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Linking flow and upper thermal limits of freshwater mussels to inform environmental flow benchmarks

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Abstract

1. Freshwater ecosystems are experiencing shifts in the natural range and variation of water temperatures due to anthropogenic activity, and these shifts can negatively affect survival, growth, and reproduction of aquatic species. Among the groups most affected are freshwater mussels of the family Unionidae. Knowledge of sublethal and lethal effects on mussels from changes in water temperature are largely unknown, especially for species from arid and semi-arid regions such as the south-western U.S.A. This limits the ability to assess, forecast, and adaptively manage this threat for those species and to understand how temperature influences population performance and community structure.
2. To determine the effects of elevated water temperature on mussels from the south-western U.S.A., we evaluated the upper thermal tolerances of adults of three species (*Amblema plicata*, *Cyclonaias necki*, and *Fusconaia mitchelli*) from the Guadalupe River. Mussels were acclimated to 27°C and then tested across a range of experimental temperatures (30–39°C) in standard acute (96-hr) and chronic (10-day) laboratory tests. The acute and chronic thresholds identified in thermal tolerance testing were then related to in situ water temperature and flows using a uniform continuous above-threshold analysis, which evaluates the duration and frequency of continuous events above a specified temperature threshold.
3. Median lethal temperature in 96-hr tests averaged 36.4°C and ranged from 33.7 to 37.5°C, while the chronic 10-day tests averaged 35.9°C and ranged from 32.4 to 37.5°C. Thermal tolerances of *F. mitchelli* were significantly lower than both *A. plicata* and *C. necki*, and the uniform continuous above-threshold analysis showed that temperature affecting 5% of the population thresholds were exceeded for *F. mitchelli* in the Guadalupe River at both acute (96-hr) and chronic (10-day) values (30.5 and 28.4°C, respectively).
4. Findings from this study indicate that freshwater mussels from the arid and semi-arid regions of the south-west U.S.A. are already at risk from rising environmental temperatures and altered hydrology. However, by incorporating laboratory thermal tolerance estimates with in situ temperature and discharge data, we provide a range of hydrologic thresholds to inform environmental flow recommendations and potentially mitigate thermal stress occurring during periods of low flow. In

addition, this method can be readily adapted to other arid regions to guide flow recommendations or assess whether flow standards are sufficient to protect freshwater mussel populations during severe droughts and low flow periods.

KEYWORDS
conservation, low flow, thermal tolerance, Unionidae, water management

1 | INTRODUCTION

Water temperature is an important abiotic driver of aquatic ecosystems and directly impacts metabolic rates, physiology, and life-history traits of ectotherms (Vannote & Sweeney, 1980). Spatial and temporal patterns in water temperature are tightly linked to flow patterns and exert a strong influence on the evolution, distribution, and ecology of aquatic organisms (Olden & Naiman, 2010). Freshwater ecosystems are experiencing shifts in the natural range and variation of water temperatures due to anthropogenic activity such as climate change, water management, riparian clearing, and thermal effluents (Caissie, 2006). These shifts can negatively affect biological endpoints such as growth, survivorship, and reproduction, which, in turn, can lead to population declines and eventually changes to species abundance and distributions, which if severe enough can result in extirpations or extinctions (Brown, Gillooly, Allen, Savage, & West, 2004; Helmuth et al., 2002).

Because water temperature is important to the ecological integrity of riverine systems and influences metabolic, physiological, and life-history traits of aquatic species, understanding the short- and long-term effects of shifts in water temperatures is critical for the successful conservation and management of riverine ecosystems (Olden & Naiman, 2010; Somero, 2010). However, knowledge of sublethal and lethal effects from changes in water temperature are unknown for several aquatic species, which limits the ability to assess, forecast, and adaptively manage this threat. Thus, this information is sorely needed, especially to prepare for a rapidly changing climate and increased human demand for water, which is expected to be severe in arid and semi-arid regions such as Texas, located within the south-western U.S.A.

Freshwater mussels are one of the most imperiled aquatic faunas, due, in part, to changes in water temperatures (Galbraith, Blakeslee, & Lellis, 2012; Pandolfo et al., 2010). As ectotherms, the influence of thermal stress on freshwater mussels (Bivalvia: Unionidae) is pervasive, and mussels are constrained in their ability to respond to changes in water temperature due to reliance on host fish for larval dispersal (Haag & Warren, 1998) and limited mobility as adults (Amyot & Downing, 1997). Elevated water temperature can directly affect mussels by causing mortality or result in sublethal effects such as altering oxygen consumption and metabolic demands (Pandolfo, Cope, & Arellano, 2009; Rodland et al., 2008; Spooner & Vaughn, 2008), which, in turn, can stimulate food web productivity in the short term, but in the long term can lead to diminished ecosystem services that may take decades to recover (e.g. nutrient cycling; DuBose, Atkinson, Vaughn, & Golladay, 2019; Howard & Cuffey, 2006). Elevated water temperature can also negatively impact growth and reproduction such as gamete development, glochidial release, and host-fish interactions (Baker & Hornbach, 2001; Galbraith & Vaughn, 2009; Gascho Landis, Mosley, Haag, & Stoeckel, 2012; Watters & O'Dee, 2000), which over time can lead to population declines.

Despite the importance of water temperature for mussels, quantitative information on lethal temperatures (LT, e.g. LT50—temperature affecting 50% of the population) is limited to only 21 of the roughly 300 mussel species in North America (Archambault, Cope, & Kwak, 2014; Dimock & Wright, 1993; Ganser, Newton, & Haro, 2013; Khan et al., 2019; Martin, 2016; Pandolfo et al., 2010). The majority of the species that have been tested are from the midwest or south-eastern U.S.A. where flow and water temperature regimes differ from those in arid and semi-arid regions. Because of this, the transferability of those results to other species, or populations of the same species but in different regions, is unknown. This concern is likely to be well supported given that recent studies have shown that mussel sensitivity to water temperature can vary widely across species (Pandolfo et al., 2010; Spooner & Vaughn, 2008).

To address the risk of elevated temperatures on mussel species from arid and semi-arid regions and to inform management and conservation efforts for mussels within Texas, the overall goal of our study was to estimate the upper thermal limits of three mussel species, two of which are species of high conservation concern, representing three tribes of the Unionidae family (Williams et al., 2017), and therefore three potentially different evolutionary and physiological adaptions to thermal stress, from the Guadalupe River of central Texas. The specific objectives of our study were to: (1) assess the acute and chronic effects of a range of water temperatures on adult survival; (2) use the resulting data along with water temperature data from our study site to determine whether thermal stress could be a threat; and (3) discuss how water temperatures could be contributing to the decline of mussel species in Texas along with management implications and potential solutions.

2 | METHODS

2.1 | Study area

Our study was conducted in the lower Guadalupe River within the floodplains and low terraces of the Western Gulf Coastal Plain

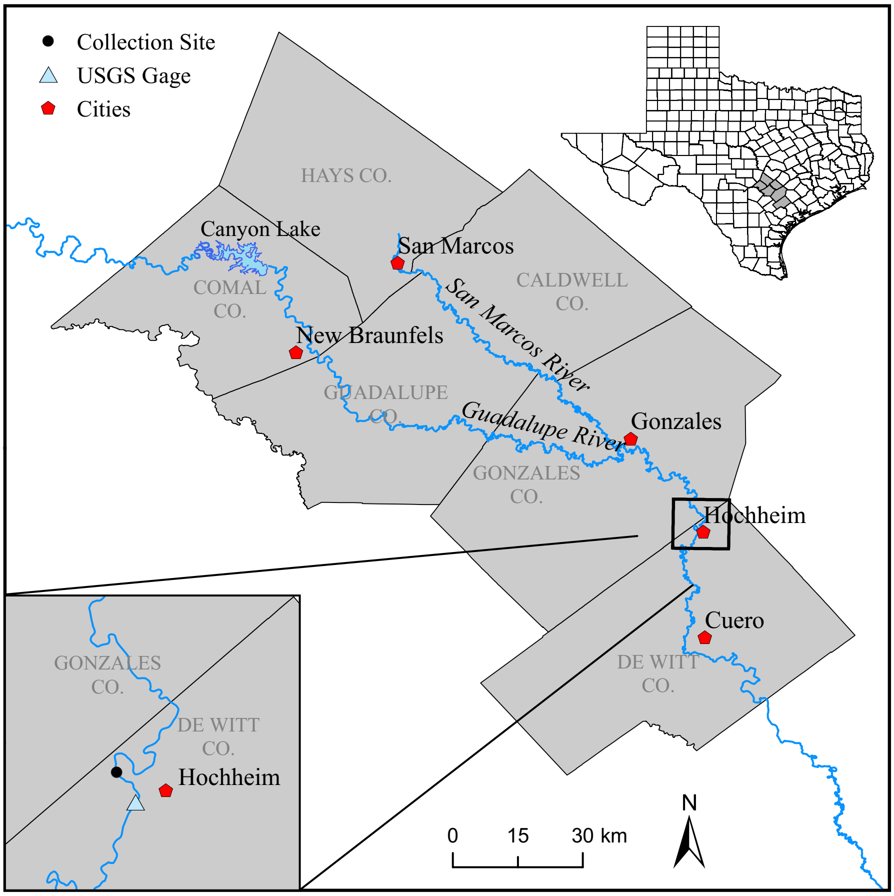


FIGURE 1 Map of study site, temperature logger location, and U.S. Geological Survey gaging station

ecoregion (Griffith, Bryce, Omernik, & Rogers, 2007; Figure 1). This portion of the Guadalupe River can experience high rates of precipation, resulting in short duration, high-magnitude flow events interspersed with longer periods of low flow (Perkin & Bonner, 2011). Baseflows are sourced from a combination of spring-fed tributaries, local groundwater inputs, upstream dam releases, and surface runoff. The flow regime in the lower Guadalupe River is modified by seven mainstem impoundments, including Canyon Lake reservoir, a deep storage bottom release reservoir (Perkin & Bonner, 2011). As a consequence, the natural flow and thermal regimes in this reach have been altered with flows becoming more homogenised due to dam releases from Canyon Lake, which account for *c*. 20% of river flow (Phillips, 2012). The remaining impoundments in the Guadalupe are low-head and run-of-river dams that have an effect on local water temperature, which can reach up to 38°C during summer months (Phillips, 2012; Young, Hannan, & Tatum, 1972). Average water temperatures in the lower Guadalupe are approximately 30°C in July and 13°C in January (SWQM, 2019).

2.2 | Study animals

We examined thermal tolerances for adults of three species representing three tribes (Amblemini, Pleurobemini, and Quadrulini) in the family Unionidae (Williams et al., 2017): *Amblema plicata*, *Cyclonaias necki*, and *Fusconaia mitchelli* (Table 1). *Amblema plicata* is considered stable throughout its range (NatureServe, 2017) and has a broad distribution in North America from Florida west to Texas and north into the Mississippi River drainage (Mulvey et al., 1997). Previous studies in Oklahoma have found *A. plicata* to be thermally tolerant relative to other co-occurring species (Spooner & Vaughn, 2008). *Cyclonaias necki* and *F. mitchelli* are Texas endemics with narrow distributions. *Fusconaia mitchelli* is currently state-listed and being considered for listing under the Endangered Species Act (TPWD, 2010; USFWS, 2009), while *C. necki* was recently separated from *Cyclonaias petrina* (Burlakova, Karatayev, Froufe, Bogan, & Lopes-Lima, 2018) and is currently being proposed for state-listing. *Cyclonaias necki* is known historically from the Guadalupe River drainage of central Texas (Burlakova et al., 2018; Johnson et al., 2018), while *F. mitchelli* historically occurred in the Brazos, Colorado, and Guadalupe River drainages (Pfeiffer, Johnson, Randklev, Howells, & Williams, 2016).

Adult mussels were collected from the lower Guadalupe River (near Hochheim, Texas) in September–December 2016 and 2017, except for *F. mitchelli*, which were collected in April 2017 (Table 1). *Amblema plicata* were collected twice: once during a winter-acclimated, non-reproductive period in December and a second collection following the end of their reproductive season in September. Water temperatures at the time of collection averaged 22.3 ± 2.5°C (mean ± *SE*) across all collection events. Following collection, adult mussels were transported to the Texas A&M AgriLife Extension and Research Center in Dallas, Texas in insulated coolers. Upon arrival in the laboratory, we held mussels at a holding temperature

TABLE 1 Summary of test species (*Amblema plicata*, *Cyclonaias necki*, and *Fusconaia mitchelli*) and water quality parameters during 10-day trials

Species	Acclimation temperatures	Collection date	*n* per chamber	Total *n*	Length (mm)	Wet weight (g)	Dissolved O_2 (mg/L)	Ammonia (mg/L)	pH	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)
A. plicata	23, 27, 30°C	December 2016	5	270	73.6 ± 0.5	81.4 ± 1.6	6.8 ± 0.01	0.02 ± 0.02	8.1 ± 0.02	246 ± 2.1	196 ± 0.9
A. plicata	27°C	September 2017	4	60	73.4 ± 1.0	80.1 ± 2.9	7.6 ± 0.01	0.04 ± 0.01	8.2 ± 0.01	257 ± 1.8	206 ± 0.7
C. necki	27°C	October 2017	4	60	48.2 ± 2.9	34.0 ± 1.1	7.7 ± 0.01	0.02 ± 0.01	8.0 ± 0.01	236 ± 1.2	191 ± 0.6
F. mitchelli	27°C	April 2017	4	60	48.3 ± 0.7	22.2 ± 0.8	7.7 ± 0.01	0.02 ± 0.01	8.1 ± 0.01	252 ± 0.7	197 ± 1.6

Note: Acclimation temperatures are the acclimation treatments under which each species was tested. Collection date is the date mussels were collected from the field. *n* per chamber is the number of mussels in each chamber in each control or experimental temperature replicate. Total *n* is the total number of mussels in all treatments. Length and wet weight are mean ± *SE* for all organisms in each thermal trial. Water quality parameters (dissolved oxygen, ammonia, hardness, alkalinity, and pH) are averaged (mean ± *SE*) across all test chambers.

of 20 ± 1°C. Mussels were fed daily with a mixture of commercial algae approximately equivalent to 3% of mean shell-free dry weight (Shellfish diet 1800 and Nanno 3600, Reed Mariculture).

2.3 | Water temperature and flow measurements

To evaluate whether water temperatures were in exceedance of estimated upper thermal tolerances, water temperatures and depth at the collection site were monitored using Hobo level loggers (Onset Computer Corporation). Water level loggers were housed in a perforated PVC pipe secured to a rebar stake, which was then anchored to the river bottom. The water level loggers were deployed from April 2016 to October 2018 and recorded at 15-min intervals to a 0.10°C resolution.

We related temperature data from our site to discharge data from the nearest U.S. Geological Survey (USGS) gaging station to ensure that the hydrologic conditions measured at the gage station reflect the conditions at our monitoring site (Larned, Arscott, Schmidt, & Diettrich, 2010). The USGS gage is located approximately 1.6 river km downstream (08174700 near Hochheim, Texas) of our site and became operational in June 2016, and while two additional gages have longer periods of record (>20 years), they are located over 50 river km from the collection site and are separated by inflowing major tributaries to the mainstem Guadalupe River. Thus, for the purposes of analysis, we limit our relationship with flow and temperature data to the Hochheim gaging station.

2.4 | Experimental design

Upper thermal limits of test organisms were determined using the LT method where test individuals are held at a specific acclimation temperature, followed by instantaneous transfer into one of a series of constant test temperatures (Beitinger, Bennett, & McCauley, 2000). We tested the effect of acclimation on thermal tolerance by randomly assigning winter-collected *A. plicata* to one of four treatment groups: a control held at 20°C (ASTM, 2006), or a 23, 27, or 30°C acclimation treatment. All other species and summer-collected *A. plicata* were assigned to one of two treatment groups: a control held at 20°C (ASTM, 2006), or a 27°C acclimation treatment (see below for rationale). Specimens were acclimated to the assigned temperature at a rate of <3°C/day, and, once the assigned temperature was reached, were held at the acclimation temperature for 96-hr. During the acclimation period, mussels were held in recirculating aquaria in refrigerated incubators maintained to ±1°C of the assigned temperature (23, 27, or 30°C treatments, or 20°C control). Following acclimation, mussels were randomly assigned to one of five different test temperatures (Figure 2) based on acclimation temperature. The acclimation and test temperatures were informed by: (1) the breadth of temperatures encountered by mussels in the Guadalupe River during the

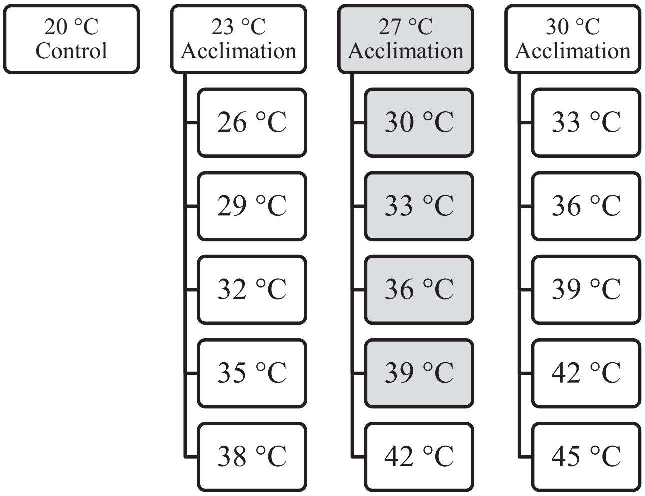


FIGURE 2 Experimental design showing acclimation and experimental temperatures following Pandolfo et al. (2010). Mussels were acclimated to one of three acclimation temperatures for 96-hr before transfer into a constant experimental temperature for 240-hr. Winter-collected *Amblema plicata* were tested at all acclimation treatments and test temperatures. Summer-collected *A. plicata*, *Fusconaia mitchelli*, and *Cyclonaias necki* were tested only at one acclimation temperature and a subset of test temperatures (grey shaded boxes). All experimental temperatures were assessed alongside a non-acclimated 20°C control

warmest months (range: 24.4–33.4°C, based on 10 years [2007–2016] of Texas Commission of Environmental Quality data from the Surface Water Quality Monitoring Information System); (2) the inclusion of higher temperatures where mortality was likely to be sufficient for the calculation of LT values; and (3) designs of previously published studies (Archambault et al., 2014; Pandolfo et al., 2010).

Following acclimation, mussels were placed in fibreglass tanks (91 × 61 × 46 cm) each partitioned into three watertight chambers isolated by 0.56-cm acrylic sheets. The chambers within each tank functioned independently of one another with separate water supplies and temperature controllers; thus, each tank represented one test temperature replicated three times. Experimental chambers were filled with *c*. 38 L reconstituted hard water, which was maintained at a given experimental temperature with a 300-W titanium heater (Finnex) using a temperature controller (Aqua Logic). Within each chamber, individuals were placed in separate 946-ml food storage containers fitted with 6-mm plastic netting to allow water circulation ($n = 5$ per chamber for winter-collected *A. plicata* and $n = 4$ for all others; Table 1). Chambers were aerated with air stones to ensure that the saturation of dissolved oxygen remained above 6 mg/L. Water temperature and dissolved oxygen were monitored daily with a YSI ProODO (YSI Inc.). Mussels were exposed to experimental conditions for 10 days (240 hr), with 50% water renewal at 96 and 168 hr, including the control. Mussel survival was assessed at 3-hr intervals for the first 24 hr and at 12-hr intervals for the remainder of the experiment. Mussels showing gaped behaviour or those unresponsive to gentle probing to elicit foot retraction or valve closure were considered moribund.

Our rationale for using two different acclimation treatment regimens was to determine if acclimation temperature influences thermal tolerance. Analyses of upper thermal limits across the three acclimation treatments for winter-collected *A. plicata* showed no significant differences. Thus, subsequent trials for summer-collected *A. plicata*, *F. mitchelli*, and *C. necki* included a single acclimation treatment (27°C), smaller sample sizes ($n = 4$ individuals per temperature treatment), and a reduced series of experimental temperatures (four temperatures ranging from 30 to 39°C).

2.5 | Statistical analyses

Lethal temperatures resulting in 50 and 5% mortality (LT50 and LT05, respectively) and their 95% confidence intervals (CIs) were determined by fitting two-parameter logistic regression curves to survival data. We calculated LT50 and LT05 estimates based on observed mortality at 24, 48, 96, and 240-hr (10-day). Statistical comparisons of LT50 and LT05 values across and within species were conducted using the confidence interval ratio test (Wheeler, Park, & Bailer, 2006). This method compares the ratio of two LT50s (or any other ratio of lethality by temperature, i.e. LT05) with one, or the log(LT50 ratio) with zero. A 95% CI is constructed for the ratio based on the variance of each LT50 estimate, 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 the R program (R Core Team, 2017).

To evaluate whether water temperatures in the lower Guadalupe exceeded estimated upper thermal limits, we performed a frequency and duration analysis following methods presented in Castelli, Parasiewicz, and Rogers (2012) using our LT50 and LT05 estimates, in situ water temperature data, and discharge data from the associated USGS gaging station. This approach, known as the uniform continuous above-threshold (UCAT) method evaluates the duration and frequency of continuous events in which water temperature is higher than a threshold value (e.g. LT50 and LT05 values) by plotting cumulative temperature–duration frequency curves. To create the UCAT plots, a time series of temperature for the bioperiod of interest (e.g. summer for our study) is obtained for a given stream, and temperature events are identified. A temperature event is defined by a continuous duration above a selected temperature threshold (e.g. LT50 or LT05). Each separate exceedance duration is summed and divided by the total duration of the period of interest. For example, if 3 events exceed the threshold for 10 days each, this equals 30 total days. Those 30 days are then divided by the length of the bioperiod (e.g. 90-day summer period), giving a frequency of 33%. The frequencies are summed and then plotted on the x-axis of the UCAT graph as a cumulative frequency, while the duration of events (e.g. 10 days) is plotted on the y-axis (Castelli et al., 2012).

In our study, we converted three years (April 2016–October 2018) of sub-daily (every 15 min) water temperature data to daily mean data and assessed two bioperiods that are likely to be important for survival and reproduction in the face of thermal stress: a summer period (May–September), which tests overall physiological tolerance, and a reproductive period (March–July), when our focal species are actively brooding (Dudding et al., 2020). From these data, we identified the cumulative duration of continuous events that were above estimated LT50 and LT05 values determined from the acute (96-hr) and chronic (10-day) trials. The resulting data were then plotted by calculating the cumulative frequency of the proportion of events of the same duration that exceeded a given threshold on the *x*-axis and the duration of a continuous event on the *y*-axis.

Using the *segmented* package in R, a three-segment, piecewise linear regression was fitted to our plotted UCAT values to identify two inflection points indicating changes in the frequency of continuous durations. We used the two breakpoints to categorise events based on duration as *typical* (shortest duration), *persistent*, and *catastrophic* (longest duration) with respect to acute (96-hr) and chronic (10-day) LT05 and LT50 thermal thresholds. We also calculated associated daily flow statistics for each persistent and catastrophic event using discharge data from the Hochheim USGS gage.

3 | RESULTS

3.1 | Lethal temperature exposures

The 24-, 48-, and 96-hr and 10-day LT50 and LT05 values and their 95% confidences limits for all species are summarised in Table 2. Water quality data were within ranges recommended by ASTM (2006) and were similar among temperature treatments and between tests (see Table 1), and control survival was 100% across all trials on day 10. Acute 96-hr LT50s ranged from 33.7 to 37.5°C with a mean of 36.4 ± 1.4°C (LT50 ± 95% CI), while acute LT05s ranged from 30.5 to 37.2°C with a mean of 35.4 ± 2.5°C. Chronic 10-day LT50s ranged from 32.4 to 37.5°C with a mean of 35.9 ± 1.8°C, while LT05s ranged from 28.4 to 37.2°C with a mean of 34.8 ± 3.2°C, respectively.

Of the 3 species tested, *Fusconaia mitchelli* had significantly lower chronic (10-day) LT50 and LT05 as well as acute (96-hr) LT05 estimates than *A. plicata* or *C. necki* (Tables 3 and 4; Figure 3). No significant differences were detected between *A. plicata* or *C. necki* at either acute or chronic durations. Additionally, acute and chronic LT50s and LT05s for *F. mitchelli* declined with time and were lower on day 10. For *A. plicata*, LT50 and LT05s were not significantly different between winter- or summer-collected individuals, nor were they significant between acclimation treatments (i.e. 23, 27, or 30°C). For *C. necki*, acute and chronic LT50s and LT05s were also not significantly different (Tables 3 and 4). For *A. plicata* and *C. necki*, very little mortality was observed at 36°C and no mortality occurred in the 30 or 33°C experimental temperature treatments. In contrast, for *F. mitchelli* the 36°C treatment resulted in 100% mortality within 48 hr, and by day 10 low to moderate mortality (25 and 42%, respectively) had occurred for the 30 and 33°C treatments. The mean difference between LT50 and LT05 values within species was 0.9°C and ranged from 0.2 to 3.9°C.

TABLE 2 Lethal temperatures for 50% (LT50) and 5% (LT05) and their associated 95% confidence intervals (where given by logistic regression) for all test species (winter- and summer-collected *Amblema plicata*, *Cyclonaias necki*, and *Fusconaia mitchelli*) and acclimation treatments (23, 27, and 30°C) at 24, 48, 96, and 10-day (240-hr) time intervals

		LT50				LT05			
Species	Acclimation temperature	24-hr	48-hr	96-hr	10-d	24-hr	48-hr	96-hr	10-d
Winter *A. plicata*	23	36.5 (35.0–38.0)[a]	36.5 (35.0–38.0)[a]	36.5 (35.0–38.0)[a]	36.5 (35.0–38.0)[a]	36.1 (35.0–38.0)[a]	36.1 (35.0–38.0)[a]	36.1 (35.0–38.0)[a]	36.1 (35.0–38.0)[a]
	27	37.5 (36.0–39.0)[a]	37.5 (36.0–39.0)[a]	36.6 (33.6–39.7)	36.3 (34.5–38.0)	37.2 (36.0–39.0)[a]	37.2 (36.0–39.0)[a]	35.9 (35.3–36.5)	35.5 (32.3–38.7)
	30	38.7 (36.8–40.6)	37.5 (36.0–39.0)[a]	37.5 (36.0–39.0)[a]	37.5 (36.0–39.0)[a]	37.9 (30.8–45.1)	37.2 (36.0–39.0)[a]	37.2 (36.0–39.0)[a]	37.2 (36.0–39.0)[a]
Summer *A. plicata*	27	37.5 (36.0–39.0)[a]	37.5 (36.0–39.0)[a]	37.5 (36.0–39.0)[a]	36.6 (33.4–39.7)	37.2 (36.0–39.0)[a]	37.2 (36.0–39.0)[a]	37.2 (36.0–39.0)[a]	35.9 (35.0–36.7)
C. necki	27	37.5 (36.0–39.0)[a]	37.5 (36.0–39.0)[a]	36.4 (33.8–39.1)	36.2 (34.5–37.9)	37.2 (36.0–39.0)[a]	37.2 (36.0–39.0)[a]	35.7 (33.5–37.9)	35.4 (30.0–40.9)
F. mitchelli	27	36.2 (34.6–37.8)	34.5 (33.0–36.0)[a]	33.7 (32.6–34.7)	32.4 (31.1–33.6)	35.4 (30.4–40.5)	34.2 (33.0–36.0)[a]	30.5 (28.6–32.4)	28.4 (26.0–30.9)

[a] The 95% confidence intervals could not be calculated due to a lack of partial mortality in any given temperature treatment at the time of observation; the lower and upper bounds represent the range of temperatures where mortality was 0 and 100%, respectively.

TABLE 3 Matrix of confidence interval (CI) ratios between estimates of lethal temperatures affecting 50% of the population (LT50) Cfor all trials

				96-hr					240-hr (10-day)						
				AP (W)	AP (W)	AP (W)	AP (S)	CN	FM	AP (W)	AP (W)	AP (W)	AP (S)	CN	FM
Duration (hr)	Species	Acclimation	LT50 (95% CI)	23	27	30	27	27	27	23	27	30	27	27	27
96	AP (W)	23	36.5 (35.0–38.0)[b]	—	0.16	1.00	1.01	0.06	2.79	0.00	0.20	1.00	0.11	0.29	4.11
96	AP (W)	27	36.6 (33.6–39.7)	0.18–1.82	—	0.85	0.85	0.22	2.94	0.16	0.36	0.85	0.04	0.45	4.26[a]
96	AP (W)	30	37.5 (36.0–39.0)[b]	-7.30–9.25	-7.30–9.25	—	0.01	1.07	3.79	1.00	1.20	0.00	0.89	1.30	5.11
96	AP (S)	27	37.5 (36.0–39.0)[b]	−0.56–2.51	−0.34–2.30	-7.57–9.57	—	1.07	3.80	1.01	1.21	0.01	0.90	1.30	5.12
96	CN	27	36.4 (33.8–39.1)	0.18–1.83	0.90–1.12	-7.68–9.74	−0.36–2.42	—	2.72	0.06	0.14	1.07	0.17	0.23	4.0[a]
96	FM	27	33.7 (32.6–34.7)	0.19–1.97	0.99–1.18	-8.31–10.53	−0.39–2.61	1.00–1.17	—	2.79	2.59[a]	3.79	2.90	2.49[a]	1.32
240	AP (W)	23	36.5 (35.0–38.0)[b]	−0.16–2.16	0.18–1.83	-7.71–9.77	−0.59–2.65	0.18–1.82	0.17–1.68	—	0.20	1.00	0.11	0.29	4.11
240	AP (W)	27	36.3 (34.5–38.0)	0.18–1.83	0.91–1.11	-7.71–9.78	−0.36–2.43	0.92–1.09	0.88–0.98	0.18–1.83	—	1.20	0.31	0.09	3.91[a]
240	AP (W)	30	37.5 (36.0–39.0)[b]	-7.30–9.25	-7.30–9.25	-10.97–12.97	-7.57–9.57	-7.25–9.20	-6.71–8.51	-7.30–9.25	-7.23–9.16	—	0.89	1.30	5.11
240	AP (S)	27	36.6 (33.4–39.7)	0.18–1.82	0.88–1.12	-7.65–9.70	−0.36–2.41	0.88–1.11	0.84–1.01	0.18–1.82	0.89–1.09	-7.65–9.70	—	0.40	4.22[a]
240	CN	27	36.2 (34.5–37.9)	0.18–1.84	0.92–1.11	-7.73–9.80	−0.36–2.43	0.92–1.09	0.88–0.98	0.18–1.84	0.94–1.07	-7.73–9.80	0.91–1.11	—	3.81[a]
240	FM	27	32.4 (31.1–33.6)	0.20–2.05	1.03–1.23	-8.64–10.96	−0.40–2.72	1.03–1.22	0.99–1.09	0.20–2.05	1.05–1.19	-8.64–10.96	1.02–1.24	1.05–1.19	—

Note: The CIs of the ratio are given below the diagonal and the difference between LT50 estimates is given above the diagonal.

[a] The CI of the ratio did not contain 1, thus LT50 comparisons are significantly different. Species are abbreviated as follows: AP (W)—winter-collected *Amblema plicata*; AP (S)—summer-collected *A. plicata*; CN—*Cyclonaias necki*, and FM—*Fusconaia mitchelli*. Acclimation is the treatment temperature (°C) to which mussels were acclimated prior to testing. Duration is the time interval for which LT50s were estimated.

[b] The 95% CIs could not be calculated due to a lack of partial mortality in any given temperature treatment at the time of observation; the lower and upper bounds represent the range of temperatures where mortality was 0 and 100%, respectively.

TABLE 4 Matrix of confidence interval (CI) ratios between estimates of lethal temperatures affecting 5% of the population (LT05) for all trials

				96-hr						240-hr (10-d)					
				AP (W)	AP (W)	AP (W)	AP (S)	CN	FM	AP (W)	AP (W)	AP (W)	AP (S)	CN	FM
Duration (hr)	Species	Acclimation	LT05 (95% CI)	23	27	30	27	27	27	23	27	30	27	27	27
96	AP (W)	23	36.1 (35.0–38.0)[b]	—	0.21	1.10	1.03	0.46	5.65	0.00	0.64	1.10	0.26	0.69	7.70
96	AP (W)	27	35.9 (35.3–36.5)	0.16–1.85	—	1.30	1.24	0.26	5.45[a]	0.21	0.43	1.30	0.06	0.48	7.50[a]
96	AP (W)	30	37.2 (36.0–39.0)[b]	-7.45–9.39	-7.37–9.30	—	0.07	1.56	6.75	1.10	1.74	0.00	1.36	1.79	8.80
96	AP (S)	27	37.2 (36.0–39.0)[b]	−0.60–2.55	−0.38–2.31	-7.76–9.77	—	1.50	6.68	1.03	1.67	0.07	1.30	1.72	8.73
96	CN	27	35.7 (33.5–37.9)	0.16–1.86	0.94–1.07	-7.98–10.06	−0.41–2.49	—	5.19[a]	0.46	0.17	1.56	0.20	0.22	7.24[a]
96	FM	27	30.5 (28.6–32.4)	0.19–2.18	1.10–1.26	-9.33–11.77	−0.48–2.91	1.07–1.27	—	5.65	5.01[a]	6.75	5.39[a]	4.96	2.05
240	AP (W)	23	36.1 (35.0–38.0)[b]	−0.18–2.18	0.16–1.83	-7.91–9.97	−0.64–2.70	0.16–1.81	0.14–1.55	—	0.64	1.10	0.26	0.69	7.70
240	AP (W)	27	35.5 (32.3–38.7)	0.16–1.87	0.92–1.11	−8.01–10.11	−0.41–2.50	0.90–1.11	0.76–0.95	0.16–1.87	—	1.74	0.37	0.05	7.06[a]
240	AP (W)	30	37.2 (36.0–39.0)[b]	-7.45–9.39	-7.37–9.30	−11.22–13.22	-7.74–9.73	-7.32–9.24	−6.26–7.89	-7.45–9.39	-7.28–9.19	—	1.36	1.79	8.80
240	AP (S)	27	35.9 (35.0–36.7)	0.16–1.85	0.97–1.03	-7.93–10.01	−0.40–2.47	0.93–1.06	0.79–0.91	0.16–1.85	0.90–1.08	-7.93–10.01	—	0.42	7.44[a]
240	CN	27	35.4 (30.0–40.9)	0.15–1.89	0.86–1.17	−8.03–10.13	−0.42–2.51	0.84–1.17	0.72–1.00	0.15–1.89	0.82–1.18	-8.03–10.13	0.85–1.17	—	7.01[a]
240	FM	27	28.4 (26.0–30.9)	0.20–2.34	1.15–1.37	−10.00–12.62	−0.51–3.13	1.12–1.39	0.96–1.19	0.20–2.34	1.09–1.40	−10.00–12.62	1.15–1.37	1.03–1.47	—

Note: The CIs are given below the diagonal and the difference between LT05 estimates is given above the diagonal.

[a] The CI of the ratio did not contain 1, thus LT05 comparisons are significantly different. Species are abbreviated as follows: AP (W)—winter-collected *Amblema plicata*; AP (S)—summer-collected *A. plicata*; CN—*Cyclonaias necki*, and FM—*Fusconaia mitchelli*. Acclimation is the treatment temperature to which mussels were acclimated prior to testing. Duration is the time interval for which LT50s were estimated.

[b] The 95% CIs could not be calculated due to a lack of partial mortality in any given temperature treatment at the time of observation; the lower and upper bounds represent the range of temperatures where mortality was 0% and 100%, respectively.

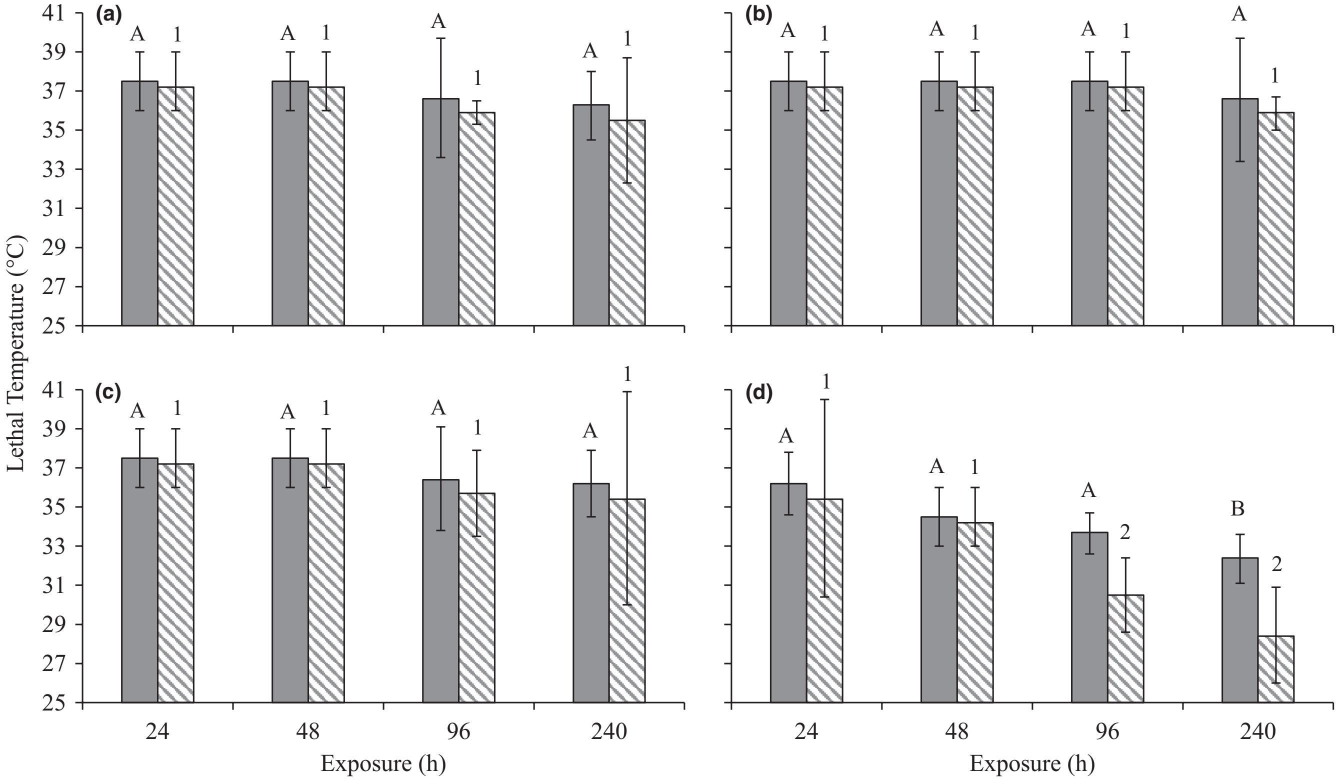
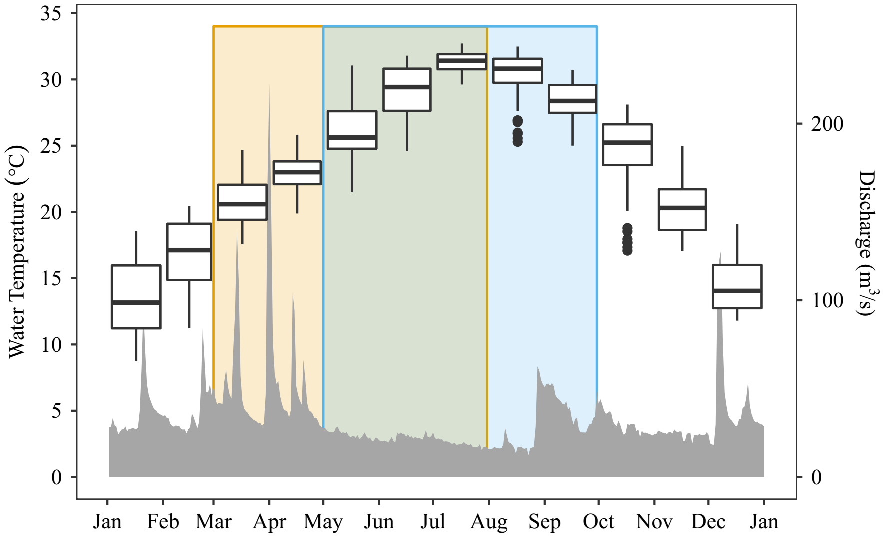


FIGURE 3 Comparison of lethal temperatures (LT) affecting 50% (solid bar) and 5% (hatched bar) of the population, and their 95% confidence intervals (error bars) at 24-, 48-, 96-, and 240-hr time intervals shown for (a) *Amblema plicata* (winter), (b) *A. plicata* (summer), (c) *Cyclonaias necki*, and (d) *Fusconaia mitchelli* at 27°C acclimation. The LT50 values with the same superscripted letter are not significantly different; LT05 values with the same superscripted number are not significantly different based on the confidence interval ratio test ($p < 0.05$)

FIGURE 4 Annual mean discharge and monthly boxplots of water temperature for the Guadalupe River near Hochheim, Texas. Flow data are from the U.S. Geological Survey gage near Hochheim, TX (08174700) from June 2016 to October 2018. Temperature data were collected from April 2016 to October 2018. Shaded regions represent the summer bioperiod (yellow polygon; time of greatest temperature stress) and reproductive bioperiod (blue polygon; time of potential brooding activity for our focal species)



3.2 | Uniform continuous above-threshold analysis

At the collection site, mean annual water temperature was 24.3°C and daily means ranged from 8.8 to 32.7°C during our study. Mean annual flow was 41.6 m^3/s and daily means ranged from 6.9 to 966.1 m^3/s. Monthly median summer temperatures (June–August, 2016–2018) ranged from 27.3–31.8°C with the highest mean daily water temperatures occurring in July and August (Figure 4). Monthly mean discharge in the summer ranged from 8.4–144.7 m^3/s with the lowest mean daily discharges occurring in July and August (Figure 4). For example, in 2018, the maximum mean daily water temperature recorded in July and August was 32.7 and 32.5°C, respectively, while monthly mean discharge during July and August of 2018 was 11.6 and 8.4 m^3/s, respectively. The highest absolute water temperature value recorded (i.e. recorded at any given 15-min interval) was 33.7°C, which occurred in July of 2018, while mean daily discharge for that day was 10.4 m^3/s. Mean daily temperatures did not exceed the LT50 threshold for any of the three species tested. However, mean daily temperatures exceeded both the 96-hr acute and 10-day chronic LT05 estimates (30.5 and 28.4°C, respectively) for *F. mitchelli* during both the summer and reproductive bioperiods (Figure 5).

We identified seven events, ranging from 1 to 38 days in duration, when the 96-hr acute LT05 threshold for *F. mitchelli* was exceeded during the reproductive period. Exceedance of the 10-day LT05 chronic threshold during the reproductive bioperiod occurred in six separate events ranging from 1 to 60 days in duration. The cumulative frequency of these events is plotted in Figure 6a in order of decreasing duration. For example, the longest consecutive duration above the acute threshold was a single 38-day event. This event made up *c*. 9% of the entire reproductive bioperiod. The second longest period lasted 32 days and accounted for *c*. 8% of the bioperiod duration. However, the frequency of each shorter duration is added to longer durations for a cumulative frequency. Thus, these two periods are plotted cumulatively to represent the total duration that the temperature was above the acute LT05 threshold for 32 days or longer and together account for *c*. 17% of the total duration of the bioperiod. Durations that did not occur in the time series (i.e. 37, 36, 35, 34, and 33 continuous days) are plotted as a vertical line, or 0% cumulative increase (see Castelli et al., 2012 for further discussion). During the summer bioperiod, the 96-hr acute threshold was exceeded in 12 separate events, ranging from 1 to 42 days in duration, while exceedance of the 10-day chronic threshold during the same period occurred in nine separate events ranging from 1 to 86 days in duration (Figure 6b).

The piecewise linear regression analysis identified two breakpoints demarcating the catastrophic, persistent, and typical categories for the summer bioperiod (both acute and chronic thresholds) and the reproductive bioperiod (chronic threshold only). However, the model for the acute threshold (96-hr LT05 = 30.5°C) during the reproductive bioperiod converged on only one breakpoint (i.e. two broken-line relationships), indicating the events identified for this bioperiod could only be divided into typical and persistent duration events. Events lasting 21 days or longer were considered persistent duration events for the acute threshold and accounted for *c*. 27% of the reproductive bioperiod. Mean discharge across all events (typical and persistent) was 20.1 ± 1.0 m^3/s (mean ± *SE*; water temperatures and discharge associated with persistent and catastrophic events are reported in Table 5). Events lasting 27 and 40 consecutive days or longer were considered persistent and catastrophic events for the chronic threshold, respectively, with a mean discharge of 22.7 ± 1.0 m^3/s across all events (Table 5). During the summer bioperiod, events exceeding the acute threshold for 17 and 39 days or longer were considered persistent and catastrophic events, while events exceeding the chronic threshold for 27 and 80 days or longer were persistent and catastrophic events, respectively (Table 5). While only 2 events were classified as catastrophic for the summer bioperiod, cumulatively these events lasted for 166 days and accounted for *c*. 36% of the summer bioperiod. Mean discharge for all acute events was 18.8 ± 0.7 m^3/s, while chronic events averaged 23.2 ± 0.8 m^3/s for the summer bioperiod.

Mean temperatures were similar for both persistent and catastrophic duration events with catastrophic events having a slightly higher mean temperature (30.7 and 31.3°C, respectively). However, catastrophic events were both longer in duration and had lower mean flows than persistent events (Table 5). Mean difference between catastrophic and persistent associated flows was 11.4 ± 3.0 m^3/s.

4 | DISCUSSION

We are the first to report lethal thermal tolerances for adult mussels occurring in the south-western U.S.A. Our results provide further evidence that freshwater mussel species can respond differently to thermal stress and suggest that geographical range and phylogeny may be important determinants of mussel thermal tolerance.

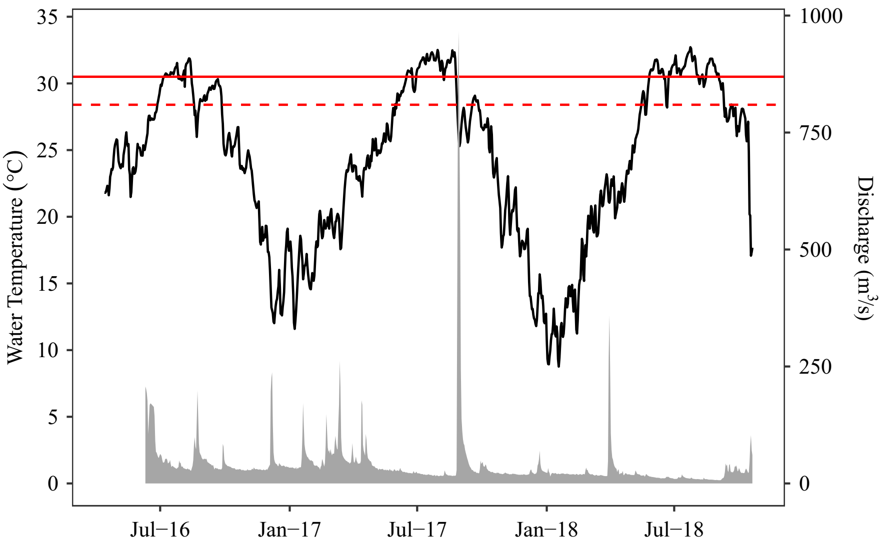


FIGURE 5 Daily mean discharge and temperature data for the Guadalupe River near Hochheim, Texas. Daily mean discharge data (grey shaded area) were from U.S. Geological Survey stream gage near Hochheim, Texas (08174700) and daily mean water temperature data were collected from April 2016 to October 2018. Red horizontal lines correspond to acute (solid line) and chronic (dashed line) 5% thermal thresholds for *Fusconaia mitchelli*

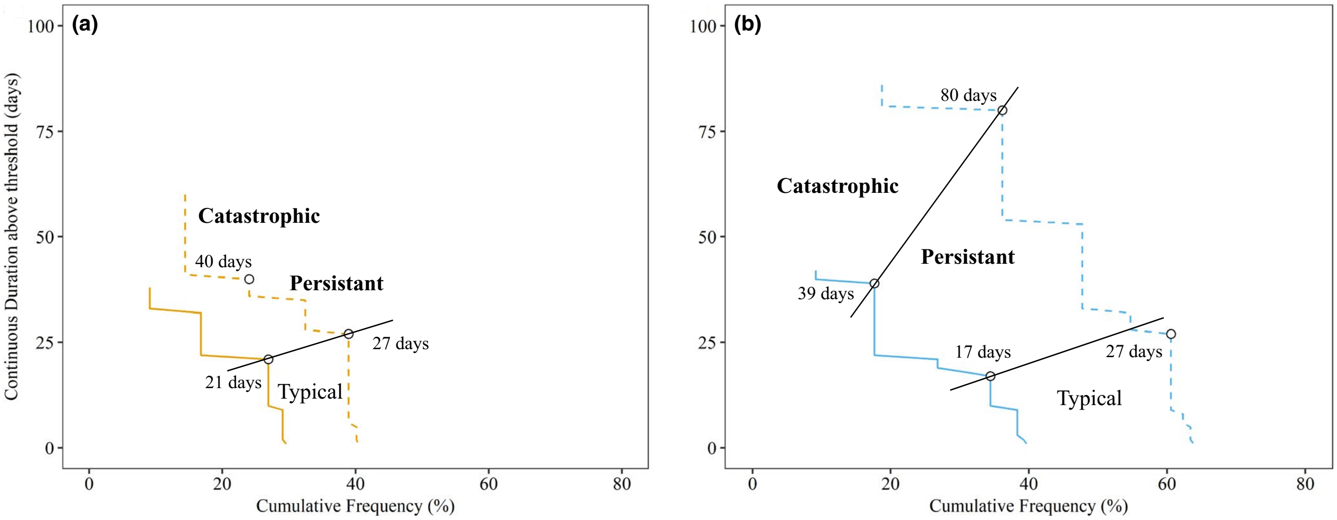


FIGURE 6 Cumulative frequency of events from April 2016–October 2018 in the Guadalupe River for both the (a) reproductive bioperiod from March–July (yellow lines) and (b) summer bioperiod from May–September (blue lines). Curves for each bioperiod represent all events exceeding the acute (solid line) and chronic (dashed line) 5% thermal thresholds for *Fusconaia mitchelli*. Breakpoints (open circles) indicate thresholds between typical, persistent, and catastrophic duration events as identified by piecewise linear regression. Boundaries between hypothetical zones experiencing typical, persistent, or catastrophic duration events are denoted with a solid black line. A catastrophic event zone could not be demarcated for the reproductive bioperiod because the model for the acute threshold (yellow solid line) converged on only one breakpoint

We found adult *A. plicata* to be thermally tolerant with acute 96-hr LT50s ranging from 36.3–37.5°C across all acclimation and seasonal treatments, and the upper thermal limits we estimated mirror those of juveniles tested from the mid-west (96-hr LT50 = 36.4°C at 27°C acclimation; Archambault et al., 2014). This could suggest that mussel upper thermal limits may be fixed, regardless of geographic location, although size of geographical range could partially explain this result. We also found adult *C. necki* to be thermally tolerant (96-hr LT50 = 36.4°C; 95% CI: 33.8–39.1°C), and, as with *A. plicata*, our estimates for adults are similar to estimates of acute upper limits for glochidia (24-hr LT50 = 36.4°C at 27°C acclimation; Khan et al., 2019). However, in contrast to *A. plicata*, geographical range may not explain overall thermal tolerance of *C. necki*, and the role of phylogeny and geography in the conservation of upper thermal limits should be further explored. Finally, we found that *F. mitchelli* was the most thermally sensitive (96-hr LT50 = 33.7°C; 95% CI: 32.6–34.7°C) of the species we tested and, compared to *A. plicata* and *C. necki*, had the smallest overall geographic range.

Environmental temperatures play a pivotal role in determining species' distributions (e.g. Merriam, 1894; Southward, 1958), and an organism's thermal tolerance is a critical aspect of its physiological niche (Magnuson, Crowder, & Medvick, 1979). The niche breadth hypothesis posits that species that have evolved broad physiological tolerances can achieve larger geographic ranges than species with narrow tolerance ranges (Brown, 1984). Several studies have found correlations between range size and physiological tolerance breadth; however, there is no consensus across taxa. For example, Calosi, Bilton, Spicer, and Atfield (2008) found that widespread taxa of diving beetle had significantly higher thermal limits than the more restricted taxa. Among marine ectotherms, geographic range boundaries are closely matched to a species' thermal tolerances (Sunday, Bates, & Dulvy, 2012), and for freshwater fish, temperature seems to be one of the main determinants of spatial distribution (e.g. Buisson, Blanc, & Grenouillet, 2008; Heino, 2002). Among our species, both *A. plicata* and *F. mitchelli* follow this pattern. *Amblema plicata* has a wide distribution across most of the mid-west and south-east U.S.A., while *F. mitchelli* is a Texas endemic with a narrow range.

Interestingly, acclimation potential has also been linked to physiological tolerance breadth (Addo-Bediako, Chown, & Gaston, 2000), although the evidence for ectotherms is mixed (Angilletta, 2009). One theory predicts that species with broad thermal breadths may have greater acclimation ability due to selective pressure from the larger variations in temperature across seasons and geography (Angilletta, 2009). Alternatively, the *trade-off hypothesis* proposes that organisms that evolve high thermal tolerance do so at the expense of their acclimation ability (Stillman, 2003). To date, there is some evidence that seasonal acclimation can affect thermal tolerance for both adult (15 and 25°C acclimation temperatures; Galbraith et al., 2012) and juvenile freshwater mussels (7 and 23°C acclimation temperatures; Martin, 2016). However, our study did not see an effect on thermal tolerance due to acclimation treatment for *A. plicata*, a wide-ranging mussel that encounters highly variable seasonal temperatures across its distribution. Our acclimation temperatures were relatively high (i.e. 23, 27, and 30°C) and comparable to other studies where little effect from acclimation was observed (i.e. 22 and 27°C; Archambault, Cope, & Kwak, 2013; Archambault et al., 2014; Pandolfo et al., 2010). These mixed results could, in part, reflect a *trade-off* due to a relatively high thermal tolerance (LT50 range: 36.3–37.5°C), which indicates that some taxa may have a

TABLE 5 Mean daily statistics for water temperature and flow at the collection site in the lower Guadalupe River for persistent and catastrophic duration events defined using the uniform continuous above-threshold analysis based on acute and chronic LT05 thresholds for *Fusconaia mitchelli* and two bioperiods that are likely to be important for reproduction and survival. The reproductive bioperiod (time of potential brooding activity for our focal species) includes continuous duration events from March to July, while the summer bioperiod (time of greatest temperature stress) includes events from May to September

Threshold			Minimum duration	Maximum duration	Temperature (°C)			Discharge (m^3/s)		
					Mean	Minimum	Maximum	Mean	Minimum	Maximum
Reproductive Bioperiod	Chronic	Catastrophic	40	60	31.2	28.6	32.7	16.7	9.2	33.1
		Persistent	27	35	30.6	28.4	31.8	29.0	10.8	62.8
	Acute	Catastrophic	–	–	–	–	–	–	–	–
		Persistent	21	38	31.3	30.5	32.7	21.1	9.2	52.9
Summer Bioperiod	Chronic	Catastrophic	80	86	31.1	28.6	32.7	15.3	6.9	33.1
		Persistent	27	53	30.3	28.4	31.9	32.3	10.8	81.6
	Acute	Catastrophic	39	42	31.6	30.6	32.7	14.9	9.2	25.2
		Persistent	17	21	31.2	30.5	32.5	19.9	7.0	52.9

limited capacity to acclimate to higher temperatures and respond to future climate change.

Physiological limits, such as thermal tolerance, are often phylogenetically constrained (Chown, Addo-Bediako, & Gaston, 2002) and, depending on the species, upper thermal limits can show little latitudinal geographic variation (Addo-Bediako et al., 2000). Among freshwater mussels, closely related congeners have similar life-history and reproductive strategies (Haag, 2012), which could mean that upper thermal limits show a similar pattern. Previous studies of thermal sensitivity in *Cyclonaias pustulosa* have found this species tolerant of short duration exposure to high water temperatures and emersion (Bartsch, Waller, Cope, & Gutreuter, 2000). Similarly, Spooner and Vaughn (2008) demonstrated that clearance, biodeposition, and nutrient excretion rates for *C. pustulosa* remained high up to 35°C, but assimilation rates were low at this temperature, indicating onset of thermal stress. *Cyclonaias pustulosa* is a member of the monophyletic Pustulosa clade, which includes *C. necki*. In our study we estimated that the 96-hr LT50 for *C. necki* was 36.4°C (95% CI: 33.8–39.1°C) and the 10-day LT50 was 36.2°C (95% CI: 34.5–37.9°C), which are similar to, but slightly higher than, the temperature reported by Spooner and Vaughn (2008). This is not unexpected because their results reflect sublethal stress, whereas our estimates are measuring acute and chronic lethality. Assuming that Spooner and Vaughn's (2008) results are reflective of sublethal thresholds for *C. necki*, this would indicate that the difference between sublethal and lethal stress is narrow, mirroring thermal studies of glochidia and juveniles (Pandolfo et al., 2010), where relatively small increases in water temperature could quickly result in widespread mortality. Thermal tolerance may also be phylogenetically conserved, based on the results of physiological biomarkers for thermal stress and acute LT50s for *Villosa delumbis* and *Villosa vibex* (Fritts, Peterson, Hazelton, & Bringolf, 2015; but see Payton, Johnson, & Jenny, 2016, which found geographic range to be a better predictor for related *Villosa* species). These results combined with the present study provide initial evidence that overall physiological tolerances may be set at and conserved across major taxonomic groupings, while specific limits are likely to be established through local adaptation (Hoffmann, Chown, & Clusella-Trullas, 2013; Somero, 2010). Thus, for species whose thermal tolerance has not been tested, phylogeny could potentially be used to make predictions about their thermal tolerance, although laboratory and in situ field studies should be used to formally evaluate these predictions.

While we identified upper thermal limits for three mussel species, there were a few limitations in our study, which we adapted from other published studies and guidelines (ASTM, 2006; e.g. Archambault et al., 2014; Pandolfo et al., 2010). First, the effects of high water, temperature, and other stressors, such as dissolved oxygen (but see Galbraith et al., 2012), and the effect of holding conditions on thermal tolerance have not been fully investigated. In our study, experimental units were aerated to control for dissolved oxygen but because we used a static system water quality may not have been optimum and as a result may have biased our thermal tolerance estimates. For example, mussels are more sensitive to ammonia relative to other invertebrates and fish (Augspurger et al., 2003) and respond to elevated temperatures by excreting more ammonium (Spooner & Vaughn, 2008). Thus, in our case high water temperature treatments could have resulted in elevated concentrations of ammonia, which could have been toxic to test individuals, resulting in biased thermal tolerance estimates. This is unlikely to be the case because we measured ammonia levels in our experiment and compared those results against published mussel ammonia toxicity studies (Augspurger et al., 2003; Newton, Allran, O'Donnell, Bartsch, & Richardson, 2003) and renewed test water twice during the 10-day trial. That said, ammonia toxicity of mussels has not been studied for our species and so it could be that subtle increases in ammonia, even below published sublethal limits, may have been toxic. Additionally, adult mussels were fed an artificial diet, which may not have been sufficient to maintain optimal physiological performance, but we doubt this is an issue given the diet we used follows published studies and guidelines. Second, our threshold for sublethal effects is based on an estimate of 5% mortality in a population and not a causal linkage between temperature and suspension of growth and reproduction (Pörtner, 2001). Thus, future studies should use more physiologically based thresholds for sublethal effects such as respiration and/or metabolic activity, which would probably improve sublethal estimates. Plus, it would provide endpoints in advance of onset of mortality, which ideally is what decision-makers and conservationists should be managing for. Third, the 10-day chronic duration of our study may not be sufficient for long-lived species such as mussels. Both *C. necki* and *F. mitchelli* are moderately long-lived (>10 years; Dudding et al., 2020), while *A. plicata* can live upwards of 50 years (Haag & Rypel, 2011), and so, relative to their longevity, the 10-day duration of this experiment is still an acute measure. Unfortunately, this issue is challenging to address as optimal holding conditions for mussels in the laboratory are difficult to achieve and so longer duration experiments may be influenced by other variables besides elevated temperatures (see Ganser et al., 2013; Gatenby, Neves, & Parker, 1996). Finally, our laboratory exposures consisted of a constant temperature, which does not reflect natural thermal patterns in streams. Recent research has demonstrated that diel fluctuations could provide some measure of thermal relief for freshwater mussels. In ramped temperature exposures, which mimic a diel thermal pattern, LT50s were 2–6°C higher for *Lampsilis siliquoidea* and *Megalonaias nervosa* (Martin, 2016) than those previously reported (Pandolfo et al., 2010), indicating that LT50s from constant temperature experiments may underestimate actual lethal thermal thresholds.

4.1 | Conservation implications

Our analysis of thermal events in the lower Guadalupe River combined with our estimates of upper thermal tolerance limits demonstrate mussels are probably experiencing sublethal thermal stress at acute and chronic levels during summer months. Depending on when thermal stress occurs the resulting sublethal effects could affect population performance, and therefore long-term viability, by redirecting energy normally allocated to growth and reproduction towards maintenance (Pörtner, 2001). Theoretically, these shifts in energy allocation could result in decreased fecundity and/or complete inhibition of reproduction as well as reduced and/or complete cessation of individual growth (Petes, Menge, & Murphy, 2007). Over time, these effects would be likely to compound, leading to population decline and, if severe enough, extirpation and ultimately extinction.

Stakeholders, managers, and conservationists can avoid impacts to species and develop proactive measures to mitigate thermal stress by merging laboratory thermal tolerance estimates with in situ temperature data. The approach we used follows that of Castelli et al. (2012) but other methods are available (see Maloney, Lellis, Bennett, & Waddle, 2012). Through the UCAT analysis, we demonstrate that temperatures exceeding *c.* 31°C are likely to be detrimental to *F. mitchelli*, a species of high conservation concern with limited distribution in the Guadalupe River. For *A. plicata* and *C. necki*, upper thermal thresholds were higher (96-hr LT05 = 35.9 and 35.7°C, respectively), indicating that thermal stress may not be as frequent, but still probably an issue, especially with expected increases in population growth and climate change predictions for this region (Loaiciga, Maidment, & Valdes, 2000). These impacts are likely to exacerbate the natural drought cycle in semi-arid rivers such as the Guadalupe River, leading to a decrease in available water and elevated water temperatures in already stressed systems. However, our results from the UCAT analysis may not be entirely accurate because they are derived from flow and water temperature data during three consecutive *wet* years. Prior to this study, the lower Guadalupe River experienced a severe 4-year drought that rivalled the drought of record (Verdon-Kidd, Scanlon, Ren, & Fernando, 2017). The results from our 3-year study period show that onset of catastrophic water temperature events occur at flows around 15.6 m^3/s and correspond with the longer durations (39–86 consecutive days) of thermal exceedance. In comparison, discharge during the 2011 drought (recorded at a gage located *c.* 51.8 river km downstream of our collection site) averaged even lower (12.6 ± 0.4 m^3/s) and was below our identified catastrophic threshold for 219 days. Through the duration of the drought (2011–2014), flows were less than our 15.6 m^3/s at this downstream gage for nearly 73% of the time, suggesting that temperature thresholds were exceeded for all three species during this period.

Environmental flow standards have been developed for a select number of gaging stations within Texas, including some arid and semi-arid rivers. These flow standards address both water quantity and the timing of flow in support of a sound ecological environment, and have thus far been adopted for two USGS gaging stations near our collection site in the lower Guadalupe River: an upstream station located *c.* 58 river km from our collection site (08173900 near Gonzales, TX) and a second located *c.* 51.8 river km downstream (08175800 near Cuero, TX). Adopted flow standards for these gages include two seasonal minimum streamflow components that inform river management during dry periods and drought: a summer subsistence flow and a summer base flow. The subsistence flow component provides a minimum streamflow that maintains tolerable water quality and habitat conditions during critical low flow periods, while the summer base flow describes seasonal, normal flow conditions that still support native aquatic communities. For the Gonzales gage, adopted flow standards for summer subsistence and summer base flows are 5.95 and 20.59 m^3/s, respectively; while summer subsistence and base flows for the Cuero gage are 3.68 and 22.65 m^3/s, respectively. However, the summer subsistence flows for both gages are well below our estimated catastrophic threshold, and thermal tolerances of mussels were not explicitly considered in the adoption of flow standards for the Guadalupe River, thus flow standards are probably not sufficient to protect freshwater mussel populations during severe droughts and low flow periods.

In this study, we identified a range of hydrologic thresholds for the lower Guadalupe basin that integrate water temperature and flow that could serve as a guide for environmental flow recommendations for mussels. We have also shown that current environmental flow standards in this river may not be sufficient to protect freshwater mussels during periods of low flow, which suggests that these standards should be revised. The thresholds we identify could serve as a starting place for this but, to provide a holistic picture of mussel habitat, additional studies are needed to examine the effects of low and high flows on mussel habitat persistence (i.e. areas that remain stable during high flow periods and thermally buffered during low flows). Information on host-fish behaviour during high flow events and thermal tolerances during low flow could be integrated to further refine specific flow recommendations. Finally, long-term water temperature datasets at locations with significant mussel populations are scant and the availability of sub-daily thermal records is limited. Thus, to ensure that environmental flow recommendations for mussels are accurate and to better understand how mussel population performance and persistence are influenced by water temperature, more robust temperature monitoring and long-term monitoring sites are sorely needed.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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