Received: 11 September 2018 | Revised: 10 April 2019 | Accepted: 13 April 2019

DOI: 10.1002/aqc.3154

RESEARCH ARTICLE

WILEY

Reproductive ecology of the threatened and endemic freshwater mussel *Lampsilis bracteata*

L. Ashley Seagroves[1] | M. Christopher Barnhart[2] | Thom Hardy[1] | Astrid N. Schwalb[1]

[1] Biology Department, Texas State University, San Marcos, Texas

[2] Department of Biology, Missouri State University, Springfield, Missouri

Correspondence
Astrid N. Schwalb, Department of Biology, Texas State University 601 University Drive San Marcos, TX 78666, USA.
Email: schwalb@txstate.edu

Funding information
Texas Department of Transportation, Grant/Award Number: 06882

Abstract

1. The Texas fatmucket, *Lampsilis bracteata*, is a unionid mussel endemic to the Colorado and upper Guadalupe River basins of Central Texas and a candidate for federal listing under the Endangered Species Act. There is increased interest in propagation and population restoration of threatened mussels in Texas as a potential conservation method, but still little is known about their life histories and how local populations may differ in aspects of their reproductive ecology, e.g. timing of brooding and potential local adaptations to host fish.
2. The purpose of this study was to compare host fish compatibilities and seasonality of reproduction between two populations: (a) by cross-infesting fish from the San Saba and Llano Rivers in the Colorado River basin with sympatric and allopatric mussel larvae in the laboratory (hatchery-produced Guadalupe bass and largemouth bass were also infested); and (b) by assessing gamete concentration, brooding period, viability of larvae and sex ratios using monthly sampling events between February 2017 and February 2018.
3. Reproduction varied with season and between populations. The proportion of females brooding tended to be lower in the summer and the autumn, and higher during winter and spring months before peak water temperatures were reached. The sex ratio in both populations did not significantly differ from 1:1. Fecundity and larvae viability were higher in the Llano River population compared with the San Saba population. Trematode flatworms were found in several female gonad samples from the San Saba population and in a few samples from the Llano population.
4. The highest metamorphosis success occurred on wild green sunfish and largemouth bass, and hatchery-produced largemouth and Guadalupe bass. The average metamorphosis success tended to be higher for some mussel–fish pairings originating from the same tributary, suggesting that mussels may be locally adapted to host fish, which should be considered in conservation efforts.

KEYWORDS

captive propagation efforts, endemic species, host fish adaptation, parasites, unionid mussels

1 | INTRODUCTION

The Texas fatmucket, *Lampsilis bracteata*, is a unionid mussel endemic to the Colorado and upper Guadalupe River basins of Central Texas. The widespread imperillment of unionid bivalves has drawn great interest in the conservation of these ecologically important organisms (Strayer, 2008; Williams, Warren, Cummings, Harris, & Neves, 1993). *Lampsilis bracteata* is one of 15 threatened mussel species in Texas that is also a candidate for federal listing under the Endangered Species Act. Human impacts are largely to blame for the massive decline in freshwater mussel populations, and have imposed pressures upon catchments both globally and locally, i.e. within the geographical distribution of *L. bracteata* (Bogan, 1993; Hansen et al., 2016; Howells, 2015; Lydeard et al., 2004).

Female unionid mussels are fertilized by spermcasting males and brood the developing eggs within their marsupial gills (Jirka & Neves, 1992). Like most other *Lampsilis* species, *L. bracteata* are thought to be long-term brooders which spawn in the summer and brood until the following spring or summer (Howells, 2000). Brooding mussels have previously been found between July and October in the San Saba River (Johnson, Caccavale, Randklev, & Gibson, 2012). Glochidia of unionid mussels remain on host fish for days to months, depending on the water temperature and on the species, before detaching from the host as juvenile mussels.

Unionid mussels have developed a fascinating variety of strategies to attract and infest host fish (Barnhart, Haag, & Roston, 2008). Female mussels of *L. bracteata* display a mantle lure, mimicking the appearance and movement of a small fish. Predatory host fish become infected with glochidia by attacking the lure and rupturing the mussel marsupial gill. Known hosts of *L. bracteata* include four species of Centrarchidae: *Lepomis cyanellus* (green sunfish), *Lepomis macrochirus* (bluegill sunfish), *Micropterus salmoides* (largemouth bass) and *Micropterus treculii* (Guadalupe bass, Johnson et al., 2012).

Host fish species are defined by physiological compatibility and also by ecological association. Laboratory experiments that examine the metamorphosis to juvenile mussels are the most common form of host fish study and provide insight into the physiological compatibility among mussel and host fish. Immunological resistance to glochidia may be acquired in fish with previous exposure to mussels, and smaller or hatchery fish may show a weaker immune response compared with larger or wild fish (Bauer & Vogel, 1987; Dodd, Barnhart, Rogers-Lowery, Fobian, & Dimock, 2005; Rogers & Dimock, 2003). Laboratory studies on host fish are ideal precursors to captive culture because they indicate which mussels can be propagated in a laboratory setting (Ćmiel, Zając, Lipińska, & Zając, 2018; Hove et al., 2011; Johnson et al., 2012; Levine, Lang, & Berg, 2012). One drawback of such studies is that they provide no information on the frequency of encounters among glochidia and the host fish in the wild. This information could be obtained from observations of natural infestations, but such studies require the collection of large numbers of fish and are impractical if natural infestations are rare (Barnhart et al., 2008; Bauer, 1994).

Captive culture has been widely used as a conservation measure to augment declining populations and to reintroduce mussels to areas where they were previously extirpated (Thomas, Taylor, & Garcia de Leaniz, 2010, but see also Gum, Lange, & Geist, 2011). There is increased interest in the propagation of threatened mussels in Texas as a potential conservation method, but still little is known about their life histories. Increasing the knowledge base of life-history information is important in planning for captive propagation and reintroduction (Haag, 2013; McMurray & Roe, 2017). One concern is the possibility that populations may exhibit local adaptations in timing of spawning and brooding, host fish requirements or other aspects of reproduction. For example, local adaptations to genetically distinct populations of host fish may sometimes make glochidia more compatible with sympatric than with allopatric host populations (Eckert, 2003; Rogers, Watson, & Neves, 2001; Taeubert, Denic, Gum, Lange, & Geist, 2010; Zanatta & Wilson, 2011).

The objective of this study was to compare life-history data between two isolated populations of *L. bracteata*, including seasonal variation in gamete development, brooding period and viability of glochidia, and compatibility among mussel and host populations.

2 | METHODS

2.1 | Sampling sites

A fully crossed study design was used with *L. bracteata* and host fish originating from two of the major tributaries of the Colorado River in Texas: The Llano River and San Saba River (Figure 1) These tributaries are separated by a stretch of the main-stem Colorado River that includes two major dams, Buchanan and Inks dams (both constructed in 1938); thus, fish and mussels cannot move freely between these tributaries.

Mussels were monitored monthly from February 2017 to February 2018. Additional preliminary monitoring occurred in the Llano River from April to November 2016 (Table 1A, 2A). Higher water clarity in the Llano River more often permitted visual searches compared with the San Saba River, which required tactile searches. On each date, sampling continued until 10 unmarked *L. bracteata* individuals were located (unmarked mussels had not been previously sampled). All mussels were sampled for gonadal fluid and were assessed for brooding and glochidia viability. All sampled mussels were uniquely marked with a Floy® Shellfish Tag (shell tag) to avoid accidental re-sampling for gonadal fluid, as mussels may experience stress from handling which could affect reproductive success (Peredo, Parada, Valdebenito, & Peredo, 2005).

Fish for host fish experiments were collected from the two mussel field sites using backpack electroshocking and seine netting methods. Fish were transported in aerated coolers filled with site water in a 0.18% NaCl solution to reduce stress of handling and transport (Carneiro & Urbinati, 2001). Fish were thermally acclimated in the laboratory overnight before being transferred to 10 gallon (45.5 L) holding tanks. The fish received pellet food and/or bloodworms (maximum of 2–3 mL per fish) daily along with weekly water changes and regular water quality testing.

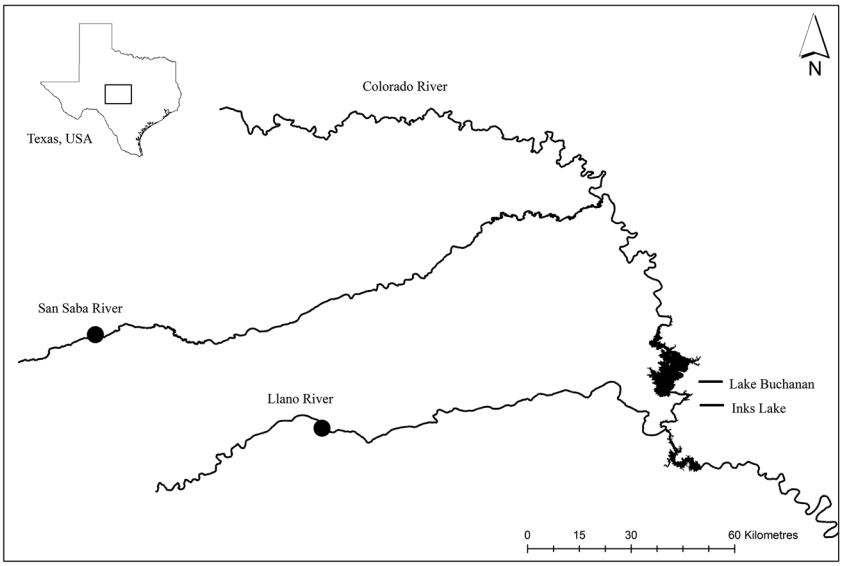

FIGURE 1 Map of sampling sites in two tributaries of the Colorado River Basin. Sampling sites (black circles) in the San Saba River near Menard, Texas, USA and the Llano River near Mason, Texas, USA. Rivers are separated by two major dams (black bars): Buchanan Dam (upstream) and Inks Dam (downstream)

2.2 | Field environmental parameters

Temperature at each field site was recorded with a temperature logger (HOBO Pro v2 and HOBO 64 K). Temperature was logged hourly from February to mid-May 2017 and from mid-November 2017 to early February 2018, and every 12 h (12 a.m. and 12 p.m.) from mid-May to mid-November 2017. Daily maximum and minimum temperatures at the two sites were derived from the hourly readings. For the days for which temperatures were only measured twice, the maximum and minimum temperatures were estimated based on the hourly data with linear regressions of the temperature measured at noon vs. maximum temperatures (R^2-values 0.94 and 0.99 for the Llano and San Saba rivers, respectively) and vs. minimum temperature (R^2 = 0.98 for both rivers). For each sampling date mean values were calculated for maximum and minimum temperatures that mussels had experienced since the last sampling.

Specific conductivity (μS cm^{-1}), dissolved oxygen (mg L^{-1}) and pH were measured in the thalweg at each mussel sampling site during monthly trips using a YSI 556 MPS. Water samples were collected for analysis of chlorophyll-*a* and total suspended solids (TSS).

2.3 | Sex ratio and gamete analysis

The numbers of male and female *L. bracteata* detected during each sampling event were recorded based on shell morphology, which is sexually dimorphic. Gonadal fluid was sampled (Tsakiris, Randklev, & Conway, 2016) from 10 *L. bracteata* (regardless of sex). Samples of 0.1–3.2 mL were extracted using a 20-gauge hypodermic needle (BD 5 mL syringe Luer-Lok™ with BD PrecisionGlide ™ needle) inserted into the visceral mass (approximately at the intersection of mid-length and mid-width of the shell). Gamete samples were fixed with 10% formalin, dyed with 0.01% methylene blue and transported to the laboratory for analysis. Sperm were quantified in 10 μL subsamples (transferred with micropipette, Fisherbrand Elite) with a compound microscope (400×) and Improved Neubauer haemocytometer (INCYTO DHC-N01–5). The sperm concentration (number per mL of gonadal fluid) was extrapolated from subsamples using equations 1 and 2:

$$\text{Number mL}^{-1} = \text{number of sperm in 5 small centre squares}^{*} \text{5 squares}^{*}\text{dilution factor}^{*}10^{4}\ \text{volume factor} \quad (1)$$

$$\text{Dilution factor} = \text{total volume (containing ethanol and methyl blue)} {}^{*}(\text{initial sample volume (gonadal fluid)})^{-1} \quad (2)$$

Egg concentration was estimated by counting the number of eggs in a 10 μL subsample at 100× magnification on a glass slide and extrapolating the number of eggs to 1 mL of gonadal fluid, similar to sperm concentration calculations, accounting for sample dilution. When egg quantities permitted, the diameters of 50 eggs were measured.

Gonad fluid sample volumes varied between 0.1 and 3.2 mL. Gamete concentration in the samples declined with sample volume, and the relationship was approximately linear between the logarithm of concentration and the sample volume, as would be expected from a dilution curve. It was concluded that gonad samples were increasingly diluted with haemolymph as more fluid was drawn; sample volume was therefore multiplied by concentration, yielding a total number of gametes per sample. The number of gametes per sample was defined as gamete abundance. This measure was not significantly correlated with sample volume and was used for comparisons between sites and among sampling dates.

Parasites are relatively common in unionid mussels, and it is important to monitor their population because of their potential for adverse impacts on the fitness of mussels (Grizzle & Brunner, 2009). Potential parasites discovered incidentally during gamete analysis were photographed for identification. Trematodes were identified to family level based on external morphology of cercaria larvae.

2.4 | Brooding, glochidia viability and fecundity

Female mussels were considered to be brooding when gills were swollen and opaque (Hove & Neves, 1994). A sample of the brood was obtained from each brooding female by flushing one or two water tubes (of the marsupium) with a 20 gauge hypodermic needle. If mature glochidia were present, their viability was determined in the field by observing the closing response of ~100 glochidia to a saturated salt solution:

$$\text{Glochidia viability} = \frac{(\text{open glochidia} - \text{open glochidia after NaCl addition})}{(\text{total glochidia})}$$

Glochidia that remained within egg membranes after flushing from the marsupium were considered to be immature and were not included in the measures of viability. Fecundity was estimated from the females used in the host fish experiments (see below). The entire contents of the marsupial gills of each mussel were flushed into separate beakers, diluted with water, and then all glochidia were counted in subsamples from the stirred suspension and the total number estimated volumetrically.

2.5 | Host fish experiment

Brooding mussels were collected from the Llano River in March to provide glochidia for testing wild host fish, and in April 2017 for testing hatchery host fish. Mussels from the San Saba River were collected in July 2017 for host fish experiments with both wild and hatchery fish. Mussels were transported in aerated coolers filled with a small layer of substrate and water from the collection site and transferred to flow-through tanks (Living Streams) containing natural gravel substrate and artesian well water from the Edward's Aquifer. Mussels were kept in the laboratory for 5–7 days before harvesting glochidia and were fed daily with manually administered Rotifer Shellfish Diet 1800 (Pentair Aquatic Eco-Systems) at about 5 μL of the diet per litre of water in the mussel tank. Following host fish inoculation, mussels were returned to the sampling site.

The following centrarchid fish species were inoculated with *L. bracteata* glochidia: *Lepomis auritus* (redbreast sunfish, only Llano mussels), green sunfish, *Lepomis gulosus* (warmouth), bluegill sunfish, *Lepomis megalotis* (longear sunfish), and largemouth bass (sample sizes are shown in Table A3). Hatchery-reared largemouth bass and Guadalupe bass were also inoculated with glochidia from mussels from both the Llano and San Saba rivers. Glochidia used for host tests were extracted from females and their viability was tested. For host fish experiments with San Saba mussels, viability was >90% (95 ± 1%, mean ± SE, n = 4); for Llano mussels, viability was >79% (88 ± 4%, n = 3) and > 76% (82 ± 4%, n = 3) for experiments with wild and hatchery fish, respectively. The combined glochidia sample was distributed between inoculation chambers, so that the concentration was ~4000 viable glochidia L^{-1}. Glochidia were kept in suspension using continuous turbulent mixing with several air-stones. Fish were exposed to glochidia for 25 min before being transferred to randomly selected individual tanks (1.5, 3 and 10 L) in the flow-through system (Douda, Martin, Glidewell, & Barnhart, 2018). Water temperature ranged between 19 and 23.9°C.

Detached glochidia and juveniles were collected from the flow-through tanks by flushing them for a 10 min interval. This collection was made at 12 and 24 h after inoculation, and on every second day thereafter. Survivorship of juveniles was determined by observing foot and valve movement, and length and height (μm) of a subset of juveniles were measured (n = 557 from the Llano River, n = 256 from the San Saba River). Any fish that died during the experiment were dissected, and the gills were checked for the presence of encapsulated glochidia (Österling & Larsen, 2013). None of the dissected fish contained encapsulated glochidia, and fish that died during the experiment were excluded from all further analyses with the exception of warmouth that died after juvenile detachment had ceased for that species. Total length (mm) and weight (g) were measured for each fish upon conclusion of the experiment. The remaining fish were stocked into a private pond for neighbourhood fishing.

2.6 | Statistical analysis

Differences in water quality data between the sites in the Llano River and in the San Saba River were compared with a paired t-test. In order to test whether sex ratios deviated from 1:1, a chi-square goodness-of-fit test was used. Differences in fecundity between rivers were analysed with an ANCOVA with length as a covariate (as fecundity is known to increase with the size of mussels; Haag & Staton, 2003). Assumptions of homogeneity were tested with a Levene's test, and for both the paired t-test (above) and the ANCOVA, assumptions of normality were tested with a Shapiro–Wilk test; all assumptions were met.

The success of metamorphosis (%) was computed by dividing the number of live juveniles recovered from each individual fish by the total number of glochidia and dead juveniles captured from a tank. As data were not normally distributed even after transformation of the data, a two-way ANOVA with permutation test (Anderson, 2001) was used to determine whether metamorphosis success differed significantly between fish species and between fish originating from different rivers. An unrestricted permutation of observations was used, which is preferred when sample sizes are low (Anderson, 2001).

For comparisons between two groups (e.g. to examine whether the rates of metamorphosis differed significantly between the same fish species obtained from different rivers), Welch's t-tests instead of Student's tests were used when sample sizes differed between groups. For the pairwise comparisons, data were root-transformed when necessary to meet the criteria of normality and homogeneity of variances. All analyses were done in R (R Core Team, 2017).

3 | RESULTS

3.1 | Environmental parameters

Conductivity and chlorophyll-*a* concentrations were significantly higher at the San Saba River site, and pH was significantly higher at

TABLE 1 Summary of water quality data collected at both sites in the Llano River and the San Saba River as mean values of all sampling events with the range given in parentheses. An asterisk indicates that there was a significant difference between sites ($P \leq 0.01$)

	Llano River	San Saba River	*P*-Value
pH*	8.3 (8.2–8.5)	8.1 (7.9–8.2)	<0.001
Specific conductivity* ($\mu S\ cm^{-1}$)	375 (338–410)	505 (453–547)	<0.001
Chlorophyll-*a** ($\mu g\ L^{-1}$)	0.6 (0.2–2.8)	1.3 (0.5–2.9)	0.01
Total suspended solids ($mg\ L^{-1}$)	0.05 (0.02–0.06)	0.05 (0.04–0.07)	0.98
Dissolved oxygen ($mg\ L^{-1}$)	9.5 (5.6–14.8)	8.3 (6.6–12.6)	0.13

the Llano River site (Table 1, Figure 2g, h). There was no significant difference in dissolved oxygen or TSS (Table 1).

Mean monthly temperatures ranged between 8 and 33°C at the Llano River site and between 9 and 31°C at the San Saba River site. Temperatures peaked in early August at both sites, and reached minima in February. Mean daily minimum temperatures were consistently lower, and mean maximum temperatures higher, at the Llano River site, than at the San Saba site (Figure 2G, H).

3.2 | Reproductive monitoring

Sex ratios of males:females for *L. bracteata* collected between February 2017 and February 2018 were not statistically different from 1:1 with 0.9 males per female (n = 110) in the Llano River [χ^2 (1) = 0.58, P=0.45] and 1.2 males per female (n = 120) in the San Saba River [χ^2 (1) = 1.2, P=0.27].

Gamete abundance in gonad fluid samples varied seasonally (Figure 2a–d). Sperm and egg abundance were generally high in October–February and declined to a minimum in midsummer, before increasing again before spawning in late autumn–early winter (Figure 2a, b). At the Llano site, the minimum egg abundance occurred

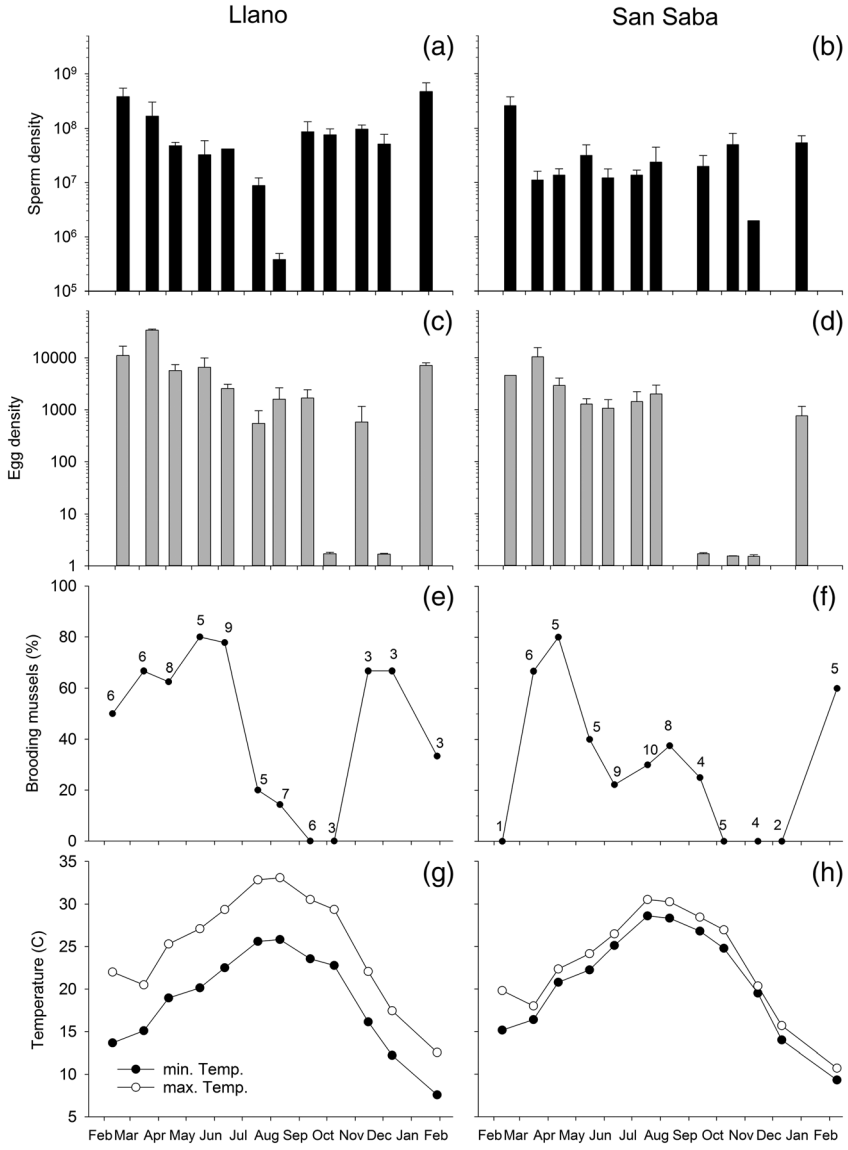

FIGURE 2 Seasonal variation of gamete abundance (mean number of gametes ± SE) for sperm (a, b) and eggs (c, d), the proportion of brooding mussels (e, f), and average minimum and maximum water temperatures (g, h) at the Llano (a, c, e, g) and the San Saba site (b, d, f, h). Numbers above data points indicate sample size. Note that the sperm abundance in August 2017 was low, but not zero

about a month earlier than the minimum sperm abundance; this seasonal pattern was less clear at the San Saba site.

Egg diameters in gonad fluid samples varied between 32 and 331 μm and a wide range of diameters was present throughout the year (Figures A1, A2 in the Supporting Information). The largest size classes of eggs were least abundant in July–September at the Llano site and in July at the San Saba site. The largest size classes were most abundant in November at the Llano site and in October at the San Saba site.

Brooding mussels were observed throughout the year except in the September–October samples at the Llano River site, and in the October–December samples at the San Saba site. After this barren period in the autumn, brooding resumed, at least a month earlier at the Llano River site than at the San Saba site (Figure 2e, f). A few glochidia samples (one out of six mussels in March 2017, and two out of eight mussels in August 2017) contained a large proportion of undeveloped eggs together with a few glochidia. Preliminary monitoring in the Llano River between April and November 2016 found no brooding mussels in July 2016 (n = 21), whereas brooding mussels were found in April (n = 2) and June (n = 8). Also in contrast to the sampling in 2017, no brooding mussels were found in November 2016 (n = 10).

Most of the brooding mussels (20 of 23 mussels) found in the Llano River had a high glochidia viability (>80%), with the exception of March 2017 (viability <40% in two of the six brooding mussels) and August 2017 (67% in the only brooding mussel found). In contrast, in the San Saba River about half of the brooding mussels (13 of 24 mussels) had dead glochidia or <5% viability (found in March, June, August and September 2017). Mussels with high glochidia viability (>80%) were found in April, May and July 2017, and in February 2018. Mean glochidia length was 217.9 ± 1.3 μm, and height was 272.2 ± 1.6 μm (n = 343).

Apart from temperature, there was no obvious correlation between gamete abundance, brooding or glochidia viability and other environmental parameters, such as chlorophyll-a or TSS (data not shown).

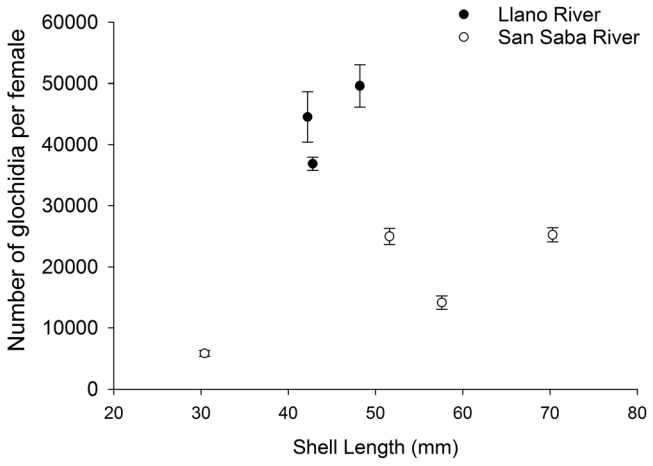

FIGURE 3 Fecundity (number of glochidia per female) in relation to shell length of mussels in the Llano River (black circles) and the San Saba River (white circles)

Bucephalid trematodes (Bucephalidae) were found in three out of five female gamete mussels collected in February and March 2017 in the San Saba River, and in two out of eight females from the Llano River. During the remaining sampling period other unidentified parasites were found in four out of 50 females from the Llano River (two in August, one in November and one in December) and in eight out of 44 females from the San Saba River (one per month between May 2017 and February 2018 except for September). Gametes were present in most infected samples, but two out of eight infected samples found between June and November 2017 contained no gametes. No trematodes were found in male mussels in either system (n = 55 in the Llano River, n = 67 in the San Saba River).

Fecundity was higher at the Llano site compared with the San Saba site and was generally higher for larger mussels (Figure 3). The ANCOVA detected a significant effect of both length (F = 10.3, P=0.03) and river (F = 18.6, P=0.01). The estimated number of glochidia per female mussel ranged from 36,900 ± 1100 to 49,600 ± 3500 (rounded to the nearest 100) in the Llano River with an average of 43,700 ± 3700 (mean ± SE, n = 3, collected in March, length of mussels ranged from 28 to 42 mm, Figure 3). In contrast, the fecundity of mussels from the San Saba River was significantly lower with 5,800 ± 500 for the smallest female (30 mm length) compared with 25,200 ± 1100 glochidia per female for the largest female (70 mm) with an average of 17,500 ± 4700 (n = 4, collected in July, Figure 3).

3.3 | Host fish experiment: wild fish

Metamorphosis success differed significantly between host fish species for glochidia from both the Llano and the San Saba rivers (Figure 4). There was also considerable variation between individual host fish. Overall metamorphosis success tended to be highest on green sunfish. For example, glochidia from the Llano River mussels had the highest average metamorphosis success on green sunfish collected from the Llano River (45% average), which ranged between 27 and 76% (or 72 vs. 167 juveniles produced). Metamorphosis was also high on largemouth bass (11–54%), but was significantly lower for bluegill sunfish and longear sunfish (<12 and <1% mean metamorphosis success respectively, Figure 4), and 0 or <1% for redbreast sunfish and warmouth, respectively.

The permutation ANOVA detected significant effects of fish origin and fish species on metamorphosis success for the Llano mussels, whereas only fish species was a significant factor for the Saba mussels (Figure 4). Metamorphosis success (mean values) was higher for sympatric than allopatric mussel–fish pairs in four of seven comparisons, i.e. for the Llano River green sunfish (26% higher), largemouth bass (21% higher) and bluegill sunfish (4% higher), and for the San Saba River largemouth bass (20% higher). Individual variation was high, however, and differences were not statistically significant for the Llano River mussels—green sunfish (Student's t-test, T_8 = 1.8,

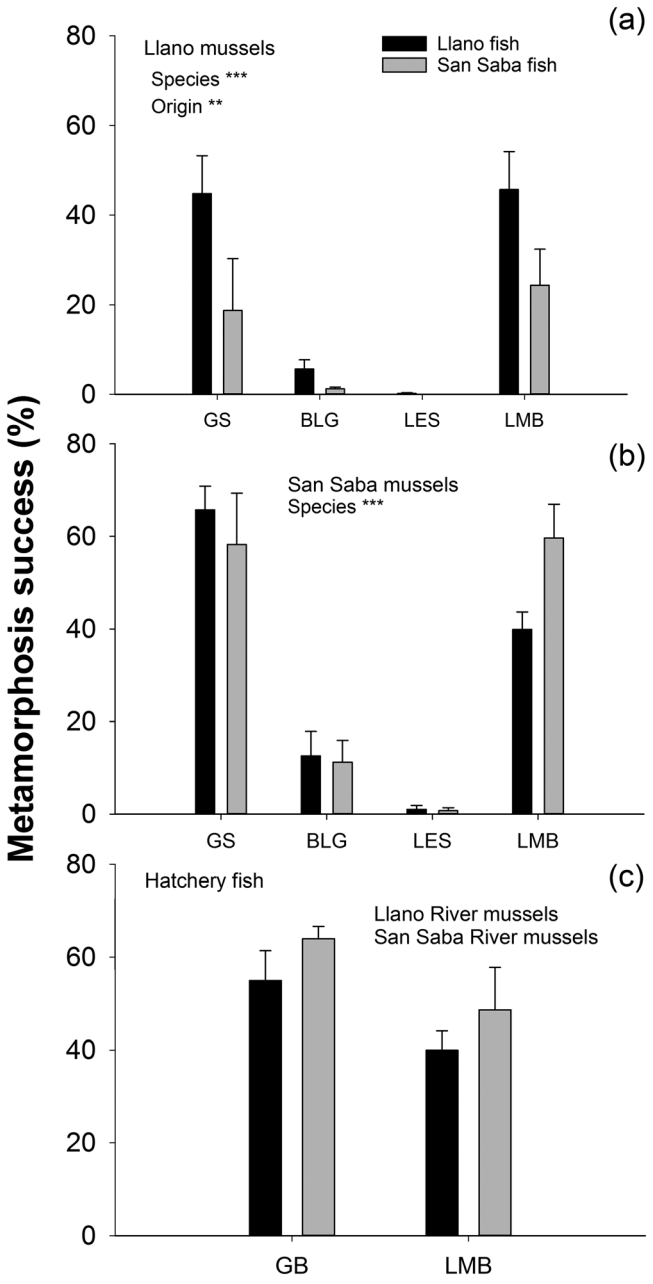

FIGURE 4 Metamorphosis success (mean ± SE) as the percentage of glochidia that successfully metamorphosed into juvenile mussels on different fish species and fish from different origin. (a) Llano River glochidia on wild fish; (b) San Saba River glochidia on wild fish; and (c) Llano River and San Saba River glochidia on hatchery fish. Species codes: RBS, redbreast sunfish; GS, green sunfish; WM, warmouth; BLG, bluegill sunfish; LES, longear sunfish; LMB, largemouth bass; GB, Guadalupe bass. Significant effects detected by the ANOVA are indicated with asterisks: ** $P \leq 0.01$; *** $P \leq 0.001$. Sample sizes were n = 5 fish, except in (a) LES from the San Saba (n = 3) and LMB from the Llano (n = 2), in (b) n = 4 for WM and GS from the San Saba, LES and LMB from the Llano, and n = 3 for WM from the Llano and LMB from the San Saba, and in (c) Guadalupe bass from the hatchery (n = 4)

P=0.11) and bluegill sunfish (T_8 = 1.7, P=0.13)—and marginally significant for the metamorphosis success of San Saba River mussels on largemouth bass from different origins (Welch's t-test $T_{5.6}$ = 2.4, P=0.06). (Note that n = 2 for largemouth bass from the Llano River, but the fish died, which hindered a statistical comparison.)

The sloughing of undeveloped or dead glochidia was highest on day 2 (Figure A3, A4) except for green sunfish infested with San Saba mussel glochidia (especially on green sunfish from the Llano River), which peaked later (Figure A4). Juvenile detachment peaked between day 18 (San Saba mussels, 4009 juveniles) and day 23 (Llano mussels, 766 juveniles). Green sunfish and Guadalupe bass had similar temporal patterns of detachment with the great majority of juveniles detaching at around ~15 days post-inoculation. Recovery of both glochidia and juveniles from green sunfish and Guadalupe bass was complete after 40 days. In contrast, juveniles detached from largemouth bass over a much longer period, up to 48 (Llano mussels) and 62 (San Saba mussels) days post-inoculation.

3.4 | Host fish experiment: hatchery fish

Both Llano and San Saba mussels had a high metamorphosis success on Guadalupe and largemouth bass from the hatchery (Figure 4). For Llano mussels, the average metamorphosis success on hatchery fish was similar to that of wild green sunfish from the same river (44 and 60% on hatchery vs. 46% on wild fish, Figure 4). For San Saba mussels, the metamorphosis success on hatchery fish was similar to the success rate on wild largemouth bass from the same river and green sunfish from both rivers (59 and 68% on hatchery fish vs. 73, 69 and 70% on wild fish, Figure 4). Metamorphosis success on hatchery Guadalupe bass and hatchery largemouth bass was similar in mussels from both rivers (San Saba, Student's t-test, T_8 = 1.6, P=0.15; Llano, T_8 = 2.0, P=0.09, Figure 4). There were no significant differences in metamorphosis success between largemouth bass from wild fish (from the Llano and San Saba rivers) vs. largemouth bass of hatchery origin for Llano mussels (Welch's t-test, $T_{9.2}$ = 1.1, P=0.28) or San Saba mussels (Welch's t-test, $T_{7.2}$ = 0.3, P=0.74).

4 | DISCUSSION

This study provides much needed information on the reproductive ecology of *L. bracteata* and is the first study to investigate host fish specificity between populations of *L. bracteata* using a fully crossed study design. Metamorphosis success of glochidia was higher on some but not all mussel–fish pairings from the same river (sympatric) vs. different rivers (allopatric). Numerous previous studies have examined host fish suitability for mussels with artificial infestation in the laboratory, but fewer studies have investigated differences in host fish compatibility of mussels and fish of sympatric and allopatric river origin (e.g. Bingham, 2002; Caldwell, Zanatta, & Woolnough, 2016; Eckert, 2003; Riusech & Barnhart, 2000; Schneider, Nilsson, Höjesjö, & Österling, 2017; St. John White, Ferreri, Lellis, Wicklow, & Cole, 2017). Only one other study examined mussel–fish pairings of different populations within the same drainage basin, but it looked at variation of infection success rather than metamorphosis success (Douda et al., 2014). Hence, to the best of our knowledge this is the first study

to look at differences in metamorphosis success of mussels from different tributaries within a single river basin.

A higher compatibility with sympatric host populations compared with allopatric host populations may be expected where different species or genetically distinct populations of fish are available to mussel populations, so that local adaptations might evolve (Eckert, 2003; Riusech & Barnhart, 2000; St. John White et al., 2017; Strange & Burr, 1997). Compatibility has been shown to decrease as isolation between the mussel source and fish source increased (St. John White et al., 2017) and host suitability was found to be higher on host fish within the natural distribution range of the mussel (Taeubert et al., 2010), but this tendency towards higher success on sympatric host fish is not universal. *Margaritifera margaritifera* in southern Norway were more abundant on allopatric than sympatric brown trout (Österling & Larsen, 2013), and a study on *Unio crassus* in two geographically separated rivers of southern Sweden suggested that not all populations of a species may show the same adaptive tendencies in respect to host fish compatibility (Schneider et al., 2017). No differences in host suitability between sympatric and allopatric mussel–fish pairings were found for *Epioblasma triquetra* in the Great Lakes basin (Caldwell et al., 2016). Host mobility was found to play a role for metacommunity structuring of mussels (Schwalb, Morris, & Cottenie, 2015) and this may also affect the degree of co-evolution and local adaptation to host fish in addition to geography, but this remains to be studied.

Other studies have explored population-specific differences of *Margaritifera margaritifera* and found differences in host species suitability in Fennoscandian rivers between populations in large main channels and small tributaries (Salonen et al., 2017). They have suggested that populations in north-west Scotland may differ in their cues for attachment to a host (Clements, Thomas, & Adams, 2018). Population-specific differences may also exist between mussel populations in the Llano River, which may be more closely adapted to green sunfish from the same river and mussels in the San Saba River, which may be more closely adapted to largemouth bass from the same river; however, further research is needed to explore this hypothesis. It should be noted that dispersal between the tributaries in the study area has been restricted by the construction of major dams in the main-stem Colorado River in the 1930s, which is considered recent over evolutionary timescales.

Higher metamorphosis success should be expected from fish without previous exposure to mussels (i.e. higher in hatchery fish compared with wild fish), as laboratory experiments found that fish may acquire an immune resistance to glochidia upon exposure (Chowdhury, Salonen, Marjomäki, & Taskinen, 2017; Dodd et al., 2005; Dodd, Barnhart, Rogers-Lowery, Fobian, & Dimock, 2006). Only minor differences were found between metamorphosis success on hatchery and wild largemouth bass, which suggests a lack of exposure to glochidia in the field. It is interesting to note that parent fish of hatchery Guadalupe bass originated from the South Llano River, and metamorphosis success was higher on Guadalupe bass compared with largemouth bass where the parents originated from a different basin (the Red River basin). Unfortunately, we were not able to catch a sufficient number of Guadalupe bass from the wild to make an experimental comparison between wild Guadalupe bass and other host fish. Thus, future experiments will be necessary to determine whether the differences between hatchery Guadalupe and largemouth bass resulted from differences in species or the origin of the parents.

Based on metamorphosis success alone, both wild and hatchery fish could be used for captive propagation of *L. bracteate*; however, using hatchery fish for captive propagation and reintroduction may have ecological risks, as domestication of juvenile mussels via (accidental) artificial selection may occur (Hoftyzer, Ackerman, Morris, & Mackie, 2008; Jones, Hallerman, & Neves, 2006). Such effects should be considered, as glochidia that metamorphose well on hatchery fish may not necessarily metamorphose well on wild fish, and local adaptations may be lost. Although beneficial for retaining local adaptations in juvenile mussels for reintroduction, wild fish may already be infested with glochidia when collected and should therefore be collected well in advance of experiments to allow for detachment of wild juveniles.

With only a few host fish species from a single family, *L. bracteata* appears to have more specialized host requirements than mussels with more general host use such as native and non-threatened yellow sandshell, *L. teres*, in central Texas, which can use host fish from at least five fish families (Ford & Oliver, 2015). The present study found both largemouth bass and green sunfish (and hatchery Guadalupe bass) to be the best host fish, whereas juveniles also metamorphosed on bluegill sunfish, but in smaller numbers. Like piscivorous green sunfish and basses, bluegill sunfish will opportunistically consume a variety of prey, but are more limited by gape size. Thus, green sunfish and basses are more likely to attack a lure that resembles a darter (such as the lure of *L. bracteata*) than bluegill sunfish, which probably feeds on smaller prey items (Carlander, 1977; Mittlebach, 1981). This may have facilitated a stronger adaptation of *L. bracteata* to green sunfish and the basses tested in this study. In a previous study, green sunfish produced the greatest number of juvenile mussels, followed by bluegill sunfish, and were considered good hosts for *L. bracteata*, whereas largemouth and Guadalupe bass—which produced 50% fewer juveniles than green sunfish in the study—appeared as less suitable hosts (Johnson et al., 2012). The longer observational timeframe (70 vs. 26 days post-inoculation) used in the present study compared with Johnson et al. (2012) may have contributed to the different findings. Largemouth bass in our study produced fewer juveniles compared with green sunfish during the peak detachment period, but live juveniles continued to detach over a longer period of time (i.e. 45 vs. 26 days, Figure A3, A4).

Lampsilis bracteata is a long-term brooder, and brooding females were observed throughout the year except in September–October at the Llano River site, and in the October–December samples at the San Saba site. Most *Lampsilis* species spawn in late summer/autumn and brood until the following spring or early summer (Barnhart et al., 2008). Some *Lampsilis* species have been reported to brood most of the year (*Lampsilis hydiana;* Howells, 2000) or even throughout the year (*Lampsilis cardium* and *Lampsilis fasciola*; Lefevre & Curtis, 1912; Stagliano, 2001). The observed brooding period of *L. bracteata* in this study appears to be much longer than previously suggested

(July–October, Johnson et al., 2012). Resumption of brooding was observed at least a month earlier at the Llano River site. The decline in the proportion of brooding females in summer coincided with rising temperatures, but further research is needed to understand better the driving factors of the seasonal variation.

Seasonal variation in gamete concentration reflects variation in gamete production only if it is assumed that the gamete fluid volume does not vary seasonally. Nevertheless, there were some interesting patterns that could be related to seasonal variation in gamete production. Similar to brooding, variation in gamete concentration appeared to be at least partly related to temperature, as declines in egg concentration (in both rivers) and sperm concentration in the Llano River coincided with increasing temperatures, consistent with previous research (Galbraith & Vaughn, 2009; Jirka & Neves, 1992). The results also indicated that sampling volume should be restricted to small amounts (<100 μL), as larger amounts may dilute the gamete samples by pulling haemolymph from the haemocoel in addition to the gonads. Such dilution could explain the logarithmic decline between gamete concentration and sampling volume.

It is possible that lower gamete densities, brooding, fecundity and glochidia viability in San Saba mussels could at least in part be associated with the gonadal parasites, which are known to castrate mussels (Haag & Staton, 2003). A higher prevalence was detected in female mussels from the San Saba compared with the Llano River. A higher prevalence of female mussels was also found in a study in Finland on *Anodonta piscinalis*, where glochidial production was reduced in infected mussels (Taskinen & Valtonen, 1995). Trematodes (Family Bucephalidae) were also detected in a congener, *Lampsilis rafinesqueana*, in Missouri, USA (Shiver, 2002), but the present study is the first to document bucephalid trematodes in *L. bracteata*.

Environmental differences may also play a role or interact with the presence of the trematodes. For example, temperature has been shown to affect the host immunity of fish and parasite virulence (Scharsack et al., 2016). Elevated summer temperatures are likely to decrease brooding duration and glochidia viability (Zimmerman & Neves, 2002). The Llano River had much higher discharges (range 1–48 $m^3\ s^{-1}$) than the San Saba River (range 0.4–1.3 $m^3\ s^{-1}$) during the survey period, which may have contributed to the lower thermal minima seen in the Llano and allowed mussels to remain brooding and maintain glochidia viability for a longer period (Zimmerman & Neves, 2002). The lower flows in the San Saba may have also contributed to the infection rate of *L bracteata* with larvae of bucephalid trematodes, because lower flows may allow parasites to accumulate in higher densities (D.G. Huffman, personal communication). Tsakiris et al. (2016) reported a high incidence (>20%) of digenetic trematodes in *Cyclonaias petrina* and *Cyclonaias houstonensis* in the San Saba River between July 2012 and July 2013 during an exceptional drought in Texas, whereas a more recent study in 2017 found only ~5% of *Cyclonaias* to be infected (A.N.S., unpublished data). In addition, mussels may have also been more stressed by high temperatures in the San Saba River, making them more susceptible to parasitic infection (Scharsack et al., 2016). *Lampsilis bracteata* was found to be more intolerant to higher temperatures compared with other unionids in a recent study (Mitchell, McGuire, Abel, Hernandez, & Schwalb, 2018).

This study provides necessary background information for conservation actions that may involve captive propagation and population restoration of *L. bracteata*. Introduction or reintroduction of threatened species at sites where they are absent is generally considered to be a less risky action than the augmentation of existing populations (McMurray & Roe, 2017). The differences observed in metamorphosis success in this study suggest that host populations at reintroduction sites should be tested for compatibility with source mussel populations. Future studies should consider the possible adverse and beneficial effects of population mixing. Although outbreeding depression is a legitimate concern (Denic et al., 2015; Hoftyzer et al., 2008), the effects of inbreeding are also a concern in small populations, and heterosis in bivalves is often dramatically positive (Meyer & Manahan, 2010). Future studies should consider longer-term survival of juvenile mussels in relation to host fish origin, as mussel propagation may require host fish from a particular location based on where the mussels originated. Monitoring juveniles through the most sensitive portion of the mussel life-cycle (the early post-parasitic stage) could better explain the relationships between the origin of fish hosts and mussels (Buddensiek, Engel, Fleischauer-Rössing, Olbrich, & Wächtler, 1993).

ACKNOWLEDGEMENTS

We thank Somerley Swarm, Jaclyn McGuire and Don Apodaca for laboratory assistance, the A. E. Wood State Fish Hatchery for fish donations, Elizabeth Glidewell for sharing her insights into host fish experiments and David Huffman for identifying the trematodes detected in this study. This research was funded by the Texas Department of Transportation (grant no. 06882).

ORCID

Astrid N. Schwalb https://orcid.org/0000-0001-8567-5012

REFERENCES

Anderson, M. J. (2001). Permutation tests for univariate or multivariate analysis of variance and regression. *Canadian Journal of Fisheries and Aquatic Sciences*, *58*, 626–639. https://doi.org/10.1139/f01-004

Barnhart, M. C., Haag, W. R., & Roston, W. N. (2008). Adaptations to host infection and larval parasitism in *Unionoida*. *Journal of the North American Benthological Society*, *27*, 370–394. https://doi.org/10.1899/07-093.1

Bauer, G. (1994). The adaptive value of offspring size among freshwater mussels (Bivalvia: Unionoidea). *Journal of Animal Ecology*, *63*, 933–944. https://doi.org/10.2307/5270

Bauer, G., & Vogel, C. (1987). The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.) I. Host response to glochidiosis. *Archiv für Hydrobiologie*, Supplementband, Monographische Beitrage, *76*, 393–402.

Bingham, S. E. (2002). Host specificity of freshwater mussels: A critical factor in conservation (MS thesis). Missouri State University, MO.

Bogan, A. E. (1993). Freshwater bivalve extinctions (Mollusca: Unionoida): A search for causes. *Integrative and Comparative Biology*, *33*, 599–609. https://doi.org/10.1093/icb/33.6.599

Buddensiek, V., Engel, H., Fleischauer-Rössing, S., Olbrich, S., & Wächtler, K. (1993). Studies on the chemistry of interstitial water taken from defined horizons in the fine sediments of bivalve habitats in several northern German lowland waters. II: Microhabitats of *Margaritifera margaritifera* L., *Unio crassus* [Philipsson] and *Unio tumidus* Philipsson. *Archiv für Hydrobiologie*, *127*, 151–166.

Caldwell, M. L., Zanatta, D. T., & Woolnough, D. A. (2016). A multi-basin approach determines variability in host fish suitability for unionids in tributaries of the Laurentian Great Lakes. *Freshwater Biology*, *61*, 1035–1048. https://doi.org/10.1111/fwb.12756

Carlander, K. D. (1977). *Handbook of freshwater fishery biology* (3rd ed.). Ames, IA: Iowa State University Press.

Carneiro, P. C. F., & Urbinati, E. C. (2001). Salt as a stress response mitigator of matrinxã, *Brycon cephalus* [Günther], during transport. *Aquaculture Research*, *32*, 297–304. https://doi.org/10.1046/j.1365-2109.2001.00558.x

Chowdhury, M. M. R., Salonen, J. K., Marjomäki, T. J., & Taskinen, J. (2017). Interaction between the endangered freshwater pearl mussel *Margaritifera margaritifera*, the duck mussel *Anodonta anatina* and the fish host (*Salmo*): Acquired and cross-immunity. *Hydrobiologia*, *810*, 273–281.

Clements, E. A., Thomas, R., & Adams, C. E. (2018). An investigation of salmonid host utilization by the endangered freshwater pearl mussel (*Margaritifera margaritifera*) in north-west Scotland. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *28*, 764–768. https://doi.org/10.1002/aqc.2900

Ćmiel, A. M., Zając, K., Lipińska, A. M., & Zając, T. (2018). Glochidial infestation of fish by the endangered thick-shelled river mussel *Unio crassus*. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *28*, 535–544. https://doi.org/10.1002/aqc.2883

Denic, M., Taeubert, J. E., Lange, M., Thielen, F., Scheder, C., Gumpinger, C., & Geist, J. (2015). Influence of stock origin and environmental conditions on the survival and growth of juvenile freshwater pearl mussels (*Margaritifera margaritifera*) in a cross-exposure experiment. *Limnologica*, *50*, 67–74. https://doi.org/10.1016/j.limno.2014.07.005

Dodd, B. J., Barnhart, M. C., Rogers-Lowery, C. L., Fobian, T. B., & Dimock, R. V. (2005). Cross-resistance of largemouth bass to glochidia of unionid mussels. *Journal of Parasitology*, *91*, 1064–1072. https://doi.org/10.1645/GE-511R.1

Dodd, B. J., Barnhart, M. C., Rogers-Lowery, C. L., Fobian, T. B., & Dimock, R. V. (2006). Persistence of host response against glochidia larvae in *Micropterus salmoides*. *Fish & Shellfish Immunology*, *21*, 473–484. https://doi.org/10.1016/j.fsi.2006.02.002

Douda, K., Sell, J., Kubíková-Peláková, L., Horký, P., Kaczmarczyk, A., & Mioduchowska, M. (2014). Host compatibility as a critical factor in management unit recognition: Population-level differences in mussel–fish relationships. *Journal of Applied Ecology*, *51*, 1085–1095. https://doi.org/10.1111/1365-2664.12264

Douda, K., Martin, M., Glidewell, E., & Barnhart, C. (2018). Stress-induced variation in host susceptibility to parasitic freshwater mussel larvae. *Hydrobiologia*, *810*, 265–272. https://doi.org/10.1007/s10750-016-2895-3

Eckert, N. L. (2003). Reproductive biology and host requirement differences among isolated populations of *Cyprogenia aberti* (Conrad, 1850) (MS thesis). Missouri State University, USA.

Ford, D., & Oliver, A. M. (2015). The known and potential hosts of Texas mussels: Implications for future research and conservation efforts. *Freshwater Mollusk Biology and Conservation*, *18*, 1–14.

Galbraith, H. S., & Vaughn, C. C. (2009). Temperature and food interact to influence gamete development in freshwater mussels. *Hydrobiologia*, *636*, 35–47. https://doi.org/10.1007/s10750-009-9933-3

Grizzle, J. M., & Brunner, C. J. (2009). Infectious diseases of freshwater mussels and other freshwater bivalve mollusks. *Reviews in Fisheries Science*, *17*, 425–467. https://doi.org/10.1080/10641260902879000

Gum, B., Lange, M., & Geist, J. (2011). A critical reflection on the success of rearing and culturing juvenile freshwater mussels with a focus on the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). *Aquatic Conservation: Marine and Freshwater Ecosystems*, *21*, 743–751. https://doi.org/10.1002/aqc.1222

Haag, W. R. (2013). The role of fecundity and reproductive effort in defining life-history strategies of North American freshwater mussels. *Biological Reviews*, *88*, 745–766. https://doi.org/10.1111/brv.12028

Haag, W. R., & Staton, J. L. (2003). Variation in fecundity and other reproductive traits in freshwater mussels. *Freshwater Biology*, *48*, 2118–2130. https://doi.org/10.1046/j.1365-2427.2003.01155.x

Hansen, A. T., Czuba, J. A., Schwenk, J., Longjas, A., Danesh-Yazdi, M., Hornbach, D. J., & Foufoula-Georgiou, E. (2016). Coupling freshwater mussel ecology and river dynamics using a simplified dynamic interaction model. *Freshwater Science*, *35*, 200–215. https://doi.org/10.1086/684223

Hoftyzer, E., Ackerman, J. D., Morris, T. J., & Mackie, G. L. (2008). Genetic and environmental implications of reintroducing laboratory-raised unionid mussels to the wild. *Canadian Journal of Fisheries and Aquatic Sciences*, *65*, 1217–1229. https://doi.org/10.1139/F08-024

Hove, M. C., & Neves, R. J. (1994). Life history of the endangered James spiny mussel *Pleurobema collina* (Conrad, 1837) (Mollusca: Unionidae). *American Malacological Bulletin*, *11*, 29–40.

Hove, M. C., Sietman, B. E., Bakelaar, J. E., Bury, J. A., Heath, D. J., Pepi, V. E., … Kapuscinski, A. R. (2011). Early life history and distribution of Pistolgrip (*Tritogonia verrucosa* (Rafinesque, 1820) in Minnesota and Wisconsin. *American Midland Naturalist*, *165*, 338–354. https://doi.org/10.1674/0003-0031-165.2.338

Howells, R. G. (2000). Reproductive seasonality of freshwater mussels (Unionidae) in Texas. In R. Tankersley, G. Warmolts, B. Watters, B. Armitage, P. Johnson, & R. Butler (Eds.), *Freshwater mollusk symposia proceedings*. Ohio Biological Survey, Columbus, OH.

Howells, R. G. (2015). *Field guide to Texas freshwater mussels*. Kerrville, TX: BioStudies.

Jirka, K. J., & Neves, R. J. (1992). Reproductive biology of four species of freshwater mussels (Mollusca: Unionidae) in the New River, Virginia and West Virginia. *Journal of Freshwater Ecology*, *7*, 35–44. https://doi.org/10.1080/02705060.1992.9664668

Johnson, M. S., Caccavale, P. D., Randklev, C. R., & Gibson, J. R. (2012). New and confirmed fish hosts for the threatened freshwater mussel *Lampsilis bracteata* (Gould, 1855), the Texas Fatmucket (Bivalvia: Unionidae). *The Nautilus*, *126*, 148–149.

Jones, J. W., Hallerman, E. M., & Neves, R. J. (2006). Genetic management guidelines for captive propagation of freshwater mussels (Unionoidea). *Journal of Shellfish Research*, *25*, 527–535. https://doi.org/10.2983/0730-8000(2006)25[527:GMGFCP]2.0.CO;2

Lefevre, G., & Curtis, W. (1912). Experiments in the artificial propagation of fresh-water mussels. Proceedings of the Fourth International Fishery Congress, Washington. *Bulletin of the Bureau of Fisheries*, *28*, 617–626.

Levine, T. D., Lang, B. K., & Berg, D. J. (2012). Physiological and ecological hosts of *Popenaias popeii* (Bivalvia: Unionidae): Laboratory studies identify more hosts than field studies. *Freshwater Biology*, *57*, 1854–1864. https://doi.org/10.1111/j.1365-2427.2012.02844.x

Lydeard, C., Cowie, R. H., Ponder, W. F., Bogan, A. E., Bouchet, P., Clark, S. A., … Thompson, F. G. (2004). The global decline of nonmarine mollusks. *Bioscience*, *54*, 321–330. https://doi.org/10.1641/0006-3568(2004)054[0321:TGDONM]2.0.CO;2

McMurray, S. E., & Roe, K. J. (2017). Perspectives on the controlled propagation, augmentation, and reintroduction of freshwater mussels (Mollusca: Bivalvia: Unionoida). *Freshwater Mollusk Biology and Conservation*, *20*, 12.

Meyer, E., & Manahan, D. T. (2010). Gene expression profiling of genetically determined growth variation in bivalve larvae (*Crassostrea gigas*). *Journal of Experimental Biology*, *213*, 749–758. https://doi.org/10.1242/jeb.037242

Mitchell, Z. A., McGuire, J., Abel, J., Hernandez, B. A., & Schwalb, A. N. (2018). Move on or take the heat: Can life history strategies of freshwater mussels predict their physiological and behavioural responses to drought and dewatering? *Freshwater Biology*, *63*, 1579–1591. https://doi.org/10.1111/fwb.13187

Mittlebach, G. G. (1981). Foraging efficiency and body size: A study of optimal diet and habitat use by bluegills. *Ecology*, *62*, 1370–1386. https://doi.org/10.2307/1937300

Österling, M. E., & Larsen, B. M. (2013). Impact of origin and condition of host fish (*Salmo trutta*) on parasitic larvae of *Margaritifera margaritifera*. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *23*, 564–570. https://doi.org/10.1002/aqc.2320

Peredo, S., Parada, E., Valdebenito, I., & Peredo, M. (2005). Relocation of the freshwater mussel *Diplodon chilensis* (Hyriidae) as a strategy for its conservation and management. *Journal of Molluscan Studies*, *71*, 195–198. https://doi.org/10.1093/mollus/eyi024

R Core Team (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/

Riusech, F. A., & Barnhart, M. C. (2000). Host suitability and utilization in *Venustaconcha ellipsiformis* and *Venustaconcha pleasii*. In R. A. Tankersley, T. Watters, B. Armitage, & D. Warmolts (Eds.), *Proceedings of the captive care, propagation, and conservation of freshwater mussels symposium* 6–8 March 1998, Columbus, OH (pp. 83–91). Columbus, OH: Ohio University Press.

Rogers, C. L., & Dimock, R. V. (2003). Acquired resistance of bluegill sunfish *Lepomis macrochirus* to glochidia larvae of the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) after multiple infections. *Journal of Parasitology*, *89*, 51–56. https://doi.org/10.1645/0022-3395(2003)089[0051:AROBSL]2.0.CO;2

Rogers, S. O., Watson, B. T., & Neves, R. J. (2001). Life history and population biology of the endangered tan riffleshell (*Epioblasma florentina walkeri*) (Bivalvia: Unionidae). *Journal of the American Benthological Society*, *20*, 582–594. https://doi.org/10.2307/1468089

Salonen, J. K., Luhta, P.-L., Moilanen, E., Oulasvirta, P., Turunen, J., & Taskinen, J. (2017). Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) differ in their suitability as hosts for the endangered freshwater pearl mussel (*Margaritifera margaritifera*) in northern Fennoscandian rivers. *Freshwater Biology*, *62*, 1346–1358. https://doi.org/10.1111/fwb.12947

Scharsack, J. P., Franke, F., Erin, N. I., Kuske, A., Büscher, J., Stolz, H., … Kalbe, M. (2016). Effects of environmental variation on host–parasite interaction in three-spined sticklebacks (*Gasterosteus aculeatus*). *Zoology*, *119*, 375–383. https://doi.org/10.1016/j.zool.2016.05.008

Schneider, L. D., Nilsson, P. A., Höjesjö, J., & Österling, E. M. (2017). Local adaptation studies and conservation: Parasite–host interactions between the endangered freshwater mussel *Unio crassus* and its host fish. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *27*, 1261–1269. https://doi.org/10.1002/aqc.2816

Schwalb, A. N., Morris, T. J., & Cottenie, K. (2015). Dispersal abilities of riverine freshwater mussels influence metacommunity structure. *Freshwater Biology*, *60*, 911–921. https://doi.org/10.1111/fwb.12544

Shiver, M. A. (2002). Reproduction and production of the Neosho mucket, *Lampsilis rafinesqueana* (MS thesis). Missouri State University, MO.

Stagliano, D. M. (2001). Special animal abstract for *Lampsilis fasciola* (wavy-rayed lampmussel). Michigan Natural Features Inventory. Lansing, MI. https://mnfi.anr.msu.edu/abstracts/zoology/Lampsilis_fasciola.pdf [3 October 2017]

St. John White, B., Ferreri, C. P., Lellis, W. A., Wicklow, B. J., & Cole, J. C. (2017). Geographic variation in host fish use and larval metamorphosis for the endangered dwarf wedgemussel. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *27*, 909–918. https://doi.org/10.1002/aqc.2782

Strange, R. M., & Burr, B. M. (1997). Intraspecific phylogeography of North American highland fishes: A test of the Pleistocene vicariance hypothesis. *Evolution*, *51*, 885–897. https://doi.org/10.1111/j.1558-5646.1997.tb03670.x

Strayer, D. L. (2008). *Freshwater mussel ecology: A multifactor approach to distribution and abundance*. Berkeley, CA: University of California Press.

Taeubert, J. E., Denic, M., Gum, B., Lange, M., & Geist, J. (2010). Suitability of different salmonid strains as hosts for the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). *Aquatic Conservation: Marine and Freshwater Ecosystems*, *20*, 728–734. https://doi.org/10.1002/aqc.1147

Taskinen, J., & Valtonen, E. T. (1995). Age-, size- and sex-specific infection of *Anodonta piscinalis* (Bivalvia: Unionidae) with *Rhipidocotyle fennica* (Digenea: Bucephalidae) and its influence on host reproduction. *Canadian Journal of Zoology*, *73*, 887–897. https://doi.org/10.1139/z95-104

Thomas, G. R., Taylor, J., & Garcia de Leaniz, C. (2010). Captive breeding of the endangered freshwater pearl mussel *Margaritifera margaritifera*. *Endangered Species Research*, *12*, 1–9. https://doi.org/10.3354/esr00286

Tsakiris, E. T., Randklev, C. R., & Conway, K. W. (2016). Effectiveness of a nonlethal method to quantify gamete production in freshwater mussels. *Freshwater Science*, *35*, 958–973. https://doi.org/10.1086/687839

Williams, J. D., Warren, M. L., Cummings, K. S., Harris, J. L., & Neves, R. J. (1993). Conservation status of freshwater mussels of the United States and Canada. *Fisheries*, *18*, 6–22. https://doi.org/10.1577/1548-8446(1993)018<0006:CSOFMO>2.0.CO;2

Zanatta, D. T., & Wilson, C. C. (2011). Testing congruency of geographic and genetic population structure for a freshwater mussel (Bivalvia: Unionoida) and its host fish. *Biological Journal of the Linnean Society*, *102*, 669–685. https://doi.org/10.1111/j.1095-8312.2010.01596.x

Zimmerman, L. L., & Neves, R. J. (2002). Effects of temperature on duration of viability for glochidia of freshwater mussels (Bivalvia: Unionidae). *American Malacological Bulletin*, *17*, 31–35.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Seagroves LA, Barnhart MC, Hardy T, Schwalb AN. Reproductive ecology of the threatened and endemic freshwater mussel *Lampsilis bracteata*. *Aquatic Conserv: Mar Freshw Ecosyst*. 2019;29:1216–1226. https://doi.org/10.1002/aqc.3154