T. cheatumi*. A better understanding of the factors that influence reproduction and population size is needed for effective conservation of *T. cheatumi*.

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REGULAR ARTICLE

EVALUATION OF HOST FISHES FOR THE BROOK FLOATER (*ALASMIDONTA VARICOSA*) FROM POPULATIONS IN MASSACHUSETTS AND MAINE, USA

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ABSTRACT

The Brook Floater (*Alasmidonta varicosa*) mussel is globally vulnerable and has disappeared from much of its historical range. Information on Brook Floater host fish use is needed for ecological and conservation purposes, but previous laboratory studies provide conflicting results. We evaluated host fish use by Brook Floater from populations in Massachusetts and Maine, USA. We conducted three experiments using a total of 10 fish species from six families, and we estimated glochidial attachment rate and juvenile metamorphosis rate. Across fish species, attachment ranged from 51.0% to 84.6% and metamorphosis ranged from 4.9% to 80.9%. Fish species and inoculation density (viable glochidia/mL) only weakly predicted attachment, and the number of glochidia that attached to fish did not affect metamorphosis rate. Juvenile metamorphosis was successful on all fish species tested, supporting evidence that Brook Floater is a host generalist. Fish species was an important factor in predicting metamorphosis rates in all experiments. The highest metamorphosis was on Slimy Sculpin (*Cottus cognatus*) (80.9% ± 2.6 SD) and Brook Trout (*Salvelinus fontinalis*) (71.6%), but metamorphosis on Brook Trout varied according to source and was lowest on hatchery-raised fish (12.8% ± 0.3 SD). These data contribute to our understanding of the life history of Brook Floater by identifying potential host fishes, and our results can inform propagation efforts for this species in the northeastern USA.

KEY WORDS: *Alasmidonta varicosa*, Brook Floater, glochidia, host fish, host generalist, propagation

INTRODUCTION

Captive propagation of freshwater mussels is an important tool to support the conservation and restoration of imperiled species (FMCS 2016; Cowie et al. 2017; Strayer et al. 2019). Captive propagation typically requires the identification of suitable host fishes that can facilitate the development of parasitic mussel larvae (glochidia). Glochidia of a particular mussel species often can parasitize multiple fish species, but fishes vary in suitability (Riusech and Barnhart 2000; McNichols et al. 2011), and host use can vary across geographic regions (Douda et al. 2014). Cost-effective propagation requires the identification of host fishes that consistently produce large numbers of juvenile mussels, and knowledge of host use has other important applications for conservation and understanding of mussel ecology (Barnhart et al. 2008; Douda et al. 2014). Consequently, the identification of host fishes is considered a research priority (Ferreira-Rodríguez et al. 2019).

The Brook Floater (*Alasmidonta varicosa*) occurs in Atlantic Coast rivers of North America from Georgia to New Brunswick and Nova Scotia, but it has disappeared from much of its former range and is considered vulnerable

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(NatureServe 2011). The largest declines have occurred in the central part of its range from Virginia to New Hampshire, and eight of 11 northeastern U.S. states designate Brook Floater as critically imperiled (NatureServe 2011). Captive propagation is proposed as a tool to recover and restore Brook Floater populations in the northeastern USA, and identification of host fishes is needed to support these efforts (Roy et al. 2022).

Two previous laboratory studies of Brook Floater host use identified 20 suitable host fish species, characterizing it as a host generalist (Eads et al. 2007; Wicklow et al. 2017). In North Carolina, Brook Floater glochidia metamorphosed on nine of 13 fish species tested, but measures of metamorphosis rate were not provided, and host use was inconsistent between experiments (Eads et al. 2007). In New Hampshire, Brook Floater glochidia metamorphosed on 12 of 17 fish species tested, but these experiments were conducted with low inoculation densities (< 41 glochidia/fish) and few individuals of each fish species (1–5), leaving questions about which fishes are robust hosts and suitable for large-scale propagation (Wicklow et al. 2017). Furthermore, suitable hosts differed between the two studies: Margined Madtom (*Noturus insignis*) and Tessellated Darter (*Etheostoma olmstedi*) were suitable hosts in New Hampshire but not in North Carolina, and Redbreast Sunfish (*Lepomis auritus*) was a suitable host in North Carolina but not in New Hampshire (Eads et al. 2007; Wicklow et al. 2017). Additional information about Brook Floater host use is needed to inform propagation efforts and other conservation and ecological questions.

We evaluated host fish use in the laboratory for Brook Floater from populations in Massachusetts and Maine. We estimated glochidial attachment and juvenile metamorphosis rates on 10 fish species across three different experiments. We evaluated how well attachment and metamorphosis rates were predicted by inoculation density, density of glochidia on fish, and fish species. Finally, we compare our results with other studies of Brook Floater host use and discuss considerations for selecting the most effective hosts for propagation of Brook Floater in the northeastern USA.

METHODS

We conducted three laboratory experiments in which we tested various combinations of potential hosts under different conditions (see subsequent description of each experiment). All experiments were conducted at the U.S. Fish and Wildlife Service's Richard Cronin Aquatic Resource Center (CARC) in Sunderland, Massachusetts.

Host Fish Collection

Fish species and numbers varied by experiment based on our ability to collect fishes in the wild in early spring and on fish availability at hatcheries. We obtained salmonids from the following fish hatcheries: Nashua National Fish Hatchery, Nashua, New Hampshire (Atlantic Salmon, *Salmo salar*); Silvio O. Conte Anadromous Fish Research Laboratory, Turners Falls, Massachusetts (Brook Trout, *Salvelinus fontinalis*); and Massachusetts Division of Fisheries and Wildlife, Sandwich, Massachusetts (Brook Trout; Brown Trout, *Salmo trutta*; Rainbow Trout, *Oncorhynchus mykiss*). We collected all other fishes by seining and backpack electrofishing in the Fall River, Massachusetts (Slimy Sculpin, *Cottus cognatus*; Longnose Dace, *Rhinichthys cataractae*; Blacknose Dace, *Rhinichthys atratulus*; White Sucker, *Catostomus commersonii*) or the Connecticut River, Massachusetts (Banded Killifish, *Fundulus diaphanous*; Bluegill, *Lepomis macrochirus*). We collected fishes from river sections where mussels were absent or rare to avoid removing potential hosts and to reduce the chances that fishes had immunity from prior exposure to glochidia (O'Connell and Neves 1999; Rogers and Dimock 2003). We maintained fishes in aquaria and fed them black worms until the start of experiments.

Mussel Broodstock Collection and Glochidia Extraction

We collected Brook Floater broodstock from streams by snorkeling. We collected one gravid mussel from the Nissitissit River in Middlesex County, Massachusetts, in March 2017 (Experiment 1); three gravid mussels from Wesserunsett Stream in Somerset County, Maine, in April 2017 (Experiment 2); two gravid mussels from the West Branch Farmington River in Berkshire County, Massachusetts; and three gravid mussels from Wesserunsett Stream in October 2018 (Experiment 3). We transported mussels to the laboratory individually in aerated 3.7-L glass jars of water in a cooler. We maintained mussels in an environmental chamber at CARC at a temperature similar to stream temperatures at the time of broodstock collection (∼5°C) to inhibit glochidia release. We conducted experiments within 6 wk of broodstock collection. Immediately before extraction of glochidia for the experiments, we acclimated broodstock to 10°C, an approximate temperature at which glochidia are released in the wild (about 14°C; Wicklow et al. 2017).

We extracted glochidia for Experiments 1 and 2 by puncturing one or both gills with a 1-mL syringe and sterilized 18-gauge needle and flushing glochidia from the gills with water into a beaker. In Experiment 3 we used aquaria to immerse mussels in a water bath with serotonin (23 mg/L) for 2–3 h (Eads et al. 2010; Patterson et al. 2018) to induce the release of glochidia and avoid gill trauma associated with gill punctures. Glochidia from the serotonin bath were collected on a 150-μm screen and then resuspended in water in a beaker.

We determined glochidia viability for each mussel by evenly suspending glochidia in a 1000-mL beaker and collecting five 200-μL subsamples with a pipette. We placed all five subsamples together in a Petri dish, added a sodium chloride (NaCl) solution, and under a dissecting microscope counted the number of open and closed

glochidia before and after exposure to NaCl. We calculated glochidia viability as

$$\text{Glochidia viability} = \frac{(\text{No. open glochidia} - \text{No. open glochidia after NaCl})}{\text{No. total glochidia}} \times 100.$$

Glochidia viability across all broodstock individuals was 88%–93%; based on consistently high viability we used all broodstock in the experiments (see Hove et al. 2000). For each experiment, we combined glochidia from all broodstock, evenly suspended the glochidia, and then divided the total volume into equal stock solutions for each replicate inoculation based on target inoculation densities (see subsequent). We decanted water in each stock solution until there was only enough water to suspend glochidia in a Petri dish and then photographed the Petri dish containing the glochidia with a digital camera and macro lens (5D Mark 3S camera, 100 mm f2.8/L Macro IS USM Lens, Canon U.S.A. Inc., Huntington, New York, USA). Photographing allowed us to count glochidia added to each inoculation bath, resulting in a more accurate quantification of glochidia than volumetric estimates alone; these numbers were used to calculate attachment rates.

Experiment 1

In Experiment 1, we tested the host suitability of three fish species: Slimy Sculpin, Longnose Dace, and Atlantic Salmon. We inoculated Slimy Sculpin (mean length = 72 mm ± 5.0 SD) and Longnose Dace (87 mm ± 7.0) by placing six individuals of each species in 200 mL of water in a McDonald-type hatching jar (similar to those produced by Global Aquaculture Supply Co., Sioux Falls, South Dakota, USA; hereafter, McDonald jar). Our target inoculation density was 200 glochidia/fish; however, counts of glochidia in photographs indicated true inoculation densities of 121 glochidia/fish (3.64 viable glochidia/mL; Table 1) for Slimy Sculpin and 150 glochidia/fish (4.50 viable glochidia/mL; Table 1) for Longnose Dace. Air injected into the bottom of the McDonald jars suspended the glochidia, facilitating glochidia contact with fishes. We exposed fishes for 20 min, removed the fish, and then filtered the water over a 150-μm mesh sieve to collect unattached glochidia. We counted the number of unattached glochidia and subtracted this number from the estimated number of glochidia in the inoculation bath to estimate the attachment rate (the percentage of viable inoculated glochidia that attached to each fish; Table 2).

Atlantic Salmon (mean length = 180 mm ± 1.0 SD) were too large for the McDonald jars; therefore, we pipetted glochidia directly onto the gills of two individuals. Before inoculating fish, we photographed the Petri dish containing the glochidia that we pipetted onto the gills of each fish. We anesthetized fish with tricaine methanesulfonate (MS 222) and pipetted the entire glochidia stock solution onto the left or right gills to obtain a target inoculation density of 300 glochidia/fish. We conducted the inoculation over a tray to collect unattached glochidia, and we counted glochidia in the tray to estimate the number of glochidia that attached to each fish by subtracting the number counted in the tray from the number counted in the photographs (Table 1).

After inoculation, we placed Slimy Sculpin and Longnose Dace in 3-L Aquatic Habitat (AHAB) tanks (Pentair Aquatic Ecosystems, Apopka, Florida, USA), for a total of three tanks/species (two individuals/tank). We placed individual Atlantic Salmon in separate 9-L AHAB tanks. We inspected the contents of each tank every 1–3 d, beginning the day after inoculation. We collected sloughed glochidia or juveniles by increasing the flow in the AHAB tanks for 10 min and collecting flushed material on a 150-μm filter. We placed sloughed glochidia or juveniles from each tank and collection event in a Petri dish and counted glochidia and juveniles under a dissecting microscope. Starting day 7 postinoculation, most juveniles exhibited a foot and two adductor muscles but lacked movement; thus, we left material in Petri dishes overnight at room temperature (~18°C) and inspected it the next morning. Mussels that exhibited foot movement the next morning were considered metamorphosed juvenile mussels, and all other individuals were considered sloughed glochidia. We estimated the metamorphosis rate of attached glochidia by dividing the total number of live juveniles recovered from tanks by the total number of glochidia collected from tanks (Rogers et al. 2001).

If no juveniles were collected after 5 d, we inspected a subsample of fish, and if no glochidia were attached, we terminated the experiment. We sacrificed all fishes at the completion of all experiments and inspected the fishes under a compound microscope for remaining glochidia. The duration of the experiments was 37–40 d. Using room-controlled temperature we slowly increased the water temperature in the AHAB tanks from 13°C to 19°C (average rate = 1°C/d for 6 d) to facilitate glochidia metamorphosis. The initial AHAB temperature (13°C) was chosen to reduce thermal stress during transfer of glochidia and fishes from the holding and inoculation chambers. We measured dissolved oxygen in a subset of the AHAB tanks every 3 d with a YSI Professional Plus multiparameter water quality meter (Xylem, Inc., Yellow Springs, Ohio, USA); dissolved oxygen was >7.0 mg/L for all measurements.

Experiment 2

In Experiment 2, we retested Slimy Sculpin (mean length = 72 mm ± 10 SD) and Longnose Dace (72 mm ± 11) using different individuals than in Experiment 1 and tested five new fish species: Blacknose Dace (mean length = 67 mm ± 7 SD), Banded Killifish (75 mm ± 9), Bluegill (77 mm ± 2), White Sucker (122 mm ± 5), and Brook Trout (375 mm ± 109). We inoculated 12 individuals each of Longnose Dace, Blacknose Dace, and Banded Killifish, with each species divided into two replicate inoculations in separate McDonald jars with six individuals/jar. We inoculated six Slimy Sculpin together in a single McDonald jar. We inoculated three White Sucker and four Bluegill, with each species in a single McDonald jar.

Table 1. Inoculation methods for three host identification experiments for Brook Floater (*Alasmidonta varicosa*). Fish species without entries under "Replicate" were held in a single chamber. Water volume is the volume of the inoculation bath. The stock solution represents the glochidia solution used to inoculate fishes. The target inoculation density was determined volumetrically. The actual inoculation density and stock solution glochidia density were determined later by counting glochidia in photographs of the inoculation stock to which fishes were exposed. Scientific names for fishes are in Table 4.

Species	Replicate	Inoculation method	Water volume (mL)	Stock solution glochidial density (viable glochidia/mL)	Target inoculation density (glochidia/fish)	Actual inoculation density (glochidia/fish)
Experiment 1						
Slimy Sculpin		McDonald	200	3.64	200	121
Longnose Dace		McDonald	200	4.50	200	150
Atlantic Salmon		Direct	n/a	n/a	300	326
Experiment 2						
Slimy Sculpin		McDonald	250	5.73	250	239
Longnose Dace	A	McDonald	250	5.14	250	214
	B	McDonald	250	5.36	250	223
Blacknose Dace	A	McDonald	250	4.72	250	197
	B	McDonald	250	4.45	250	185
Banded Killifish	A	McDonald	250	4.55	250	190
	B	McDonald	250	4.80	250	200
White Sucker		McDonald	250	2.82	300	235
Bluegill		McDonald	250	1.97	200	123
Brook Trout		Bucket	4,000	4.27	1,000	743
Experiment 3						
Brook Trout	A	Bucket	4,000	1.01	200	270
	B	Bucket	4,000	0.75	200	200
	C	Bucket	4,000	0.81	200	217
Brown Trout	A	Bucket	4,000	0.93	200	247
	B	Bucket	4,000	0.87	200	232
	C	Bucket	4,000	1.18	200	315
Rainbow Trout	A	Bucket	4,000	1.12	200	299
	B	Bucket	4,000	0.84	200	224
	C	Bucket	4,000	0.90	200	241

Table 2. Glochidia attachment rates and juvenile metamorphosis rates of Brook Floater (*Alasmidonta varicosa*) on fishes in three experiments. Attachment rate is the percentage of inoculated glochidia that attached to fishes. Metamorphosis rate is the percentage of attached glochidia that metamorphosed into juvenile mussels. Average juveniles/fish is based on the daily number of juveniles produced/the number of fish surviving, summed across experimental days. Mean values and SD were calculated only from replicate chambers in which fishes survived to produce juvenile mussels (see Fig. 2). Scientific names for fishes are in Table 4.

		% Attachment		% Metamorphosis				
Experiment	Fish species	Mean	SD	Mean	SD	Avg. juveniles/fish	No. fish inoculated	No. fish survivors
1	Slimy Sculpin	79.7		80.9	2.6	203	6	6
1	Longnose Dace	84.0		29.1	21.9	67	6	6
1	Atlantic Salmon	78.1		35.2	13.7	69	2	1
2	Longnose Dace	61.1		24.5	6.7	70	12	4
2	Blacknose Dace	77.6		16.9	9.1	9	12	1
2	Banded Killifish	64.1		43.0	34.2	44	12	4
2	Slimy Sculpin	75.1		72.6	5.2	301	6	5
2	White Sucker	64.7		22.3	12.9	23	3	3
2	Bluegill	51.0		4.9		1	4	1
2	Brook Trout	80.3		71.6		342	23	23
3	Brook Trout	83.2	2.3	12.8	0.3	67	45	45
3	Brown Trout	84.6	0.4	62.1	6.7	316	45	45
3	Rainbow Trout	83.5	4.7	5.7	0.4	31	45	45

Water volume in all McDonald jars was 250 mL (50 mL higher than in Experiment 1). Inoculation methods and duration in McDonald jars were as described for Experiment 1 using a McDonald jar.

Our target inoculation densities were 250 glochidia/fish for Longnose Dace, Blacknose Dace, Banded Killifish, and Slimy Sculpin; 300/fish for White Sucker; and 200/fish for Bluegill. Photographic counts indicated that inoculation densities differed slightly from our targets (Table 1). For example, replicate inoculations for Longnose Dace contained 1,284 viable glochidia (214 glochidia/fish; 5.14 viable glochidia/mL; Table 1) and 1,338 viable glochidia (223 viable glochidia/fish; 5.36 viable glochidia/mL; Table 1).

We inoculated Brook Trout together in a single bucket with 23 fish in 4,000 mL of water. We exposed fish for 20 min, removed the fish, and then filtered the water over a 150-μm mesh sieve to collect unattached glochidia. Our target inoculation density was 1,000 glochidia/fish, but photographic counts indicated a density of 743 glochidia/fish (4.27 viable glochidia/mL).

After inoculations, we separated fishes into AHAB tanks that consisted of three 3-L tanks for Blacknose Dace (4 fish/tank), Longnose Dace (4 fish/tank), Banded Killifish (4 fish/tank), Slimy Sculpin (2 fish/tank), and White Sucker (1 fish/tank). We placed Bluegill (2 fish/tank) into two replicate 3-L tanks and Brook Trout into one 260-L circular tank.

We collected glochidia and juvenile mussels from AHAB tanks following methods described for Experiment 1. We collected glochidia and juveniles from the Brook Trout tank by siphoning 60 L of water from the tank bottom with a 2-cm hose every 1–3 d; we collected siphoned material on a 150-μm filter. We estimated metamorphosis rate and measured dissolved oxygen as described for Experiment 1. Experiment 2 ended on days 24–34.

Experiment 3

In Experiment 3, we tested new individuals of Brook Trout (mean length = 145 mm ± 13 SD), Rainbow Trout (146 mm ± 11), and Brown Trout (140 mm ± 10). We inoculated fishes with glochidia following methods described for Brook Trout in Experiment 2, except that we inoculated each fish species in three replicate inoculation baths, each with 15 individuals. Our target inoculation density was 200 glochidia/fish, but photographic counts indicated densities of 200–315 glochidia/fish (0.75–1.18 viable glochidia/mL; Table 1). We calculated glochidia attachment rate as in Experiments 1 and 2.

After inoculations, we transferred fishes from each bath into a 113-L circular tank with flow-through well water; we used three replicate tanks for each species, each containing 15 individuals. Unlike in Experiments 1 and 2, for Experiment 3 we kept all fish from each replicate inoculation bath in the same holding tank throughout the experiment, which allowed us to examine the relationship between attachment rate and metamorphosis rate. We increased the tank temperature from 15°C to 18°C using a heater (average increase = 1°C/d). We inspected tanks for glochidia and juveniles as described for Brook Trout in Experiment 2. We estimated metamorphosis rate as described for Experiment 1 and measured dissolved oxygen daily. Experiment 3 lasted 25 d.

Table 3. Results of generalized linear models (GLMs) assessing factors that predict Brook Floater glochidia attachment and juvenile metamorphosis rates in Experiment 3. Inoculation density is the number of viable glochidia/mL to which fishes were exposed (see Table 1). Attachment is the estimated number of glochidia attached/fish calculated as the chamber-wide attachment rate divided by the number of fish in the chamber. The top models are in bold.

Model	Δquasi-AIC	Explained deviance (%)	df
Attachment			
Inoculation density	0	28.2	2
Host species + Inoculation density	**1.0**	**54.9**	**4**
Null	1.1	0.0	1
Host species	4.2	8.3	3
Metamorphosis			
Host species	**0**	**98.7**	**3**
Host species + Attachment	1.5	98.8	4
Attachment	433.1	7.2	2
Null	465.4	0	1

Data Analysis

We created sets of generalized linear models (GLM) to assess how well attachment rate (Experiment 3) and metamorphosis rate (Experiment 1 and 3) were predicted by various factors. We did not assess metamorphosis rate for Experiment 2 because of high fish mortality resulting in insufficient replication for analysis. For Experiment 1, we created a model to assess how well metamorphosis rate was predicted by host species (fixed factor). We excluded Atlantic Salmon from these models because of insufficient replication. For Experiment 3, we compared models to assess how well attachment rate was predicted by host species and inoculation density (number of viable glochidia/mL in the inoculation bath) individually, and when both factors were modeled together as an additive term (Table 3). For Experiment 3 we also created models to assess how well metamorphosis rate was predicted by host species and attachment rate, individually and together. For this model, we expressed attachment rate as the number of glochidia attached to the fish.

For each experiment, we created a separate model for each factor or combination of factors and included a null model (a model with no explanatory factors; Table 3). We fit all models with a logit link function and a quasi-binomial error structure; this error structure accounted for overdispersion that resulted from clustering in the data. We evaluated models by fitting them twice: we first extracted the log-likelihood from the binomial model, and then we extracted the dispersion parameter from the quasi model to calculate the likelihood; these were used to calculate a quasi-corrected Akaike

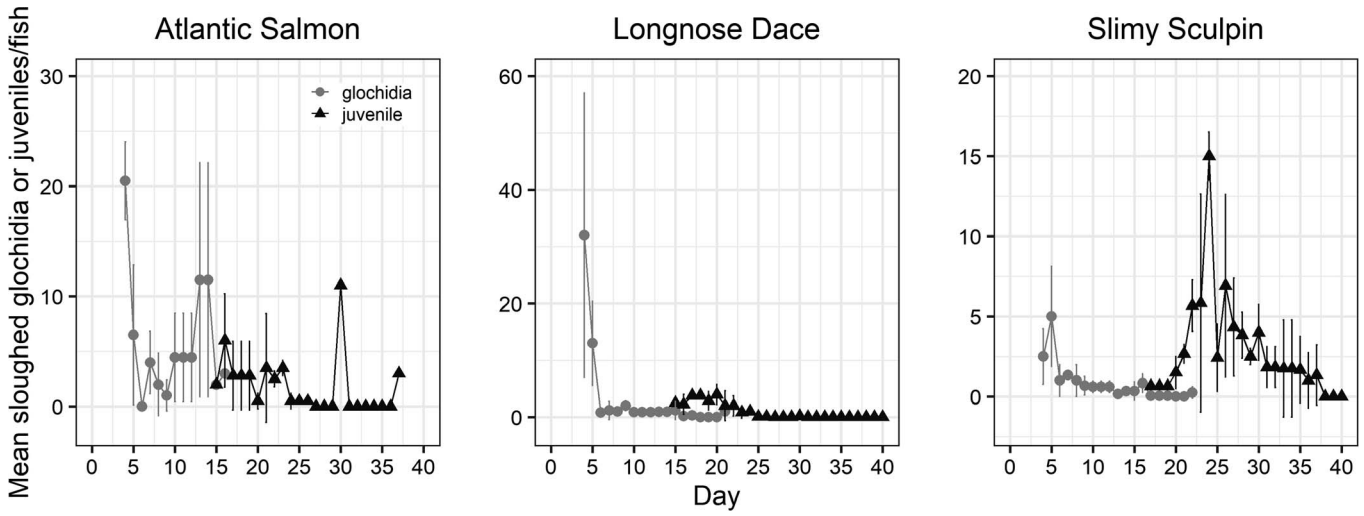

Figure 1. Number of sloughed glochidia or juvenile mussels produced by Brook Floater (*Alasmidonta varicosa*) in Experiment 1. Data points and bars represent the mean and standard deviation, respectively, among replicate fish holding chambers on each day standardized by the number of fish in each chamber.

Information Criterion (qAIC) (Bolker 2021). We calculated explained deviance by subtracting the residual deviance from the null deviance and dividing by the null deviance (Zuur et al. 2015). We selected the best model as the most parsimonious model with high explained deviance and low qAIC (Burnham and Anderson 2004; Wagenmakers and Farrell 2004). We contrasted marginal means using 95% confidence intervals to compare fixed factors in models, and we back-transformed standard error intervals from the logit scale using package "emmeans" (Length et al. 2022; R package version 1.6.0.). All data analyses and models were created in R v4.0.2 software package (R Core Team 2020, Vienna, Austria).

RESULTS

Experiment 1

Glochidia attachment rate was high for all fish species (range = 78.1%–84.0%, Table 2). For Slimy Sculpin and Longnose Dace, most sloughed glochidia appeared within 5 d of inoculation (Fig. 1). For Atlantic Salmon, large numbers of sloughed glochidia appeared within the first 5 d, but this was followed by another peak shortly before juveniles began to appear on day 15 (Fig. 1).

Mean metamorphosis rate of attached glochidia varied by host species and was highest for Slimy Sculpin (80.9% ± 2.6 SD), followed by Atlantic Salmon (35.2% ± 13.7) and Longnose Dace (29.1% ± 21.9) (Table 2). Metamorphosis rate was similar across the three replicates for Slimy Sculpin, but it varied for Atlantic Salmon and Longnose Dace (Fig. 2). Production of juveniles on Slimy Sculpin and Longnose Dace began on days 17 and 15, respectively, and Slimy Sculpin peaked on day 24; production of juveniles on Longnose Dace did not indicate a clear peak (Fig. 1). Juvenile production on Atlantic Salmon began on day 15 but appeared to occur over a more protracted period with no distinct peaks.

Fish species was a good predictor of metamorphosis rate. When comparing modeled probability of metamorphosis using 95% confidence intervals among fish species, Slimy Sculpin had a higher probability (0.81; 95% confidence interval = 0.57–0.93) than Longnose Dace (0.22; 95% confidence interval = 0.09–0.43) ($P < 0.05$); this model explained 79.5% of the deviance.

Experiment 2

Attachment rate varied among fish species (Table 2). The lowest attachment rate of glochidia was on Bluegill (51.0%) and the highest was on Brook Trout (80.3%), with the other species having attachment rates of 61.1%–77.6%. Sloughed glochidia appeared mostly in the first 5 d after inoculation for all species except for Brook Trout, which sloughed glochidia until day 10 (Fig. 3).

Metamorphosis rate varied greatly among fish species and was highest for Brook Trout (71.6%) and Slimy Sculpin (72.6% ± 5.2 SD) and lowest for Bluegill (4.9%) (Table 2). Metamorphosis rate was similar across the three replicates for Slimy Sculpin, but it varied among replicates for all other species (Fig. 2). Production of juvenile mussels began on days 10–13 for all species except Bluegill, from which one juvenile appeared on day 24. Production of juvenile mussels peaked on day 11 for Brook Trout and on days 20 and 21 for Slimy Sculpin and Banded Killifish. Juvenile production from fish species that had a low metamorphosis rate (e.g., Longnose Dace, Blacknose Dace, White Sucker) did not display conspicuous peaks (Fig. 3), and Bluegill produced only a single juvenile.

Experiment 3

Attachment rate was similarly high among the three trout species (83.2%–84.6%, Table 2). Sloughed glochidia appeared

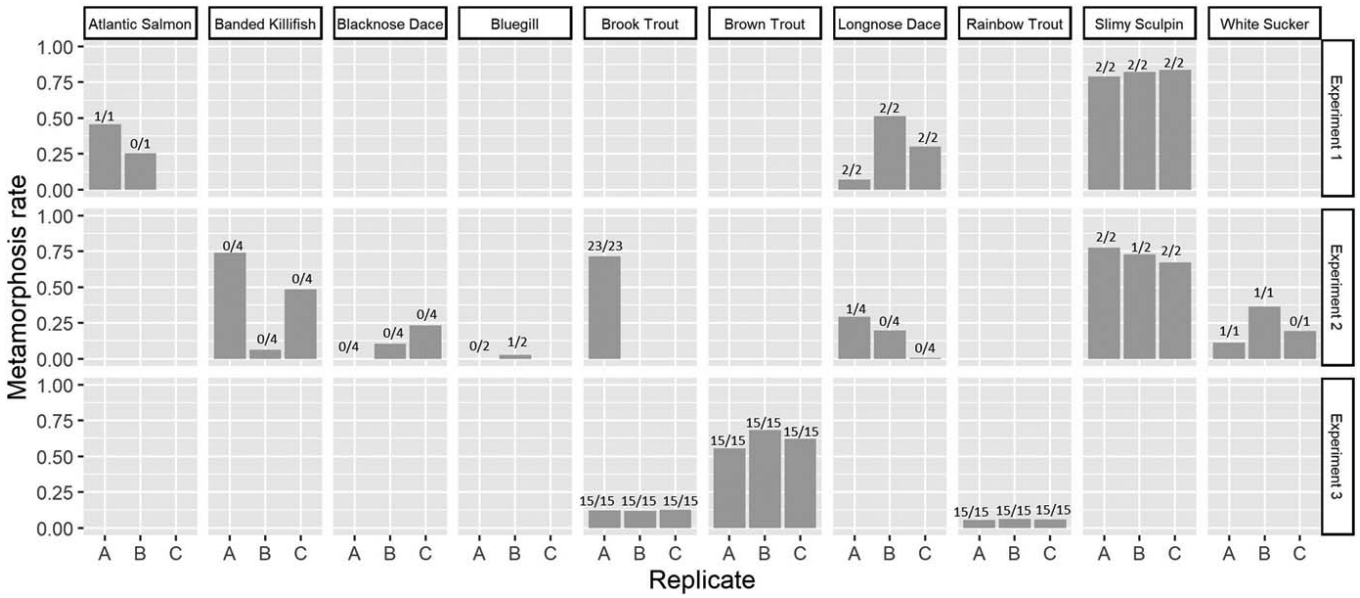

Figure 2. Juvenile metamorphosis rate (number of juveniles/number of glochidia) of Brook Floater (*Alasmidonta varicosa*) on fishes in three experiments. Replicates refer to individual fish holding chambers. Numbers above each bar refer to the number of fish in each chamber that survived (left number) out of the initial number inoculated (right number).

mostly before day 11 for Brook Trout and Brown Trout and before day 7 for Rainbow Trout (Fig. 4).

Metamorphosis rate varied widely among species and was highest for Brown Trout and lowest for Rainbow Trout (Table 2), but metamorphosis was similar among replicates for all three species (Fig. 2). Production of juvenile mussels began on days 11–12 for all three species and peaked on day 12 for Brook Trout and days 14–16 for Brown Trout and Rainbow Trout (Fig. 4).

The top model for predicting glochidia attachment included host species + inoculation density and explained 54.9% of the deviance (Table 3). In the top model, contrasts among attachment rates for host species did not differ ($P > 0.05$), and inoculation density alone was only a marginally significant factor ($P = 0.07$). The model including only host species explained 8.3% of the deviance, and the model including only inoculation density explained 28.2% of the deviance. Overall, models with host species + inoculation density and inoculation density alone were within two qAIC units of the null model, and thus models were not considered strong predictors of glochidia attachment.

The top model for predicting glochidia metamorphosis contained host species only, explained 98.7% of the deviance, and had the lowest qAIC (Table 3). Brown Trout had the highest probability of metamorphosis (0.62 ± 0.02 SD), followed by Brook Trout (0.13 ± 0.02; $P < 0.001$) and Rainbow Trout (0.06 ± 0.01; $P < 0.001$).

DISCUSSION

In our experiments, Brook Floater metamorphosed on all 10 fish species tested, which represented six fish families. Our study was the first to observe metamorphosis on Banded Killifish and the first to test salmonids. Our results support previous categorizations of the Brook Floater as a host generalist (Eads et al. 2007; Wicklow et al. 2017; Table 4). The hooked glochidia of the tribe Anodontini may contribute to their ability to use multiple host species by allowing them to attach to skin, fins, and gills (Bauer 1994; Barnhart et al. 2008). High attachment rates (51.0%–84.6% in our experiments) may offset their passive host infection strategy in which females produce glochidia in mucus strands to entangle potential hosts (Wicklow et al. 2017). Host generalists are largely restricted to the tribe Anodontini; adults of most mussel species in other tribes have specialized adaptations to lure a particular host species or feeding guild, and their glochidia attach mainly to fish gills (Haag 2012).

Slimy Sculpin had the highest glochidia metamorphosis rate, similar to a previous study of Brook Floater host use in New Hampshire (Wicklow et al. 2017; Table 4). Fishes from the family Cottidae are potential hosts for other *Alasmidonta* including the Slippershell (*Alasmidonta viridis*; Zale and Neves 1982), Dwarf Wedgemussel (*Alasmidonta heterodon*; Michaelson and Neves 1995; White et al. 2017), and Elktoe (*Alasmidonta marginata*; Bloodsworth et al. 2013).

Our results about the relative suitability as hosts of other fishes varied in their agreement with the results of previous studies. Longnose Dace was a better host in New Hampshire (51% metamorphosis; Wicklow et al. 2017) than in our study (29.1% and 24.5% in Experiments 1 and 2, respectively). Metamorphosis on White Sucker was similar in our study and in New Hampshire (22.3%, and 26%, respectively; Wicklow et al. 2017). Blacknose Dace supported glochidia metamorphosis in all three studies, but the metamorphosis rate was low (6%)

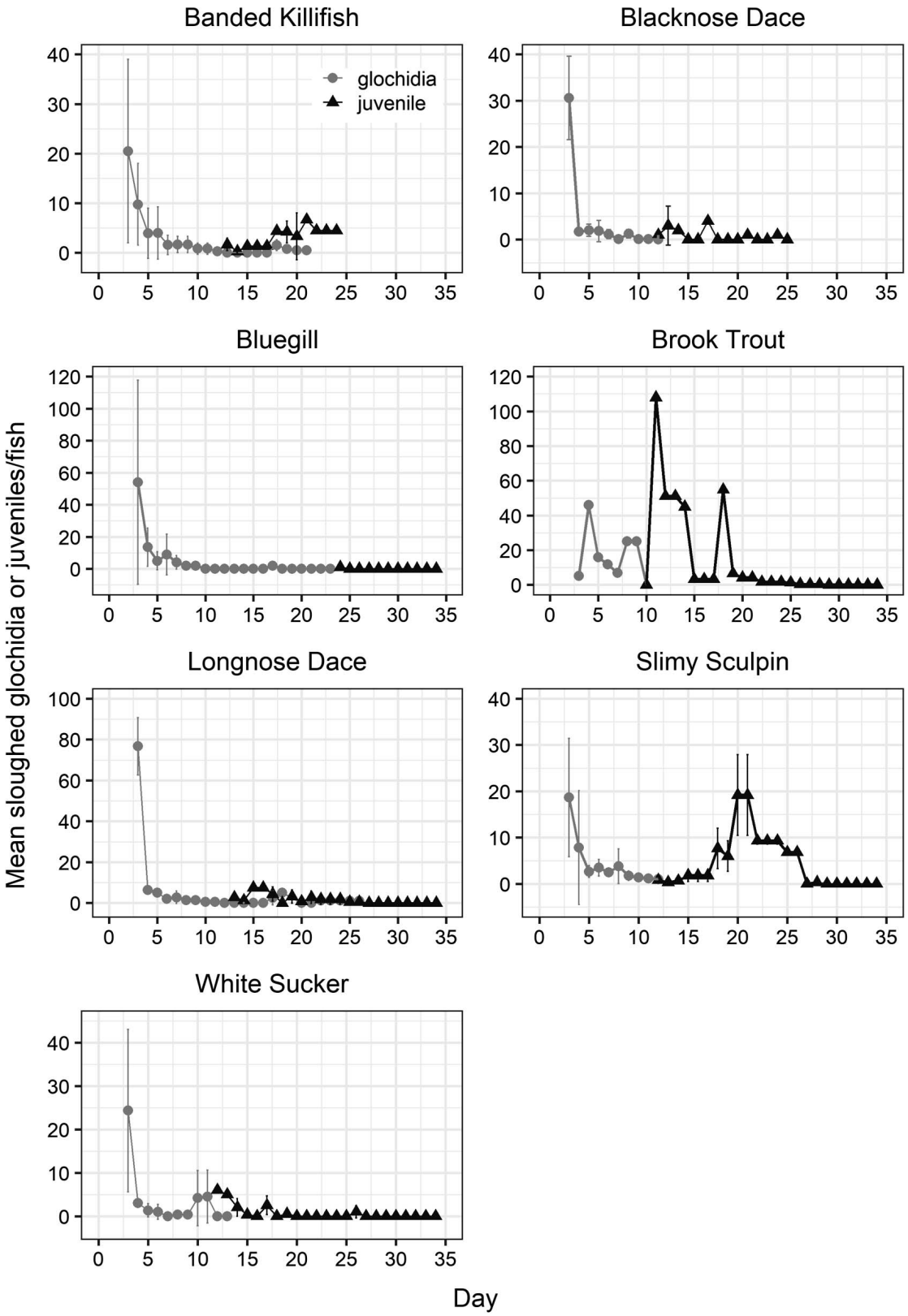

Figure 3. Number of sloughed glochidia or juvenile mussels produced by Brook Floater in Experiment 2. Data points and bars represent the mean and standard deviation, respectively, among replicate fish holding chambers on each day standardized by the number of fish in each chamber.

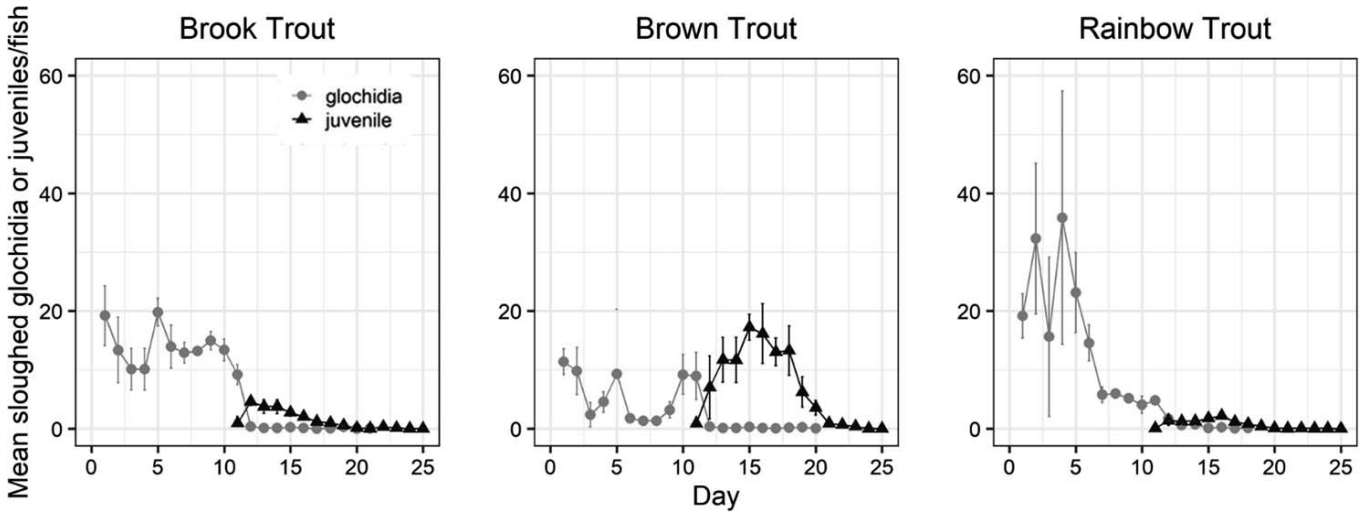

Figure 4. Number of sloughed glochidia or juvenile mussels produced by Brook Floater in Experiment 3. Data points and bars represent the mean and standard deviation, respectively, among fish holding chambers on each day standardized by the number of fish in each chamber.

in New Hampshire (Wicklow et al. 2017) and North Carolina (four juveniles produced; Eads et al. 2007, metamorphosis rate not reported) but higher in our study (16.9%). Cutlip Minnow (*Exoglossum maxillingua*) may be a host to test in future experiments since we commonly observed this species at one of our broodstock collection sites.

The most conspicuous difference in host use in our study and previous studies involved Bluegill. Bluegill produced the highest number of juveniles of any fish species tested in North Carolina in one experiment (184 juveniles produced; Eads et al. 2007, metamorphosis rate not reported), but in another North Carolina experiment Bluegill produced no juveniles (Eads et al. 2007), and it produced only one juvenile in our study. Wicklow et al. (2017) did not test Bluegill. The poor production of juveniles on Bluegill in our study may have been due to high fish mortality, warranting additional tests on Bluegill in Massachusetts.

Variability in metamorphosis rate in our study may be explained by the source of broodstock and the timing of broodstock collection. Glochidia from genetically distinct populations of the same mussel species may vary in their ability to metamorphose on host fishes (evaluated through glochidial retention in the first 96 h; Douda et al. 2014). Because of the small extant Brook Floater populations in Massachusetts, we were unable to collect all mussel broodstock from one location. Genetic differences between the three populations from which we obtained broodstock, and how they might influence host use, are unknown. Genetic information is also critical for informing decisions on where to collect broodstock for propagation to maintain genetic integrity during population augmentation (Jones et al. 2006; McMurray and Roe 2017; Lane et al. 2019). Finally, for Experiment 3, we collected glochidia from broodstock in the fall (October) instead of the spring, as in Experiment 1 (March) and Experiment 2 (April). It is unknown if the length of time that glochidia were brooded by the female mussel affected metamorphosis rate.

The source of host fish also may explain variability in metamorphosis rates between experiments. Brook Trout in Experiment 2 were a mix of wild F1 and F2 generations, whereas Brook Trout in Experiment 3 originated from a domesticated Sandwich strain raised in outdoor raceways at a hatchery; the two experiments resulted in vastly different rates of metamorphosis (71.6% in Experiment 2 vs. 12.8% in Experiment 3). The Brook Trout Sandwich strain is registered with the National Fish Strain Registry and was developed at a state fish hatchery in Montague, Massachusetts, from wild fish (Kincaid et al. 2002; Annett et al. 2012). If stocked hatchery-strain trout displace wild-strain fish, the overall recruitment rate of Brook Floater could decrease because hatchery-raised fish can act as glochidia sinks (Salonen et al. 2016). Further assessment of differences in attachment and metamorphosis rates among fishes of different origins may expand our understanding of mussel-host relationships and provide important information for propagation programs.

Lastly, inoculation density can affect the metamorphosis rate. In the Paper Pondshell (*Utterbackia imbecillis*), higher inoculation densities (2,000–8,000 glochidia/L vs. 1,000/L) resulted in higher mean metamorphosis rates (79.9% vs. 48.8%); this was attributed to increased host plasma cortisol levels and decreased fish immunity (Dubansky et al. 2011). However, another study found no relationship between inoculation densities (1,000, 4,000, and 8,000 glochidia/L) and metamorphosis rate for the Fatmucket (*Lampsilis siliquoidea*; Douda et al. 2018). In our Experiment 3, the number of glochidia that attached to fishes was not a good predictor of metamorphosis rate; rather, fish species was the most important factor in predicting Brook Floater metamorphosis. Similarly, we did not see an effect of inoculation density on glochidia attachment, although the narrow range we tested (0.75–1.18 viable glochidia/mL) limited our ability to

Table 4. Summary of glochidia metamorphosis of Brook Floater observed on fishes in three studies.

Fish species Family, common name	Scientific name	Metamorphosis Yes	Metamorphosis No	Study
Ictaluridae				
Brown Bullhead	*Ameiurus nebulosus*	■		Wicklow et al. 2017
Catostomidae				
White Sucker	*Catostomus commersonii*	■		this study, Wicklow et al. 2017
White Sucker (adult)	*Catostomus commersonii*		■	Wicklow et al. 2017
Centrarchidae				
Bluegill	*Lepomis macrochirus*	■	■	Eads et al. 2007*, this study
Largemouth Bass	*Micropterus salmoides*		■	Wicklow et al. 2017
Mixed Sunfish	*Lepomis spp.*	■		Eads et al. 2007
Pumpkinseed	*Lepomis gibbosus*	■		Wicklow et al. 2017
Redbreast Sunfish	*Lepomis auritus*	■		Eads et al. 2007
Redbreast Sunfish	*Lepomis auritus*		■	Wicklow et al. 2017
Smallmouth Bass	*Micropterus dolomieu*		■	Wicklow et al. 2017
Cottidae				
Mottled Sculpin	*Cottus bairdii*	■		Eads et al. 2007
Slimy Sculpin	*Cottus cognatus*	■		this study, Wicklow et al. 2017
Cyprinidae				
Blacknose Dace	*Rhinichthys atratulus*	■		Eads et al. 2007, this study, Wicklow et al. 2017
Common Carp	*Cyprinus carpio*		■	Wicklow et al. 2017
Common Shiner	*Luxilus cornutus*	■		Wicklow et al. 2017
Fallfish	*Semotilus corporalis*	■		Wicklow et al. 2017
Golden Shiner	*Notemigonus crysoleucas*	■		Wicklow et al. 2017
Highfin Shiner	*Notropis altipinnis*		■	Eads et al. 2007
Longnose Dace	*Rhinichthys cataractae*	■		this study, Wicklow et al. 2007
White Shiner	*Luxilus albeolus*	■		Eads et al. 2007
Whitemouth Shiner	*Notropis alborus*		■	Eads et al. 2007
Fundulidae				
Banded Killifish	*Fundulus diaphanus*	■		this study
Ictaluridae				
Margined Madtom	*Noturus insignis*		■	Eads et al. 2007
Margined Madtom	*Noturus insignis*	■		Wicklow et al. 2017
Percidae				
Fantail Darter	*Etheostoma flabellare*	■		Eads et al. 2007
Johnny Darter	*Etheostoma nigrum*	■		Eads et al. 2007
Piedmont Darter	*Percina crassa*	■		Eads et al. 2007
Roanoke Darter	*Percina roanoka*	■		Eads et al. 2007
Tessellated Darter	*Etheostoma olmstedi*		■	Eads et al. 2007
Tessellated Darter	*Etheostoma olmstedi*	■		Wicklow et al. 2017
Yellow Perch	*Perca flavescens*	■		Wicklow et al. 2017
Salmonidae				
Atlantic Salmon	*Salmo salar*	■		this study
Brook Trout	*Salvelinus fontinalis*	■		this study
Brown Trout	*Salmo trutta*	■		this study
Rainbow Trout	*Oncorhynchus mykiss*	■		this study

* Eads et al. 2007 found conflicting results from two host trials including Bluegill

evaluate density. Host fish species were not important in predicting glochidia attachment (only tested in Experiment 3); this is unsurprising because we tested species with relatively similar morphologies within the same family (Salmonidae). Host species may have a greater effect on glochidia attachment when testing fishes across families with varied morphologies.

Laboratory host studies are important for affirming fish species as physiological hosts (i.e., that can facilitate glochidia metamorphosis), but they do not confirm them as ecological hosts that are important in nature (Levine et al. 2012). To serve as a host in the wild, the habitat of the fish and mussel must overlap, and the mussels' mode of glochidia transfer must be compatible with the fishes' feeding or movement behavior (Barnhart et al. 2008). The only host for Brook Floater confirmed by both laboratory and field studies is the Margined Madtom in New Hampshire; glochidia were found on this species in the wild, and wild fish brought into the laboratory produced juveniles (Wicklow et al. 2017). However, the Margined Madtom is not native north of Connecticut (Page and Burr 1991) and is thought to have been introduced to New Hampshire in the 1930s (Hartel et al. 2002), indicating that Brook Floater glochidia can use non-native fish species as hosts in the wild. Brook Floater glochidia were found attached to Ninespine Stickleback (*Pungitius pungitius*) in New Brunswick, Canada, but glochidia inoculations in a laboratory are needed to confirm whether this fish can produce juveniles (Beaudet 2006 *in* Department of Fisheries and Oceans Canada 2016).

Cost-effective captive propagation requires selecting a host species that produces consistently high metamorphosis rates yet is easily procured in large numbers and maintained in captivity. Slimy Sculpin produced the highest metamorphosis rates in our study, but obtaining sculpins is dependent on suitable conditions for collection in streams, and these conditions may not coincide with availability of mussel broodstock. Furthermore, removing large numbers of sculpins from the wild may negatively affect those populations. Hatchery-reared Brook Trout from wild F1 and F2 generations produced a metamorphosis rate nearly as high as Slimy Sculpin (Experiment 2). The ability to easily procure large numbers of hatchery-reared Brook Trout could make them a cost-effective choice for large-scale propagation of Brook Floater in the northeastern USA; however, care must be taken to select hatchery strains that produce high metamorphosis. Brown Trout also produced relatively high metamorphosis rates, but they produced copious mucus and shed scales that entangled juvenile Brook Floater, which increased the time needed to harvest juveniles. Furthermore, use of a non-native host species like Brown Trout presents a potential for undesirable hatchery selection. These considerations highlight the need to evaluate various fish species, sources, and other factors when selecting an optimal host fish for captive mussel propagation.

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