

RESEARCH ARTICLE

Evaluating the upper thermal limits of glochidia for selected freshwater mussel species (Bivalvia: Unionidae) in central and east Texas, and the implications for their conservation

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Abstract

1. Understanding the temperature tolerances of organisms is critical because the thermal regimes of freshwater ecosystems are changing globally. Native freshwater mussels are sensitive to increasing water temperatures because of their physiology and unique life history. Detailed knowledge on lethal temperatures for mussels has been limited to less than 5% of the species known to occur in North America, and little is known about the thermal tolerances of mussel species from rivers within the south-western USA.
2. To determine the effects of elevated water temperature on mussels, the upper thermal tolerances of larvae (glochidia) for the following species across four basins in Texas (Neches, Guadalupe, San Antonio, and Colorado) were tested: *Amblema plicata*, *Cyclonaias necki*, *Fusconaia mitchelli*, *Lampsilis bracteata*, *Lampsilis hydiana*, *Lampsilis satura*, *Lampsilis teres*, and *Obovaria arkansasensis*.
3. Glochidia were acclimated to 27°C across a range of experimental temperatures (30–39°C) in 24-h standard acute laboratory tests. The median lethal temperature (LT50) among glochidia averaged 32.4°C and ranged from 26.9 to 36.4°C.
4. Thermal tolerances differed significantly among and within species, and by season. Comparing these results with current water temperatures in central and east Texas indicated that populations of the focal species studied are at risk from rising environmental temperatures and, as a consequence, their long-term viability will be challenging in future years.

KEYWORDS

climate change, invertebrates, physiology, protected species, river

1 | INTRODUCTION

Water temperature is widely recognized as a key variable for maintaining riverine biota, and is tightly linked to flow patterns (Olden & Naiman, 2010). Natural spatial and temporal variations in temperature

exert a strong influence on the evolution, distribution, and ecology of aquatic organisms (Vannote & Sweeney, 1980; Ward & Stanford, 1982); however, human activities, such as river regulation, riparian clearing, and thermal effluents can disrupt the natural thermal regime of a river. This change to the thermal regime can adversely affect

biological end points, such as growth, survivorship, and reproduction, which can lead to shifts in species distributions or abundance and, in worst-case scenarios, extirpation or extinction. Climate change is expected to amplify these impacts, as the global mean surface temperature is predicted to increase by 1.5°C (Intergovernmental Panel on Climate Change (IPCC), 2013), with certain regions likely to undergo even larger changes. Within the south-western USA, places such as Texas are considered hot spots for climate change (Diffenbaugh, Giorgi, & Pal, 2008), as surface temperatures in this region are predicted to increase between 2 and 5°C by the year 2100 (Jiang & Yang, 2012), which in turn is likely to increase the frequency and intensity of droughts and extreme flow events (Milly, Dunne, & Vecchia, 2005).

Freshwater mussels are one of the most imperilled aquatic faunas because of human impacts on water quality and quantity (Strayer et al., 2004; Williams, Warren, Cummings, Harris, & Neves, 1993). As ectotherms, the influence of water temperature on freshwater mussels (*Bivalvia: Unionidae*) is pervasive, and mussels are constrained in their ability to escape harmful temperatures because of limited mobility (Amyot & Downing, 1997) and the reliance on host fish for larval dispersal as obligate parasites (Haag & Warren, 1998). Direct impacts of elevated water temperature on mussels include altered heart rate, gape frequency, and filtration rate, which presumably corresponds to increasing oxygen consumption and metabolic demands (Pandolfo, Cope, & Arellano, 2009; Rodland et al., 2008; Spooner & Vaughn, 2008), which in turn has implications for the ecosystem services provided by mussels (e.g. nutrient cycling; Vaughn & Hakenkamp, 2001; Vaughn, Nichols, & Spooner, 2008). Changes in the natural thermal regime can also influence seasonal growth cycles and reproductive behaviour (e.g. gamete development and glochidial release) that are cued by environmental factors such as temperature and photoperiod (Baker & Hornbach, 2001; Galbraith & Vaughn, 2009; Gascho Landis, Mosley, Haag, & Stoeckel, 2012; Watters & O'Dee, 2000). In addition to direct effects, changes to the natural temperature regime may impair mussel recruitment by affecting the timing and success of glochidial release. As obligate parasites, freshwater mussels rely on fish hosts to transform into juveniles, and temperature can affect the success of transformation (Roberts & Barnhart, 1999; Taeubert, El-Nobi, & Geist, 2014), the phenological synchrony of glochidial release with host fish occurrence (Pandolfo, Kwak, & Cope, 2012; Schneider, Nilsson, & Österling, 2018), and the duration of the parasitic phase (Eybe, Thielen, Bohn, & Sures, 2015; Taeubert et al., 2014), which can in turn affect the subsequent survival of the transformed juveniles (Marwaha, Jensen, Jakobsen, & Geist, 2017). Thus, mussel-dependent ecosystem services and population resilience are tightly linked to the natural thermal regime (Gates, Vaughn, & Julian, 2015; Spooner & Vaughn, 2008), making mussel populations a model system from which to gain a better understanding of how thermal regimes in rivers shape population and community structure for aquatic biota (Allen, Galbraith, Vaughn, & Spooner, 2013).

Thermal studies for mussels have largely focused on glochidia (larvae) and juveniles because these are the most sensitive life stages; however, quantitative information on lethal temperatures for freshwater mussels has been limited to 15 species (Archambault, Cope, &

Kwak, 2014; Dimock & Wright, 1993; Ganser, Newton, & Haro, 2015; Martin, 2016; Pandolfo et al., 2010), and these thermal studies have been carried out in geographical regions (i.e. the mid-western, south-eastern, and western USA) with stream types that are probably not representative of those occurring in the south-western USA or other arid or semi-arid regions. Because of this, the applicability of these studies to other regions and species is unknown. In Texas, the lack of thermal studies is problematic, as 11 mussel species have been petitioned for protection under the US Endangered Species Act (US Fish and Wildlife Service (USFWS), 2009; 2011; 2016). For these species, there has been little consideration of how elevated water temperatures will affect them. This situation is unfortunate because many of these species occur in rivers and streams within arid or semi-arid regions of Texas, where flow is primarily maintained by groundwater and springs, which are under threat because of groundwater extraction and water infrastructure projects. In fact, there is already indirect evidence that mussels in Texas are living close to or beyond their upper thermal limits based on their current distribution. Most of the mussel fauna of Texas, including threatened species, are now primarily found in the lower reaches of large rivers or in tributaries where flow continues to remain perennial.

The overall objective of this study was to identify thermal tolerance limits for mussels from the south-western USA using glochidia (the larval life stage) of three endemic and seven widespread species, representing four tribes of the family Unionidae (Williams et al., 2017). The specific objectives of this study were: (i) to assess the effects of a range of water temperatures on glochidial survival; (ii) to use the resulting data to compare upper thermal limits between species and populations; and (iii) to discuss how these results may relate to the decline of these species, as well as the management implications and potential solutions for minimizing these threats. The resulting data from this study will help to inform the overall management and conservation efforts for mussels within Texas, and will expand the present understanding of how thermal stress affects mussels, particularly species that occur in arid and semi-arid regions such as the south-western USA.

2 | METHODS

2.1 | Study area

The present study was conducted at eight sites in seven rivers across the Colorado, Guadalupe, San Antonio, and Neches river basins of central and east Texas (Figure 1). Within central Texas, the westernmost sites were in the Llano and San Saba rivers of the Colorado River drainage. The Llano and San Saba rivers are major tributaries of the middle Colorado River, and are located within the Edwards Plateau Woodland of the Edwards Plateau ecoregion (Griffith, Bryce, Omernik, & Rogers, 2007). Baseflows within the Llano and San Saba rivers are derived primarily from spring inflows from the Edwards–Trinity Plateau and Ellenburger–San Saba aquifers (Wolaver, Cook, Scanlon, & Young, 2012). The third site, Cherokee Creek, is located near both these rivers but, in contrast, is a minor tributary of the middle

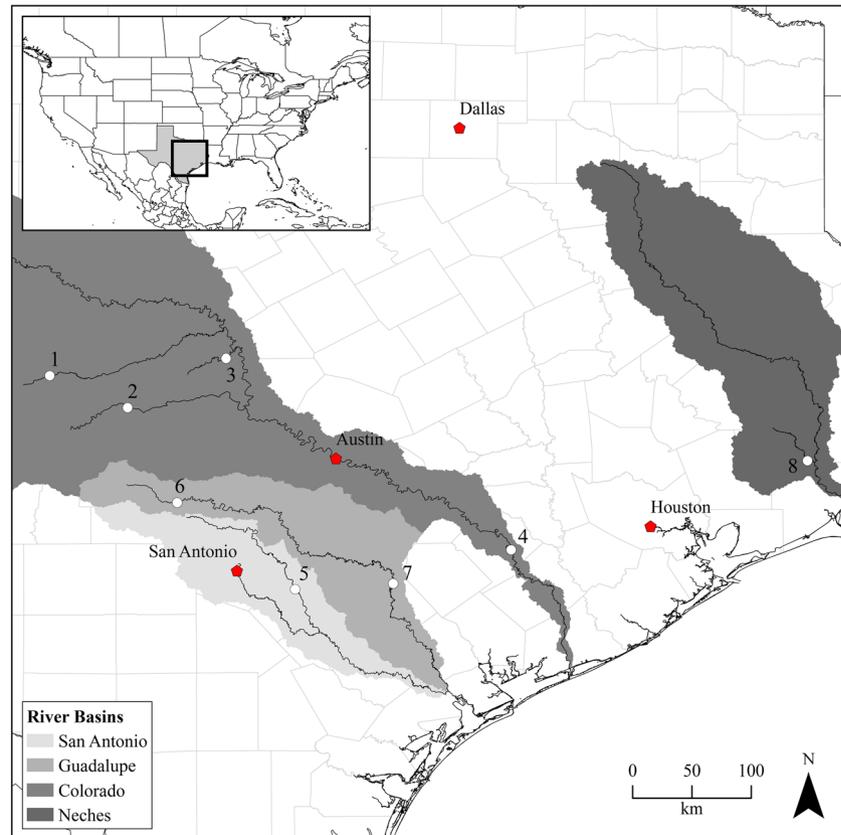


FIGURE 1 Collection sites for gravid mussel species from which glochidia were harvested for thermal experiments: 1, San Saba River; 2, Llano River; 3, Cherokee Creek; 4, Colorado River; 5, Cibolo Creek; 6, Upper Guadalupe River; 7, Lower Guadalupe River; and 8, Village Creek

Colorado River. Similar to the baseflows within the Llano and San Saba rivers, the source of the baseflow within Cherokee Creek is primarily from groundwater input and spring flows, though contributions for Cherokee Creek are largely derived from the Ellenburger–San Saba and Marble Falls aquifers. The flow regimes across all three sites, regardless of differences in stream size and length, are characterized by long periods of low flow followed by short, high-magnitude floods during heavy rainfall events (Blum, Toomey, & Valastro, 1994; Brune, 1975). Water temperatures in the Llano and San Saba rivers average 29°C and 28°C in July and 9°C and 7°C in January, respectively (Surface Water Quality Monitoring (SWQM), 2017). For Cherokee Creek, water temperature data are sparse, but average 29°C in July and 14°C in February (SWQM, 2017).

The fourth site in central Texas is located in the main stem of the lower Colorado River, which flows through the Southern Blackland Prairie of the Texas Blackland Prairies ecoregion. The flow regime within the lower Colorado River is more stable compared with the upper reaches of the drainage (Wolaver et al., 2014), and is primarily derived from surface run-off and releases from six reservoirs (Lake Buchanan, Inks Lake, Lake Lyndon B. Johnson, Lake Marble Falls, Lake Travis, and Lake Austin), although periods of drought and low flow are common (Blum & Valastro, 1994). Average water temperatures for the lower Colorado River in July and January are 30°C and 11°C, respectively (SWQM, 2017).

The fifth and sixth sites within central Texas were situated within the upper and lower reaches of the Guadalupe River, respectively. Specifically, the upstream site was located within the upper reach, which flows through the Balcones Canyonlands of the Edwards Plateau

ecoregion, whereas the downstream site was located in the lower reach, which flows through the floodplains and low terraces of the Western Gulf Coastal Plain ecoregion (Griffith et al., 2007). Both reaches can experience sudden high rates of precipitation, resulting in short, high-magnitude flow events, interspersed with longer periods of low flow. Baseflows in the upper reaches of the Guadalupe River are derived from spring inputs from the Edwards–Trinity and Trinity aquifers (Ashworth, 2005). In contrast, baseflows within the lower reaches are derived from a combination of spring-fed tributaries, local groundwater inputs, upstream dam releases, and surface run-off. The flow regime within the upper reaches of the Guadalupe River is not managed, but flow within the lower reaches is modified by seven main-stem impoundments, including Canyon Lake reservoir, which is a deep-storage bottom-release reservoir (Perkin & Bonner, 2011). As a consequence, the natural flow regime in this reach, including temperature, has been altered (Phillips, 2012). Average water temperatures in the upper and lower Guadalupe River are 28°C and 30°C in July and 10°C and 13°C in January, respectively, although there is significant spatiotemporal variability between the two reaches (SWQM, 2017).

The seventh site within central Texas was located in Cibolo Creek, which is part of the San Antonio River basin and flows through the Southern Post Oak Savannah region of the East Central Texas Plains ecoregion (Griffith et al., 2007). Baseflow in Cibolo Creek is derived from a combination of treated effluent, surface run-off, and spring flow, although historically it was derived from spring inflows from the Edwards–Trinity and Trinity aquifers. The flow regime within Cibolo Creek is now characterized by short, high-pulse events during

heavy rainfall followed by elevated baseflows (San Antonio River Authority (SARA), 2017). Water temperatures in Cibolo Creek average 29°C in July and 12°C in January (SWQM, 2017).

In east Texas, the eighth site was located on Village Creek in the Neches River drainage basin of east Texas, situated in the Flatwoods region of the South Central Plains ecoregion (Griffith et al., 2007). Village Creek is unregulated, and its baseflows are derived from groundwater inputs and surface run-off. The flow regime within this system is characterized by sluggish flow followed by prolonged periods of flooding during heavy rainfall events. The daily water temperatures for July and January averaged 29°C and 11°C, respectively (SWQM, 2017).

2.2 | Species

Thermal tolerances were evaluated for the glochidia of eight species representing four tribes (Amblemini, Lampsilini, Pleurobemini, and Quadrulini) in the family Unionidae (Williams et al., 2017): *Amblema plicata*, *Cyclonaias necki*, *Fusconaia mitchelli*, *Lampsilis bracteata*, *Lampsilis hydiana*, *Lampsilis satura*, *Lampsilis teres*, and *Obovaria arkansasensis* (Table 1). Three of these species (*A. plicata*, *L. hydiana*, and *L. teres*) are considered stable with broad distributions (NatureServe, 2017; Williams, Bogan, & Garner, 2008), whereas *F. mitchelli*, *L. bracteata*, and *C. necki* are state-listed Texas endemics (Texas Parks and Wildlife Department (TPWD), 2010). In addition, both *C. necki* and *L. bracteata* are candidates for federal protection under the Endangered Species Act (ESA), and both species plus *F. mitchelli* are currently pending review for ESA listing (USFWS, 2009; 2011). *Lampsilis satura* and *O. arkansasensis* are listed as state-threatened in Texas (TPWD, 2010), but are not under review for ESA listing.

2.3 | Thermal tolerance testing

Gravid female mussels were collected from the Guadalupe, San Antonio, Colorado, and Neches river basins from April 2017 to April 2018 (Table 1). Following collection, mussels were transported in

insulated coolers to the Texas A&M AgriLife Extension and Research Center in Dallas, Texas. Upon arrival in the laboratory, mussels were gradually adjusted (<2.5°C d⁻¹) from the transport temperature (19–23°C) to the holding temperature (20± 1°C). Mussels were fed daily with a mixture of commercial algae approximately equivalent to 3% of the mean shell-free dry weight (Shellfish diet 1800 and Nanno 3600; Reed Mariculture, Campbell, CA).

For Lampsilini species (*L. bracteata*, *L. hydiana*, *L. satura*, *L. teres*) and *O. arkansasensis*, glochidia were flushed from the gills of gravid females by puncturing the ventral margin of the marsupium with a hypodermic syringe (American Society of Testing Materials (ASTM), 2006a). For all other species, individual mussels were isolated in plastic cups and checked daily for the presence of glochidia or conglutinates, which were freed before testing by using fine forceps to gently rupture the outer conglutinate membrane. All glochidia were less than 24-h old at the start of each experimental trial, and all gravid mussels were used within 1–14 days of collection from the wild. The viability of glochidia isolated from individual females was determined by introducing a saturated NaCl solution to a subsample of suspended glochidia. Glochidia were considered viable if valve closure in response to NaCl solution was observed (ASTM, 2006a; Zale & Neves, 1982). Glochidia isolated from between one and three mussels were pooled for thermal tests.

Only glochidia isolated from female mussels with ≥80% viability were thermally tested (Ingersoll et al., 2006), except for the ESA candidate species *C. necki* (77.5 ± 7%) and *L. bracteata*, from the Llano (64.3 ± 5%) and San Saba rivers (79.6 ± 6%; Table 1). Because of the difficulty in locating gravid females of these rare species, smaller sample sizes ($n < 3$ gravid females) and lower initial viabilities (~70–80%) were used in order to provide information on the thermal tolerance of glochidia before listing.

For comparison against previous studies (Archambault et al., 2014; Pandolfo et al., 2010), glochidia were acclimated to 27°C and thermal tolerances were tested for a series of four temperatures ranging from 30°C to 39°C (Figure 2) alongside a non-acclimated control (20°C) (Pandolfo et al., 2010). The effect of acclimation on thermal tolerance

TABLE 1 A list of species from which glochidia were tested (*Amblema plicata*, *Fusconaia mitchelli*, *Lampsilis bracteata*, *Lampsilis hydiana*, *Lampsilis satura*, *Lampsilis teres*, *Obovaria arkansasensis*, and *Cyclonaias necki*), the collection date and river from which gravid mussels were collected, and the viability of glochidia at the onset of the thermal trials

Tribe	Species	Collection date	Population	<i>n</i> gravid females	Viability (%)
Amblemini	<i>A. plicata</i>	28 June 2017	Lower Guadalupe River	1	94.2
Lampsilini	<i>L. bracteata</i>	25 May 2017	Llano River	3	64.3
	<i>L. bracteata</i>	18 July 2017	San Saba River	3	79.6
	<i>L. bracteata</i>	10 April 2018	Cherokee Creek	2	91.7
	<i>L. bracteata</i>	10 April 2018	Upper Guadalupe River	3	89.4
	<i>L. hydiana</i>	25 May 2017	Lower Guadalupe River	2	79.6
	<i>L. hydiana</i>	11 April 2018	Cibolo Creek	3	92.4
	<i>L. satura</i>	19 April 2017	Village Creek	3	94.5
	<i>L. teres</i>	9 June 2017	Colorado River	3	85.2
	<i>O. arkansasensis</i>	19 April 2017	Village Creek	3	95.4
Pleurobemini	<i>F. mitchelli</i>	5 April 2017	Lower Guadalupe River	2	83.7
Quadrulini	<i>C. necki</i>	5 April 2017	Lower Guadalupe River	1	70.5
	<i>C. necki</i>	28 June 2017	Lower Guadalupe River	1	84.5

was not tested because previous studies found no difference between median lethal temperatures for glochidia acclimated to different temperatures (e.g. 22°C or 27°C; Pandolfo et al., 2010, but see Martin, 2016). At the start of each trial, glochidia were acclimated from the holding temperature (20°C) to the acclimation temperature (27°C) by increments of 1°C h⁻¹ with a 2-h acclimation period once the acclimation temperature was reached. Each temperature tested (including the control) used three replicates and was conducted for 24 h under non-aerated, static conditions in 100-mL beakers containing 80 mL of reconstituted hard water (ASTM, 2006a; 2006b). Replicate beakers were held in fibreglass water baths containing ~5 L of water and maintained at one of four experimental temperatures (Figure 2) or a 20°C control with a 300-W titanium heater (Finnex, Countryside, IL) attached to a temperature controller (Aqua Logic, San Diego, CA). The temperature in the water baths was verified using a YSI ProODO (YSI Inc., Yellow Springs, OH). At 24 h (i.e. the conclusion of the test), viability was assessed for a subsample of ~50 of the 250 glochidia in each of the three replicates per temperature by exposing them to a saturated NaCl solution and then examining their responses under an Olympus SZ51 microscope (Olympus America, Center Valley, PA). Glochidia that exhibited shell closure in response to the NaCl solution were considered viable, whereas glochidia that did not close their shell were considered non-viable (ASTM, 2006a). For a subset of four populations (three species), viability was also assessed at 12 h in addition to the 24-h subsample.

2.4 | Statistical analyses

Lethal temperatures resulting in 50% or 5% mortality (LT50 and LT05, respectively), and their 95% confidence intervals (95% CIs), were determined by a two-parameter regression model using either a logistic or Weibull distribution. The best-fit model was then selected using Akaike's information criterion (AIC). All LT50/05 estimates were calculated using adjusted survival values following Wang et al. (2007) because glochidia survival was not 100% at the onset of each trial. Adjusted survival values were also used for the calculation of control survival at the end of the 24-h and 12-h assessments. Control survival of ≥80% was considered acceptable for a temperature trial and for the calculation of LT50/05 estimates.

Statistical comparisons of LT50/05 values across and within species were conducted using the confidence interval ratio test (Wheeler, Park, & Bailer, 2006). This method compares the ratios of two LT50/05 values (or any other ratio of lethality by temperature)

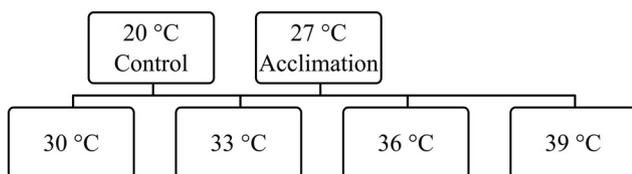


FIGURE 2 Experimental design following Pandolfo et al. (2010) showing the acclimation and experimental temperatures. Experimental temperatures were assessed alongside a non-acclimated 20°C control

with one or the log(LT50 ratio) with zero. The 95% CIs are then constructed, and if the 95% CI does not contain one (or zero if the log was used), then the hypothesis that population LTs are the same is rejected (Wheeler et al., 2006). Regression models and the confidence interval test were implemented using the DRC package in R (R Core Team, 2017; Ritz, Baty, Streibig, & Gerhard, 2015).

3 | RESULTS

3.1 | Across species

Paired comparisons of 24-h LT50 and LT05 values showed significant differences in thermal tolerances across species (Figures 3 and 4; Tables 2 and 3). *Cyclonaias necki* collected during the spring had the highest 24-h LT50 value (36.4 ± 0.4°C; LT50 ± 95% CI), whereas *A. plicata* had one of the lowest 24-h LT50 values (28.3 ± 1.0°C; Figure 5a). Among the Lampsilini species, *L. bracteata* collected during the summer from the San Saba River had the highest 24-h LT50 value (34.7 ± 0.3°C), followed by *L. hydiana*, which was collected during the summer from the Lower Guadalupe River (34.4 ± 0.3°C), followed by *O. arkansasensis*, which was collected during the spring from Village Creek (33.2 ± 0.5°C); followed by *L. satura* (32.5 ± 0.5°C), which was also collected during the spring from Village Creek; and, finally, *L. teres* (31.1 ± 0.5°C), which was collected during the summer from the lower Colorado River. For the subsample of species tested at 12 h, LT50 values also varied across species (Figure S1; Table S1). *Fusconaia mitchelli*, collected during the spring from the Lower Guadalupe, had the highest 12-h LT50 value (36.1 ± 0.4°C), followed by *L. hydiana*, which was collected during the spring from Cibolo Creek (34.1 ± 0.2°C), and finally *L. bracteata*, which was collected from the upper Guadalupe (33.9 ± 0.3°C) and Cherokee Creek (33.9 ± 0.2°C). These differences were generally not significant, however, and for *L. bracteata* and *L. hydiana* there were no differences in the corresponding LT50 estimates (Figure 5b).

Estimates for 24-h LT05 values also differed across species and generally mirrored the results for the LT50 estimates, but the rank order from highest to lowest by LT05 was different (Figure 4; Table 3). *Lampsilis bracteata* collected during the spring from Cherokee Creek had the highest LT05 value (29.7 ± 0.4°C), whereas *A. plicata* (22.2 ± 2.0°C) and *L. teres* (22.1 ± 1.2°C) had the lowest LT05 value. *Cyclonaias necki*, which had the highest estimated LT50 value, had an LT05 value of 27.4 ± 0.9°C. The 12-h LT05 estimates were similar to the 12-h LT50 estimates in that they varied by species, although this difference was generally not significant, and nor was it significantly different from the corresponding 24-h LT05 estimates (Figure S2; Table S2). Similarly, the 12-h LT05 values also mirrored the results of the 24-h LT05 values, in that there was a reversal in the rank order between Pleurobemini or Quadrulini and Lampsilini species in relation to high LT estimates. Specifically, Lampsilini species, which did not have the highest 24- or 12-h LT50 values, had the highest 24- or 12-h LT05 estimates.

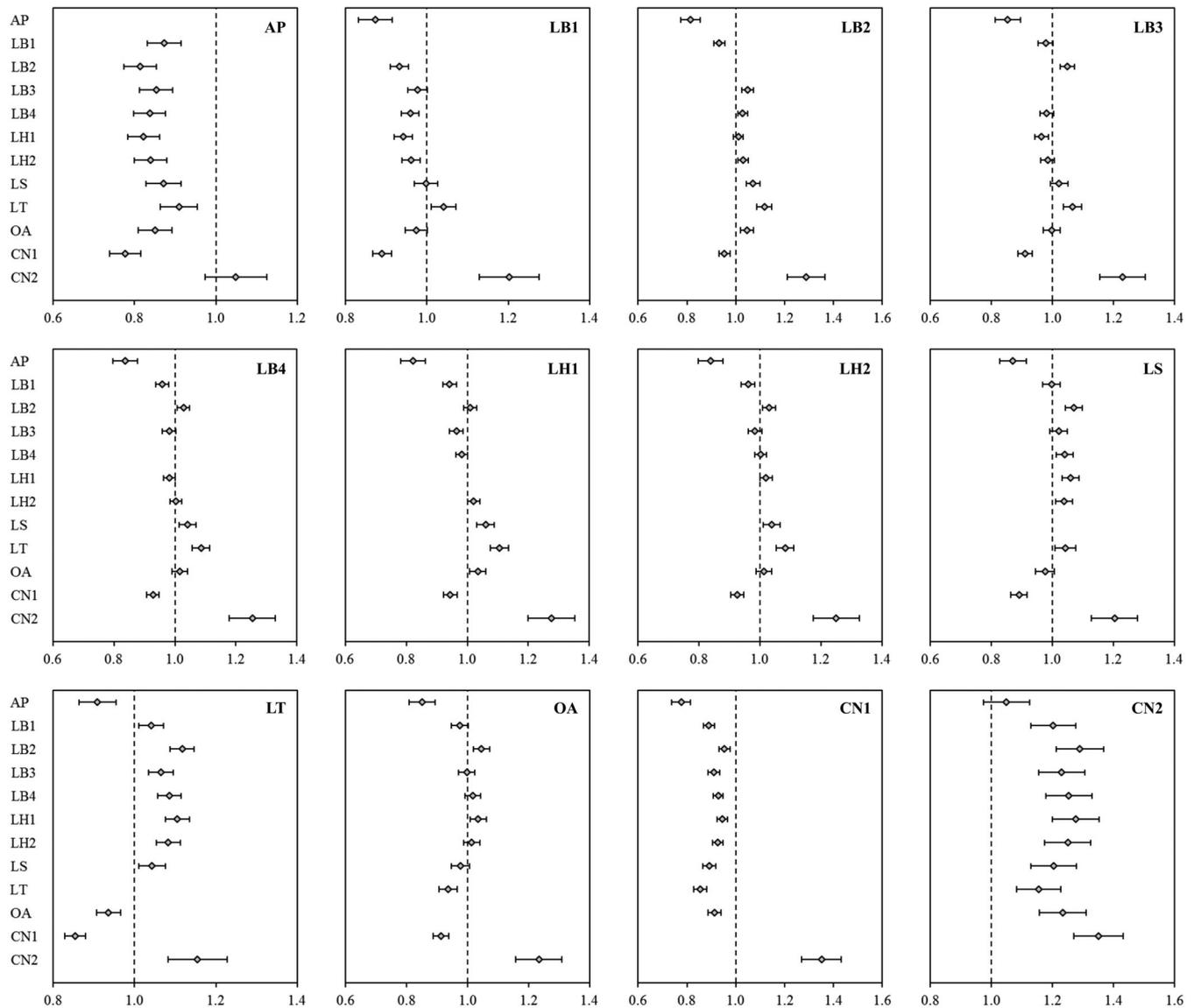


FIGURE 3 Comparison of ratios for 24-h lethal temperatures resulting in 50% mortality (LT50 values, grey diamonds) and 95% confidence intervals (error bars) for each pairwise comparison across species. Confidence intervals that do not contain 1 (dashed line) indicate that the LT50 comparisons were significantly different. Abbreviations: AP, *Amblema plicata*; FM, *Fusconaia mitchelli*; LB, *Lampsilis bracteata*; LH, *Lampsilis hydiana*; LS, *Lampsilis satara*; LT, *Lampsilis teres*; OA, *Obovaria arkansensis*; and CN, *Cyclonaias necki*

3.2 | Within species

Comparing LT50/LT05 values within species, LT50 values for *L. bracteata* were significantly different across all populations, except for comparisons between Cherokee Creek and the upper Guadalupe. The San Saba population had the highest 24-h LT50 value ($34.7 \pm 0.3^\circ\text{C}$), followed by Cherokee Creek ($33.8 \pm 0.2^\circ\text{C}$), followed by the Upper Guadalupe ($33.1 \pm 0.3^\circ\text{C}$), and finally the Llano River ($32.4 \pm 0.3^\circ\text{C}$). The 24-h LT05 values across the same populations followed a similar pattern, although the onset of mortality occurred much sooner, between 25.5°C (Llano population) and 29.7°C (Cherokee Creek population). Comparing the effect of seasonality on LT50/LT05 estimates, there were significant differences for *C. necki* between spring and summer. Specifically, the 24-h LT50 value for *C. necki* collected during the spring was $36.4 \pm 0.4^\circ\text{C}$, whereas the

same population sampled during the summer had a 24-h LT50 value of $26.9 \pm 1.4^\circ\text{C}$.

4 | DISCUSSION

This study is the first to report lethal thermal tolerances for the early life stages of mussels occurring in the south-western USA. The results of this study together with others in recent years (Archambault et al., 2014; Ganser, Newton, & Haro, 2013; Martin, 2016; Pandolfo et al., 2010) show that upper thermal limits differ between species, but overall that LT50 and LT05 values are similar across regions, with 24-h LT50 values averaging 31.6°C ($21.4\text{--}37.2^\circ\text{C}$) and 24-h LT05 values averaging 27.9°C ($15.6\text{--}36.7^\circ\text{C}$; Archambault et al., 2014; Pandolfo et al., 2010). These results suggest that although there may be some limited regional

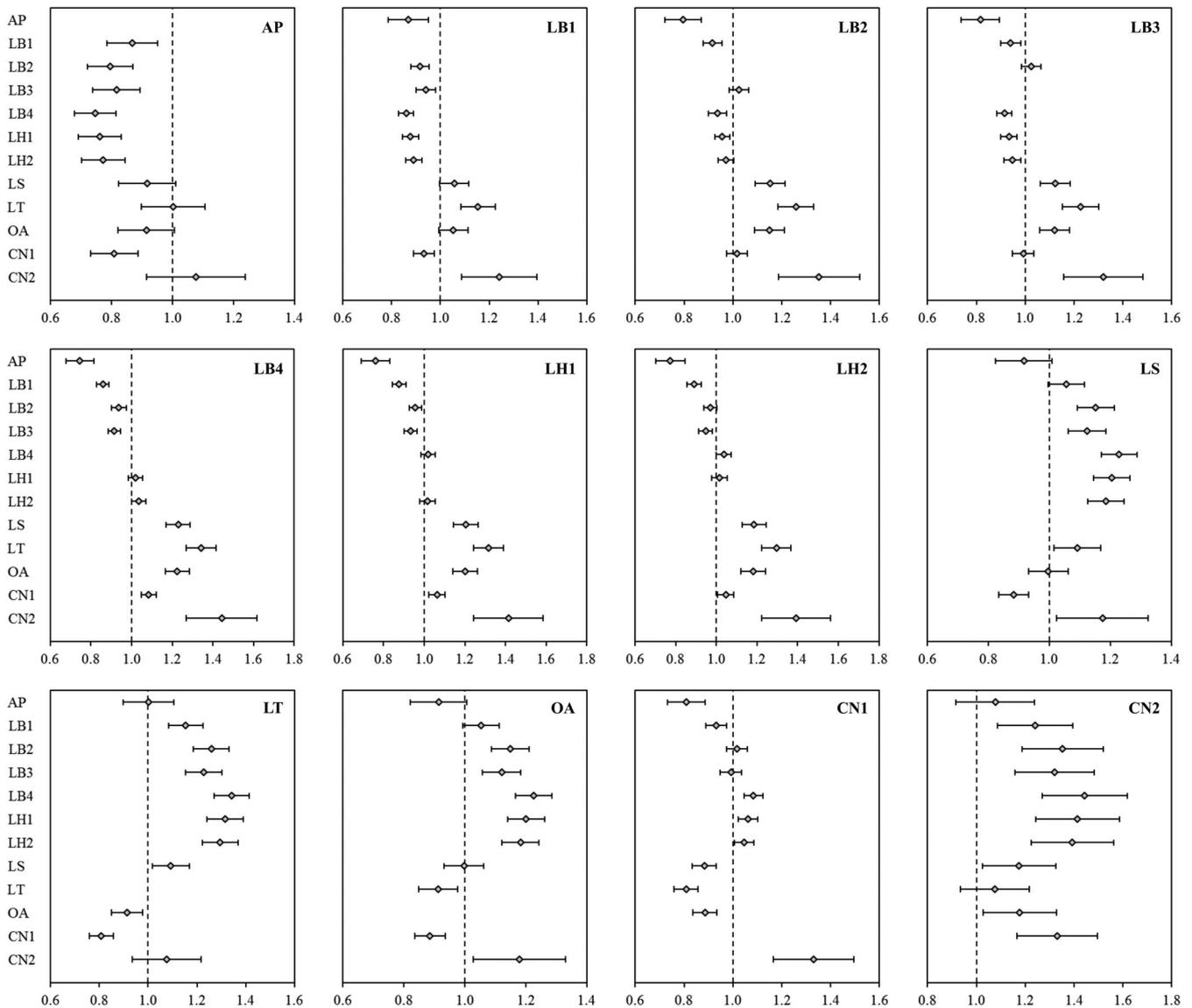


FIGURE 4 Comparison of ratios for 24-h lethal temperatures resulting in 5% mortality (LT05 values, grey diamonds) and 95% confidence intervals (error bars) for each pairwise comparison across species. Confidence intervals that do not contain 1 (dashed line) indicate that the LT05 comparisons were significantly different. Abbreviations: AP, *Amblema plicata*; FM, *Fusconaia mitchelli*; LB, *Lampsilis bracteate*; LH, *Lampsilis hydiana*; LS, *Lampsilis satura*; LT, *Lampsilis teres*; OA, *Obovaria arkansensis*; and CN, *Cyclonaias necki*

variation, the range of upper thermal limits for early life stages is generally fixed, which could be the result of the fact that glochidia are only exposed to ambient water temperatures for a short period of time (Ingersoll et al., 2006), and freshwater mussels generally occupy similar habitats among river systems (Cummings & Graf, 2009). Similar observations have been made for marine molluscs (Bayne, 1965, 1976; Zippay & Hofmann, 2010). In addition, the difference between LT50 and LT05 values by species was narrow, ranging from 3.9 to 9.0°C, suggesting that a relatively small increase in water temperature can result in mortality, which mirrors other glochidia thermal tolerance studies (Archambault et al., 2014; Pandolfo et al., 2010). Before the onset of mortality, or within the window between the LT05 and LT50 estimates, it is likely that sublethal effects are also occurring, which could reduce fitness in later life stages. For example, the larval stages of marine

invertebrates subjected to sublethal thermal stress have been shown to have decreased juvenile survival and growth, with these effects often extending into adulthood (Delorme & Sewell, 2014; Kessel & Phillips, 2018). Reversals in rank order between Pleurobemini or Quadrulini and Lampsilini species in relation to extreme LT50 and LT05 estimates were also found, which indicates that the onset of lethal and sublethal effects occurs much sooner for Pleurobemini and Quadrulini species. This difference may be explained, in part, by the fact that Lampsilini species tend to have life-history traits that allow them to occupy environments that are more stochastic in terms of flow and water temperature (Haag, 2012). Moreover, Lampsilini species are long-term brooders (i.e. they carry their brood throughout the year), and higher LT05 values may confer protection to gravid females and their brood (Jim Stoeckel, Auburn University, pers. comm).

TABLE 2 Matrix of confidence interval ratios for 24-h lethal temperature resulting in 50% mortality (LT50 estimates) for paired species comparisons acclimated to 27°C. The confidence intervals are given below the diagonal, and the difference between LT50 estimates is given above the diagonal. Asterisks indicate that the confidence interval of the ratio did not contain 1, and thus LT50 comparisons were significantly different. Species (n) indicates the number of gravid females from which glochidia were collected for each species, abbreviated as follows: AP, *Amblema plicata*; FM, *Fusconia mitchelli*; LB, *Lampsilis bracteata*; LH, *Lampsilis hyadiana*; LS, *Lampsilis satura*; LT, *Lampsilis teres*; OA, *Obovaria arkansensis*; and CN, *Cyclonaias necki*. 'Population' is the river basin from which gravid mussels were collected. 'Season' is the time of year gravid female mussels were collected from the field

Trial	Tribe	Species (n)	Population	Season	LT50	AP	LB1	LB2	LB3	LB4	LH1	LH2	LS	LT	OA	CN1	CN2
AP	Amblemini	AP (1)	Lower Guadalupe	Summer	28.3 ± 1.0	-	4.11*	6.47*	4.87*	5.51*	6.11*	5.42*	4.18*	2.83*	4.97*	8.14*	1.33
LB1	Lampsilini	LB (3)	Llano	Summer	32.4 ± 0.3	0.84-0.91*	-	2.36*	0.76*	1.40*	2.00*	1.31*	0.06	1.28*	0.85*	4.03*	5.44*
LB2	Lampsilini	LB (3)	San Saba	Summer	34.7 ± 0.3	0.78-0.84*	0.92-0.94*	-	1.60*	0.96*	0.35	1.05*	2.29*	3.63*	1.50*	1.67*	7.80*
LB3	Lampsilini	LB (2)	Upper Guadalupe	Spring	33.1 ± 0.3	0.82-0.88*	0.96-0.99*	1.03-1.06*	-	0.64	1.24*	0.55	0.70*	2.04*	0.09	3.27*	6.20*
LB4	Lampsilini	LB (3)	Cherokee	Spring	33.8 ± 0.2	0.81-0.87*	0.95-0.97*	1.02-1.04*	0.97-1.00	-	0.60*	0.09	1.33*	2.68*	0.54*	2.63*	6.84*
LH1	Lampsilini	LH (2)	Lower Guadalupe	Summer	34.4 ± 0.3	0.79-0.85*	0.93-0.95*	1.00-1.02	0.95-0.98*	0.97-0.99*	-	0.69	1.94*	3.28*	1.15*	2.03*	7.44*
LH2	Lampsilini	LH (3)	Cibolo	Spring	33.7 ± 0.2	0.81-0.87*	0.95-0.97*	1.02-1.04*	0.97-1.00	0.99-1.01	0.99-1.03	-	1.24*	2.59*	0.45	2.72*	6.75*
LS	Lampsilini	LS (3)	Village	Spring	32.5 ± 0.5	0.84-0.90*	0.98-1.02	1.05-1.09*	1.00-1.04*	1.02-1.06*	1.04-1.08*	1.02-1.06*	-	1.34*	0.79*	3.96*	5.50*
LT	Lampsilini	LT (3)	Lower Colorado	Summer	31.1 ± 0.5	0.87-0.94*	1.02-1.06*	1.10-1.14*	1.05-1.09*	1.07-1.10*	1.09-1.12*	1.06-1.10*	1.02-1.07*	-	2.13*	5.31*	4.16*
OA	Lampsilini	OA (3)	Village	Spring	33.2 ± 0.5	0.82-0.88*	0.96-0.99*	1.03-1.06*	0.98-1.01	1.00-1.03*	1.02-1.05*	1.00-1.03	0.96-1.00*	0.92-0.96*	-	3.17*	6.29*
CN1	Quadrulini	CP (1)	Lower Guadalupe	Spring	36.4 ± 0.4	0.75-0.80*	0.88-0.90*	0.94-0.97*	0.90-0.92*	0.92-0.94*	0.93-0.96*	0.91-0.94*	0.87-0.91*	0.84-0.87*	0.90-0.93*	-	9.47*
CN2	Quadrulini	CP (1)	Lower Guadalupe	Summer	26.9 ± 1.4	0.98-1.11	1.14-1.27*	1.22-1.36*	1.16-1.30*	1.19-1.32*	1.21-1.34*	1.18-1.32*	1.14-1.27*	1.09-1.22*	1.17-1.30*	1.28-1.42*	-

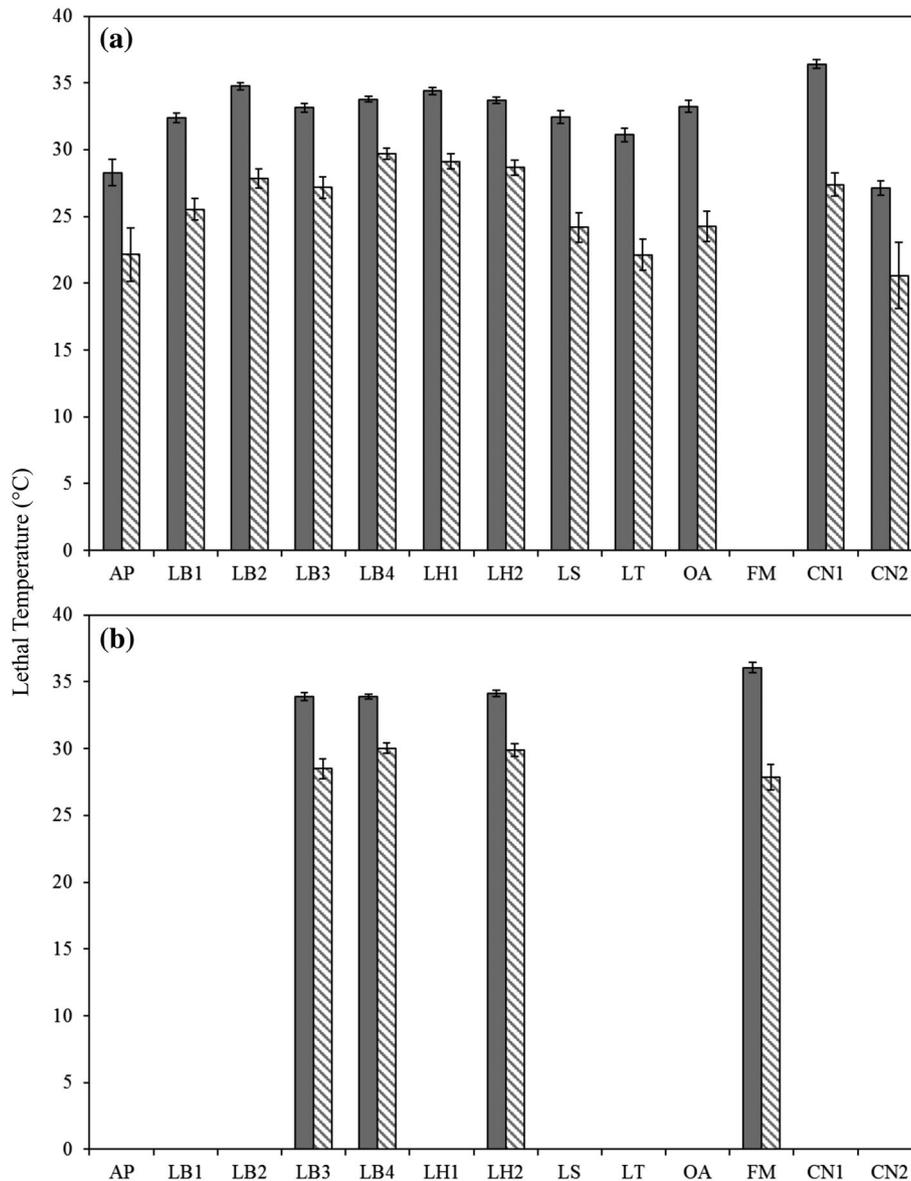


FIGURE 5 Comparison of (a) 24-h and (b) 12-h LT50 (solid bar) and LT05 (hatched bar) estimates at 27°C acclimation. Error bars denote 95% confidence intervals. Abbreviations: AP, *Amblema plicata*; FM, *Fusconaia mitchelli*; LB, *Lampsilis bracteata*; LH, *Lampsilis hydiana*; LS, *Lampsilis satura*; LT, *Lampsilis teres*; OA, *Obovaria arkansasensis*; and CN, *Cyclonaias necki*

Within-species variation in thermal tolerance has been documented for many marine and freshwater taxa (Sorte, Jones, & Miller, 2011; Urban, 1994), although it has not been well studied in unionid mussels (but see Doucet-Beaupré, Dubé, Breton, Portner, & Blier, 2010; Galbraith, Blakeslee, & Lellis, 2012). In this study, *L. bracteata* from the Llano River was, on average, 2.1°C less tolerant than the *L. bracteata* population from the San Saba River. However, the initial viability of the Llano population was low (64.4%) compared with that of the San Saba River (79.6%), and although survival data were adjusted to correct for <100% viability, reduced glochidial fitness may have affected the calculated median lethal temperature. This difference in thermal tolerance may also result from where these sites are located within these rivers (i.e. stream position) and differences in local climate and hydrology. Alternatively, this difference could be a function of evolutionary history, as there is evidence that these populations differ in genetic

structure (Kentaro Inoue, Texas A&M University, pers. comm), the morphology of their mantle lures (Howells, Randklev, & Johnson, 2011), and the timing of brooding and spawning (Seagroves, 2017).

Seasonality in water temperature is likely to play an important role in regulating mussel metabolic rates (Baker & Hornbach, 2001) and population performance (i.e. growth, survivorship, and reproduction; Doucet-Beaupré et al., 2010), and as such thermal limits should vary at different times of the year (Martin, 2016). In this study, within-species differences for glochidial LT50 and LT05 values were found based on the time of year that gravid females were collected. Specifically, for *C. necki*, both the LT50 and LT05 values were significantly different between spring (36.4°C) and summer (26.9°C). Water temperatures in the lower Guadalupe where gravid females were collected averaged 20°C in spring compared with 29°C in the summer; however, only one gravid female *C. necki* with sufficiently viable glochidia (i.e.

initial viability >70%) was collected in each season, and although control survival was similar across temperature trials, individual variability could have affected the LT50 estimate. Alternatively, this difference in thermal tolerance may also be the result of seasonal variation, at least for this species, and could stem from energetic trade-offs between survival, growth, and reproduction to maximize fitness (Cody, 1966; MacArthur & Levins, 1967). In this case, for brooding female mussels, energy normally allocated to reproduction might be tied up in offsetting the effects of elevated water temperature, resulting in reduced glochidial fitness and lower upper thermal limits during the summer months (Pörtner et al., 2006). Similarly, among marine molluscs, thermal stress can have transgenerational effects, whereby sublethal environmental factors affecting the parental generation can have adverse impacts on the survival or growth of the offspring (Kessel & Phillips, 2018).

Although upper thermal tolerances for a suite of mussel species in the south-western USA were identified, some of which are highly imperilled, there were a few shortcomings in the approach used in this study, which was adapted from other published studies and guidelines (Archambault et al., 2014; ASTM, 2006a; Pandolfo et al., 2010). First, the length of time that glochidia are exposed to thermal testing may be too long for certain species. Generally, glochidia viability following release from the female varies from hours to weeks, depending on the species (Akiyama & Iwakuma, 2007; Ingersoll et al., 2006; Zimmerman & Neves, 2002); however, for toxicity tests, a duration of 24 h is recommended (ASTM, 2006a; Cope et al., 2008). Based on the results of this study, 24 h may be too long for species belonging to the tribes Anodontini, Pleurobemini, or Quadrulini, which have been shown to have shorter glochidial longevity (Cope et al., 2008; Ingersoll et al., 2006). For example, the control viability of *F. mitchelli* was >80% at 12 h but dropped to <40% by 24 h, which precluded an accurate estimation of the 24-h median lethal temperatures. Second, species within the Pleurobemini and Quadrulini produce conglutinates, which are aggregated glochidia surrounded by a membrane that are then ingested by a fish host (Barnhart, Haag, & Roston, 2008). The viability of glochidia within a conglutinate is typically higher than the viability of free glochidia, which suggests that conglutinate membranes may confer protection (Fritts et al., 2014). For example, conglutinates of *Ptychobranthus jonesi* maintained >90% viability for 3 days and only dropped below 75% by day 10 (McLeod, Jelks, Pursifull, & Johnson, 2017). Thus, thermal tolerance testing of free glochidia for species that produce conglutinates may not be entirely accurate, indicating that thermal testing should include both conglutinates and free glochidia, not just one or the other.

4.1 | Conservation implications

It is well known that ectotherms, such as mussels, which are living close to their upper thermal limits, are more vulnerable to future environmental warming (Deutsch et al., 2008; Stillman, 2003), in part because of their inability to acclimate to rapidly changing temperature regimes. The results of this study show that several of the focal

species studied may already be experiencing temperatures near their upper thermal limits. In central Texas rivers, where most of the study sites were located, the summer maximum water temperature ranges from 30°C to 34°C, which is close to or exceeds the thermal limits of all glochidia tested in the present study, including several Texas endemics proposed for ESA listing. This result would suggest that any future increases in water temperature could be extremely challenging, which is likely to occur given that river systems within central Texas are already over-allocated, and Texas is projected to have a population increase of 73% over the next 50 years, with most of this growth occurring in central Texas (Texas Water Development Board (TWDB), 2016). Thus, future increases in population growth coupled with climate change, which is predicted to be severe for the south-western USA (2–5°C increase by the year 2100; Jiang & Yang, 2012), is likely to lead to more frequent and intense droughts and elevated stream temperatures in these systems. In fact, this may already be occurring within central Texas, as most of the mussel diversity is now located in the lower reaches of large rivers where flow remains perennial, or within tributaries where diffuse groundwater inflows maintain aquatic habitat. Thus, existing populations of endemic species in these arid and semi-arid regions will probably be further endangered as human demand for water continues to increase.

The results of this study underscore the recommendation by Olden and Naiman (2010), and others, that human society must reconcile its management of water resources to ensure the long-term viability of most aquatic ecosystems. In Texas, the response to this challenge has been a stakeholder process to identify environmental flows by coupling water quantity and, where available, water quality requirements with biological information (Opdyke, Oborny, Vaugh, & Mayes, 2014), but thermal tolerance data for mussels, and their host fish, has yet to be fully considered within this framework (but see SARA, 2017). Thus, it is likely that current environmental flow standards are not fully protective of mussels, particularly during subsistence flows when water depth and velocity are at their lowest. Thermal tolerance data from studies such as this, combined with water temperature data, could be used to identify periods of exceedance of acute LT50 or LT05 temperatures during these periods, or any other stage of flow. For the existing populations of endemic species in the arid and semi-arid regions of Texas, this approach would also mean prescribing environmental flows that protect against excessive extraction during periods of low flow. Once specific thresholds have been identified, managers could then adopt a water management programme specific to the river system in question. Flow standards have thus far only been adopted for a limited number of USGS gauges and river systems in Texas and are only applied to new water right permit applications. In effect, this would leave some river systems with little to no flow protection (e.g. San Saba River; Randklev et al., 2018). Thus, future environmental flow efforts within the state might consider developing flow standards at other USGS gauges in rivers with known endemic or threatened mussel populations. In turn, this information could provide guidance on the flows required by mussels to maintain and protect population performance (i.e. growth, survivorship, and reproduction) to ensure long-term persistence. In addition, thermal

tolerance data combined with life-history information could provide insight into population viability under various types of flow management practices (Gates et al., 2015) or as a way of evaluating the potential consequences of climate and land-use changes on mussel populations or critical habitat.

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REFERENCES

- Akiyama, Y., & Iwakuma, T. (2007). Survival of glochidial larvae of the freshwater pearl mussel, *Margaritifera laevis* (Bivalvia: Unionoida), at different temperatures: A comparison between two populations with and without recruitment. *Zoological Science*, 24, 890–893. <https://doi.org/10.2108/zsj.24.890>
- Allen, D. C., Galbraith, H. S., Vaughn, C. C., & Spooner, D. E. (2013). A tale of two rivers: Implications of water management practices for mussel biodiversity outcomes during droughts. *Ambio*, 42, 881–891. <https://doi.org/10.1007/s13280-013-0420-8>
- American Society of Testing Materials (ASTM). (2006a). Standard guide for conducting laboratory toxicity tests with freshwater mussels. E2455-06. In *Annual book of ASTM standards* (Vol. 11.06) (pp. 1393–1444). West Conshohocken, PA: American Society of Testing Materials International.
- American Society of Testing Materials (ASTM). (2006b). Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. E729-96. In *Annual book of ASTM standards* (Vol. 11.06) (pp. 79–100). West Conshohocken, PA: American Society for Testing Materials International.
- Amyot, J.-P., & Downing, J. A. (1997). Seasonal variation in vertical and horizontal movement of the freshwater bivalve *Elliptio complanata* (Mollusca: Unionidae). *Freshwater Biology*, 37, 345–354. <https://doi.org/10.1046/j.1365-2427.1997.00158.x>
- Archambault, J. M., Cope, W. G., & Kwak, T. J. (2014). Influence of sediment presence on freshwater mussel thermal tolerance. *Freshwater Science*, 33, 56–65. <https://doi.org/10.1086/674141>
- Ashworth, J. B. (2005). *Spring flow contribution to the headwaters of the Guadalupe River in western Kerr County. Report prepared for the plateau region water planning group*. Austin, Texas: LBG-Guyton Associates.
- Baker, S. M., & Hornbach, D. J. (2001). Seasonal metabolism and biochemical composition of two unionid mussels, *Actinonaias ligamentina* and *Amblema plicata*. *Journal of Molluscan Studies*, 67, 407–416. <https://doi.org/10.1093/mollus/67.4.407>
- 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>
- Bayne, B. L. (1965). Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L.). *Ophelia*, 2, 1–47. <https://doi.org/10.1080/00785326.1965.10409596>
- Bayne, B. L. (1976). *Marine mussels, their ecology and physiology*. Cambridge, UK: Cambridge University Press.
- Blum, M. D., Toomey, R. S., & Valastro, S. (1994). Fluvial response to Late Quaternary climatic and environmental change, Edwards Plateau, Texas. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 108, 1–21. [https://doi.org/10.1016/0031-0182\(94\)90019-1](https://doi.org/10.1016/0031-0182(94)90019-1)
- Blum, M. D., & Valastro, S. (1994). Late Quaternary sedimentation, lower Colorado River, Gulf Coastal Plain of Texas. *Geological Society of America Bulletin*, 106, 1002–1016. [https://doi.org/10.1130/0016-7606\(1994\)106<1002:LQSLCR>2.3.CO;2](https://doi.org/10.1130/0016-7606(1994)106<1002:LQSLCR>2.3.CO;2)
- Brune, G. (1975). *Major and historical springs of Texas. Report 189*. Austin, TX: Texas Water Development Board.
- Cody, M. L. (1966). A general theory of clutch size. *Evolution*, 20, 174–184. <https://doi.org/10.1111/j.1558-5646.1966.tb03353.x>
- Cope, W. G., Bringolf, R. B., Buchwalter, D. B., Newton, T. J., Ingersoll, C. G., Wang, N., ... Hammer, E. (2008). Differential exposure, duration, and sensitivity of unionoidan bivalve life stages to environmental contaminants. *Journal of the North American Benthological Society*, 27, 451–462. <https://doi.org/10.1899/07-094.1>
- Core Team, R. (2017). *R: A language and environment for statistical computing*. Vienna: Austria. Retrieved from <http://www.r-project.org/>
- Cummings, K. S., & Graf, D. L. (2009). Mollusca: Bivalvia. In J. H. Thorp, & A. P. Covich (Eds.), *Ecology and classification of North American freshwater invertebrates* (3rd ed.) (pp. 309–384). New York, NY: Academic Press.
- Delorme, N. J., & Sewell, M. A. (2014). Temperature and salinity: Two climate change stressors affecting early development of the New Zealand sea urchin *Evechinus chloroticus*. *Marine Biology*, 161, 1999–2009. <https://doi.org/10.1007/s00227-014-2480-0>
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 6668–6672. <https://doi.org/10.1073/pnas.0709472105>
- Diffenbaugh, N. S., Giorgi, F., & Pal, J. S. (2008). Climate change hotspots in the United States. *Geophysical Research Letters*, 35, 1–5. <https://doi.org/10.1029/2008GL035075>
- Dimock, R. V., & Wright, A. H. (1993). Sensitivity of juvenile freshwater mussels to hypoxic, thermal and acid stress. *The Journal of the Elisha Mitchell Scientific Society*, 109, 183–192.
- Doucet-Beaupré, H., Dubé, C., Breton, S., Portner, H. O., & Blier, P. U. (2010). Thermal sensitivity of metabolic enzymes in subarctic and temperate freshwater mussels (Bivalvia: Unionoida). *Journal of Thermal Biology*, 35, 11–20. <https://doi.org/10.1016/j.jtherbio.2009.10.002>
- Eybe, T., Thielen, F., Bohn, T., & Sures, B. (2015). Influence of the excitement time on the breeding success of juvenile freshwater pearl mussels (*Margaritifera margaritifera*). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 25, 21–30. <https://doi.org/10.1002/aqc.2471>
- Fritts, A. K., Barnhart, M. C., Bradley, M., Liu, N., Cope, W. G., Hammer, E., & Bringolf, R. B. (2014). Assessment of toxicity test endpoints for freshwater mussel larvae (glochidia). *Environmental Toxicology and Chemistry*, 33, 199–207. <https://doi.org/10.1002/etc.2417>
- Galbraith, H. S., Blakeslee, C. J., & Lellis, W. A. (2012). Recent thermal history influences thermal tolerance in freshwater mussel species (Bivalvia: Unionoida). *Freshwater Science*, 31, 83–92. <https://doi.org/10.1899/11-025.1>

- 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>
- Ganser, A. M., Newton, T. J., & Haro, R. J. (2013). The effects of elevated water temperature on native juvenile mussels: Implications for climate change. *Freshwater Science*, 32, 1168–1177. <https://doi.org/10.1899/12-132.1>
- Ganser, A. M., Newton, T. J., & Haro, R. J. (2015). Effects of elevated water temperature on physiological responses in adult freshwater mussels. *Freshwater Biology*, 60, 1705–1716. <https://doi.org/10.1111/fwb.12603>
- Gascho Landis, A. M., Mosley, T. L., Haag, W. R., & Stoeckel, J. A. (2012). Effects of temperature and photoperiod on lure display and glochidial release in a freshwater mussel. *Freshwater Science*, 31, 775–786. <https://doi.org/10.1899/11-082.1>
- Gates, K. K., Vaughn, C. C., & Julian, J. P. (2015). Developing environmental flow recommendations for freshwater mussels using the biological traits of species guilds. *Freshwater Biology*, 60, 620–635. <https://doi.org/10.1111/fwb.12528>
- Griffith, G., Bryce, S., Omernik, J., & Rogers, A. (2007). *Ecoregions of Texas*. Reston, VA: U.S. Geological Survey.
- Haag, W. R. (2012). *North American freshwater mussels: Natural history, ecology, and conservation*. West Nyack, NY: Cambridge University Press. <https://doi.org/10.1017/CBO9781139048217>
- Haag, W. R., & Warren, M. L. (1998). Role of ecological factors and reproductive strategies in structuring freshwater mussel communities. *Canadian Journal of Fisheries and Aquatic Sciences*, 55, 197–306. <https://doi.org/10.1139/f97-210>
- Howells, R. G., Randklev, C. R., & Johnson, M. S. (2011). Mantle flap variation in Texas fatmucket (*Lampsilis bracteata*). *Ellipsaria*, 13, 14–16.
- Ingersoll, C. G., Kernaghan, N. J., Gross, T. S., Bishop, C. D., Wang, N., & Roberts, A. (2006). Laboratory toxicity testing with freshwater mussels. In *Freshwater bivalve ecotoxicology* (pp. 95–134). Pensacola, FL: Society of Environmental Toxicology and Chemistry.
- Intergovernmental Panel on Climate Change (IPCC). (2013). In T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, et al. (Eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK and New York, NY, USA: Cambridge University Press.
- Jiang, X., & Yang, Z. Z. L. (2012). Projected changes of temperature and precipitation in Texas from downscaled global climate models. *Climate Research*, 53, 229–244. <https://doi.org/10.3354/cr01093>
- Kessel, G. M., & Phillips, N. E. (2018). Global change scenarios trigger carry-over effects across life stages and generations of the intertidal limpet, *Siphonaria australis*. *PLoS ONE*, 13, 1–15. <https://doi.org/10.1371/journal.pone.0194645>
- MacArthur, R., & Levins, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist*, 101, 377–385. <https://doi.org/10.1086/282505>
- Martin, K. R. C. (2016). Upper thermal limits of freshwater mussels (Bivalvia, Unionoida) in ramped temperature exposures (MS thesis). Springfield, MO: Missouri State University.
- Marwaha, J., Jensen, K. H., Jakobsen, P. J., & Geist, J. (2017). Duration of the parasitic phase determines subsequent performance in juvenile freshwater pearl mussels (*Margaritifera margaritifera*). *Ecology and Evolution*, 7, 1375–1383. <https://doi.org/10.1002/ece3.2740>
- McLeod, J. M., Jelks, H. L., Pursifull, S., & Johnson, N. A. (2017). Characterizing the early life history of an imperiled freshwater mussel (*Ptychobranthus jonesi*) with host-fish determination and fecundity estimation. *Freshwater Science*, 36, 338–350. <https://doi.org/10.1086/692096>
- Milly, P. C. D., Dunne, K. A., & Vecchia, A. V. (2005). Global pattern of trends in streamflow and water availability in a changing climate. *Nature*, 438, 347–350. <https://doi.org/10.1038/nature04312>
- NatureServe (2017). *NatureServe Explorer: An online encyclopedia of life [web application]*. Version 7.1. Arlington, Virginia: NatureServe. Retrieved April 19, 2018, from <http://explorer.natureserve.org>
- Olden, J. D., & Naiman, R. J. (2010). Incorporating thermal regimes into environmental flows assessments: Modifying dam operations to restore freshwater ecosystem integrity. *Freshwater Biology*, 55, 86–107. <https://doi.org/10.1111/j.1365-2427.2009.02179.x>
- Opdyke, D. R., Oborny, E. L., Vaughn, S. K., & Mayes, K. B. (2014). Texas environmental flow standards and the hydrology-based environmental flow regime methodology. *Hydrological Sciences Journal*, 59, 820–830. <https://doi.org/10.1080/02626667.2014.892600>
- Pandolfo, T. J., Cope, W. G., & Arellano, C. (2009). Heart rate as a sublethal indicator of thermal stress in juvenile freshwater mussels. *Comparative Biochemistry and Physiology, Part A*, 154, 347–352. <https://doi.org/10.1016/j.cbpa.2009.07.001>
- Pandolfo, T. J., Cope, W. G., Arellano, C., Bringolf, R. B., Barnhart, M. C., & Hammer, E. (2010). Upper thermal tolerances of early life stages of freshwater mussels. *Journal of the North American Benthological Society*, 29, 959–969. <https://doi.org/10.1899/09-128.1>
- Pandolfo, T. J., Kwak, T. J., & Cope, W. G. (2012). Thermal tolerances of freshwater mussels and their host fishes: Species interactions in a changing climate. *Walkerana*, 15, 69–82. <https://doi.org/10.31931/fmbc.v15i1.2012.69-82>
- Perkin, J. S., & Bonner, T. H. (2011). Long-term changes in flow regime and fish assemblage composition in the Guadalupe and San Marcos Rivers of Texas. *River Research and Applications*, 27, 566–579. <https://doi.org/10.1002/rra.1373>
- Phillips, J. D. (2012). Geomorphic Responses to Changes in Flow Regimes in Texas Rivers. Project Report for the Texas Water Development Board and Texas Instream Flow Program, TWDB Contract Number, 1104831147.
- Pörtner, H. O., Bennett, A. F., Bozinovic, F., Clarke, A., Lardies, M. A., Lucassen, M., ... Stillman, J. H. (2006). Trade-offs in thermal adaptation: The need for a molecular to ecological integration. *Physiological and Biochemical Zoology*, 79, 295–313. <https://doi.org/10.1086/499986>
- Randklev, C. R., Tsakris, E. T., Johnson, M. S., Popejoy, T., Hart, M. A., Khan, J., ... Robertson, C. R. (2018). The effect of dewatering on freshwater mussel (Unionidae) community structure and the implications for conservation and water policy: A case study from a spring-fed stream in the southwestern United States. *Global Ecology and Conservation*, 16, 1–15. <https://doi.org/10.1016/j.gecco.2018.e00456>
- Ritz, C., Baty, F., Streibig, J. C., & Gerhard, D. (2015). Dose-response analysis using R. *PLoS ONE*, 10, 1–13. <https://doi.org/10.1371/journal.pone.0146021>
- Roberts, A. D., & Barnhart, M. C. (1999). Effects of temperature, pH, and CO₂ on transformation of the glochidia of *Anodonta suborbiculata* on fish hosts and in vitro. *Journal of the North American Benthological Society*, 18, 477–487. <https://doi.org/10.2307/1468380>
- Rodland, D. L., Schone, B. R., Baier, S., Zhang, Z., Dreyer, W., & Page, N. A. (2008). Changes in gape frequency, siphon activity and thermal response in the freshwater bivalves *Anodonta cygnea* and *Margaritifera falcata*. *Journal of Molluscan Studies*, 75, 51–57. <https://doi.org/10.1093/mollus/eyn038>
- San Antonio River Authority (SARA). (2017). Instream flow study of the lower San Antonio River and lower Cibolo Creek. Draft report prepared for lower San Antonio River Sub-Basin workgroup. TX: San Antonio.

- Schneider, L. D., Nilsson, P. A., & Österling, E. M. (2018). Evaluating temperature- and host-dependent reproduction in the parasitic freshwater mussel *Unio crassus*. *Hydrobiologia*, 810, 283–293. <https://doi.org/10.1007/s10750-017-3217-0>
- Seagroves, L. A. (2017). Reproductive ecology of *Lampsilis bracteata* (Bivalvia: Unionidae) (Master's thesis). San Marcos, TX: Texas State University.
- Sorte, C. J. B., Jones, S. J., & Miller, L. P. (2011). Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. *Journal of Experimental Marine Biology and Ecology*, 400, 209–217. <https://doi.org/10.1016/j.jembe.2011.02.009>
- Spooner, D. E., & Vaughn, C. C. (2008). A trait-based approach to species' roles in stream ecosystems: Climate change, community structure, and material cycling. *Oecologia*, 158, 307–317. <https://doi.org/10.1007/s00442-008-1132-9>
- Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science*, 301, 65. <https://doi.org/10.1126/science.1083073>
- Strayer, D. L., Downing, J. A., Haag, W. R., King, T. L., Layzer, J. B., Newton, T. J., & Nichols, S. J. (2004). Changing perspectives on pearly mussels, North American's most imperiled animals. *BioScience*, 54, 429–439. [https://doi.org/10.1641/0006-3568\(2004\)054\[0429:CPOPMN\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2004)054[0429:CPOPMN]2.0.CO;2)
- Surface Water Quality Monitoring (SWQM). (2017). Water quality data. Retrieved April 17, 2018, from <https://www80.tceq.texas.gov/SwqmisPublic/public/default.htm>
- Taeubert, J.-E., El-Nobi, G., & Geist, J. (2014). Effects of water temperature on the larval parasitic stage of the thick-shelled river mussel (*Unio crassus*). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 24, 231–237. <https://doi.org/10.1002/aqc.2385>
- Texas Parks and Wildlife Department (TPWD). (2010). *Threatened and endangered nongame species. Texas register 35. Chapter 65. Wildlife subchapter G. 31 TAC §65.175. Adopted rules.* Austin, TX: Texas Secretary of State.
- Texas Water Development Board (TWDB). (2016). *2017 State Water Plan.* Austin, TX. Retrieved from <https://www.twdb.texas.gov/waterplanning/swp/2017/index.asp>
- Urban, H. J. (1994). Upper temperature tolerance of 10 bivalve species off Peru and Chile related to El Niño. *Marine Ecology Progress Series*, 107, 139–146. <https://doi.org/10.3354/meps107139>
- US Fish and Wildlife Service (USFWS). (2016). Species status assessment report for the Texas hornshell (*Popenaias popeii*), Version 1.0. Albuquerque, NM.
- US Fish and Wildlife Service (USFWS). (2009). Endangered and threatened wildlife and plants: 90-day finding on petitions to list nine species of mussels from Texas as threatened or endangered with critical habitat. *Federal Register*, 74, 66260–66271.
- US Fish and Wildlife Service (USFWS). (2011). Endangered and threatened wildlife and plants; 12-month finding on a petition to list Texas fatmucket, golden orb, smooth pimpleback, Texas pimpleback, and Texas fawnsfoot as threatened or endangered. *Federal Register*, 76, 62166–62212.
- Vannote, R. L., & Sweeney, B. W. (1980). Geographic analysis of thermal equilibria: A conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. *The American Naturalist*, 115, 667–695. <https://doi.org/10.1086/283591>
- Vaughn, C. C., & Hakenkamp, C. C. (2001). The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology*, 46, 1431–1446. <https://doi.org/10.1046/j.1365-2427.2001.00771.x>
- Vaughn, C. C., Nichols, S. J., & Spooner, D. E. (2008). Community and foodweb ecology of freshwater mussels. *Journal of the North American Benthological Society*, 27, 409–423. <https://doi.org/10.1899/07-058.1>
- Wang, N., Augspurger, T., Barnhart, M. C., Bidwell, J. R., Cope, W. G., Dwyer, F. J., ... Whites, D. W. (2007). Intra- and interlaboratory variability in acute toxicity tests with glochidia and juveniles of freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry/SETAC*, 26, 2029–2035. <https://doi.org/10.1897/06-520R.1>
- Ward, J. V., & Stanford, J. A. (1982). Thermal responses in the evolutionary ecology of aquatic insects. *Annual Review of Entomology*, 27, 97–117. <https://doi.org/10.1146/annurev.en.27.010182.000525>
- Watters, G. T., & O'Dee, S. H. (2000). Glochidial release as a function of water temperature: Beyond bradycticty and tachycticty. *Proceedings of the Conservation, Captive Care, and Propagation of Freshwater Mussels Symposium*, 1998, 135–140.
- Wheeler, M. W., Park, R. M., & Bailer, A. J. (2006). Comparing median lethal concentration values using confidence interval overlap or ratio tests. *Environmental Toxicology and Chemistry*, 25, 1441–1444. <https://doi.org/10.1897/05-320R.1>
- Williams, J. D., Bogan, A. E., Butler, R. S., Cummings, K. S., Garner, J. T., Harris, J. L., ... Watters, G. T. (2017). A revised list of the freshwater mussels (Mollusca: Bivalvia: Unionida) of the United States and Canada. *Freshwater Mollusk Biology and Conservation*, 20, 33–58. <https://doi.org/10.31931/fmbc.v20i2.2017.33-58>
- Williams, J. D., Bogan, A. E., & Garner, J. T. (2008). *The freshwater mussels of Alabama and the Mobile Basin of Georgia, Mississippi, and Tennessee.* Tuscaloosa, AL: University of Alabama Press.
- 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](https://doi.org/10.1577/1548-8446(1993)018<0006:CSOFMO>2.0.CO;2)
- Wolaver, B. D., Cook, C. E., Scanlon, B. R., & Young, M. H. (2012). A hydrologic-characterization approach for Texas aquatic-species studies. *Gulf Coast Association of Geological Societies Transactions*, 62, 645–651.
- Wolaver, B. D., Cook, C. E., Sunding, D. L., Hamilton, S. F., Scanlon, B. R., Young, M. H., ... Reedy, R. C. (2014). Potential economic impacts of environmental flows following a possible listing of endangered Texas freshwater mussels. *Journal of the American Water Resources Association*, 50, 1081–1101. <https://doi.org/10.1111/jawr.12171>
- Zale, A. V., & Neves, R. J. (1982). Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. *Canadian Journal of Zoology*, 60, 2535–2542. <https://doi.org/10.1139/z82-325>
- 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.
- Zippay, M. L., & Hofmann, G. E. (2010). Physiological tolerances across latitudes: Thermal sensitivity of larval marine snails (*Nucella* spp.). *Marine Biology*, 157, 707–714. <https://doi.org/10.1007/s00227-009-1354-3>

SUPPORTING INFORMATION

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

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