UPPER THERMAL LIMITS OF FRESHWATER MUSSELS IN TEXAS TO INFORM CONSERVATION AND MANAGEMENT

A Thesis

by

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ABSTRACT

Understanding the temperature tolerances of organisms is critical because thermal regimes of freshwater ecosystems are changing globally due to climate change, river regulation, and land development, which may create conflicts between the needs of humans and aquatic ecosystems. Native freshwater mussels are especially 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 thermal tolerances of mussel species from rivers within the southwestern United States. To determine the effects of elevated water temperature on mussels from the southwestern United States, I tested the upper thermal tolerances of larvae (glochidia) for the following species across 4 basins in Texas (Neches, Guadalupe, San Antonio, and Colorado): *Amblema plicata*, *Cyclonaias petrina*, *Fusconaia mitchelli*, *Lampsilis bracteata*, *Lampsilis hydiana*, *Lampsilis satura*, *Lampsilis teres*, *Leptodea fragilis*, and *Obovaria arkansasensis*. I then tested the upper thermal tolerances of adults of 3 of these species (*Amblema plicata*, *Cyclonaias petrina*, and *Fusconaia mitchelli*) from the Guadalupe River. I evaluated upper lethal limits of freshwater mussels acclimated to 27 °C across a range of experimental temperatures (30–39 °C) in standard acute laboratory tests. The results of the adult trials were then related to *in situ* water temperature and flows using a uniform continuous above-threshold (UCAT) analysis which evaluates the duration and frequency of continuous events above a specified temperature threshold.

Median lethal temperature (LT50) in 24-h tests among glochidia averaged 32.4 °C and ranged from 26.9 to 36.4 °C. The mean LT50 in acute 96-h adult tests averaged 36.4 °C and ranged from 33.7 to 37.5 °C, while the chronic 10-d adult 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. petrina*, and the UCAT analysis showed that LT05 (temperature affecting 5% of the population) thresholds were exceeded for *F. mitchelli* in the Guadalupe River at both acute (96-h) and chronic (10-d) values. Findings from my study indicate freshwater mussels from the arid and semi-arid regions of the Southwest are already at risk from rising environmental temperatures and altered hydrologic flows.

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CHAPTER I

INTRODUCTION

Anthropogenic alterations of freshwater environments has caused shifts in species distributions (Root et al. 2003, Hickling et al. 2006) and led to the global decline of species diversity in freshwater ecosystems (Dudgeon et al. 2006). A variety of abiotic and biotic stressors are responsible for this trend, but arguably the most important drivers are water temperature and stream flow (Pyne and Poff 2017). Temperature influences many aspects of an organism's existence including growth rates, metabolic rates, and behavior (Vannote and Sweeney 1980). Water temperature can be modified by a variety of anthropogenic factors (Caissie 2006), such as climate change, river regulation, riparian clearing, and thermal effluent pollution. Climate change is expected to raise the global mean surface temperature by 1.5 °C (IPCC 2013), with certain regions like the southwestern United States likely to undergo even larger changes (Diffenbaugh et al. 2008). In addition to warmer temperatures, climate change also is expected to increase the frequency and intensity of droughts and extreme flow events (Milly et al. 2005). These hydrologic changes, along with increasing temperatures, can lead to altered nutrient cycling, habitat degradation, and water quality impairment, ultimately leading to population declines and possibly even localized extinctions (Helmuth et al. 2002, Brown et al. 2004). Because water temperature directly influence the metabolic rates, physiology, and life-history traits of aquatic species, these changes will in turn affect the ecosystem services provided by aquatic ecosystems (e.g., nutrient cycling and productivity; Woodward et al. 2010).

Freshwater mussels (Bivalvia: Unionidae) are long-lived, sedentary benthic invertebrates that perform important functions in stream ecosystems, linking the benthic and pelagic zones through filtration, biodeposition of organic matter, bioturbation of sediment, and nutrient cycling (Vaughn and Hakenkamp 2001, Howard and Cuffey 2006, Spooner and Vaughn 2006). However, unionid mussels are one of the most sensitive and rapidly declining faunal groups in North America, largely due to their complex life cycle, limited mobility, reliance on fish for dispersal, and sensitivity to anthropogenic impacts (Williams et al. 1993, Strayer et al. 2004). Altered thermal regimes have the potential to exacerbate current vulnerabilities, because mussels are ectotherms and temperature affects physiological processes such as respiration, filtration, and excretion rates (Spooner and Vaughn 2008, Pandolfo et al. 2009), which can have significant effects on survival, growth, and reproductive success (Mann and Glomb 1978, Roberts and Barnhart 1999, Zimmerman and Neves 2002). In addition to increasing respiration rates (and presumably aerobic demands), high water temperatures affect the dissolved oxygen content with the solubility of oxygen decreasing as water temperature increases. Aerobic metabolism in ectotherms likely plays a key ecological role in an organism’s response to rising temperatures (Schulte 2015), with temperature limiting an organism’s oxygen capacity, or aerobic scope, which represents the energy that an organism has available to perform critical functions such as growth, mobility, and reproduction (Portner 2001, 2002, Portner and Knust 2007). Elevated water temperatures also can negatively affecting burrowing behavior and a mussel’s ability to right itself after settling (Bartsch et al. 2000, Archambault et al. 2013). Low-flow conditions can result in both lethal and sub-lethal effects, due to low dissolved oxygen concentrations and higher water temperatures (Gagnon et al. 2004, Galbraith et al. 2010). Sub-lethal stress from stranding or desiccation reduces reproductive potential and overall fitness (McMahon and Bogan 1991), while prolonged drought periods may result in mass mortality (Gagnon et al. 2004, Golladay et al. 2004, Haag and Warren 2008). Water temperature also affects the timing of mussel reproductive behaviors (e.g., gamete development and glochidial release; Watters and O’Dee 2000, Galbraith and Vaughn 2009, Gascho Landis et al. 2012). As obligate parasites, freshwater mussels rely on fish hosts to transform into juveniles, and temperature can affect the availability and abundance of potential hosts (Schwalb et al. 2011), the success of transformation (Roberts and Barnhart 1999), and phenological synchrony of glochidial release with host fish occurrence (Pandolfo et al. 2012, Schneider et al. 2018). Shifting thermal regimes among fish hosts might lead to coextirpation among certain species of mussels (Spooner et al. 2011), because the geographic ranges and limits of many aquatic species are defined by temperature (Poff et al. 2002). While the latitudinal shifts of marine ectotherms in response to climate change have been well documented (Barry et al. 1995), freshwater mussels may not be able to respond to altered thermal regimes by shifting their geographic distribution or range boundaries in the same way due to limited mobility and patchy distribution (Huey and Stevenson 1979, Strayer et al. 2004).

Unfortunately, quantitative information on lethal temperatures for freshwater mussels has been limited to 15 species (Dimock and Wright 1993, Pandolfo et al. 2010, Archambault et al. 2014, Ganser et al. 2015, Martin 2016), and relatively little is known about the thermal tolerance of any Texas mussel species. Texas has approximately 52 native species of freshwater mussel, and in recent decades, mussel populations have been declining (Howells et al. 1996). Texas Parks and Wildlife Department (TPWD) has listed 15 species as state-threatened (TPWD 2010), including 12 that are currently being considered for federal protection under the Endangered Species Act (ESA; USFWS 2011). It also is likely that many species may already be living close to or beyond their upper thermal limits (Pandolfo et al. 2010). In fact, there is increasing evidence that changes in water temperature, such as those caused by altered flow regimes, are leading to population declines, shifts in mussel assemblages, and changes in rates of mussel-contributed ecosystem services (Haag and Warren 2008, Spooner and Vaughn 2008, Allen et al. 2013, Vaughn et al. 2015). This is problematic as climate change is expected to increase the frequency and intensity of droughts, especially for states located in the southwest like Texas. For example, it has been suggested that annual average temperatures will increase across Texas anywhere from 2–5 ºC over the 21st century (IPCC 2007). In addition to warmer average temperatures, the frequency and magnitude of minimum and maximum temperature extremes also are expected to increase (Shafer et al. 2014). These changes, coupled with reductions in precipitation or shifts in precipitation patterns, are likely to amplify current and future anthropogenic pressures on aquatic ecosystems, particularly stressors like impoundments and groundwater extraction that affect stream flows (Bond et al. 2008).

The overall objective of my study was to identify thermal tolerance limits for mussels from the southwestern United States using glochidia and adults of species with both narrow and broad distributions. Glochidia of 8 species of mussels were tested: *Amblema plicata*, *Cyclonaias petrina*, *Fusconaia mitchelli*, *Lampsilis bracteata*, *Lampsilis hydiana*, *Lampsilis satura*, *Lampsilis teres*, and *Obovaria arkansasensis*. Three of these species (*Amblema plicata*, *Cyclonaias petrina*, and *Fusconaia mitchelli*) also were tested as adults. The specific objectives of my study were to: (1) assess the effects of a range of water temperatures on glochidial and adult survival, (2) use the resulting data to compare upper thermal limits between species and populations, (3) use water temperature and flow data from the Guadalupe River to determine whether thermal stress could be a threat; and (4) discuss how water temperatures could be contributing to the decline of mussel species in Texas along with management implications and potential solutions. The resulting data from my study will likely help inform overall management and conservation efforts for mussels within Texas and expand current understanding of how thermal stress affects mussels, particularly species occurring in arid and semiarid regions of the southwestern United States.

CHAPTER II

UPPER THERMAL TOLERANCES OF GLOCHIDIA FOR SELECT FRESHWATER MUSSELS FROM CENTRAL AND EAST TEXAS

Introduction

Water temperature is widely recognized as a key variable for maintaining riverine biota and is tightly linked to flow patterns (Olden and Naiman 2010). Natural spatial and temporal variations in temperature exert a strong influence on the evolution, distribution and ecology of aquatic organisms (Vannote and Sweeney 1980, Ward and Stanford 1982). However, anthropogenic activities, such as climate change, river regulation, riparian clearing, and thermal effluents can disrupt the natural thermal regime of a river. This change to the thermal regime can negatively affect biological endpoints 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 (IPCC 2013), with certain regions likely to undergo even larger changes. Within the southwestern United States, places such as Texas are considered hotspots for climate change (Diffenbaugh et al. 2008), as surface temperatures in this region are predicted to increase between 2–5 °C by 2100 (Jiang and Yang 2012), which in turn will likely increase the frequency and intensity of droughts and extreme flow events (Milly et al. 2005).

Freshwater mussels are one of the most imperiled aquatic faunas due to anthropogenic impacts on water quality and quantity (Williams et al. 1999, Strayer et al. 2004). 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 due to limited mobility (Amyot and Downing 1998) and reliance on host fish for larval dispersal as obligate parasites (Haag and 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 (Spooner and Vaughn 2008, Pandolfo et al. 2009, Rodland et al. 2009), and which has implications for the ecosystem services provided by mussels (e.g., nutrient cycling; Vaughn and Hakenkamp 2001, Vaughn 2008). Changes in the natural thermal regime also can influence seasonal growth cycles and reproductive behaviors (e.g., gamete development and glochidial release) that are cued by environmental factors, such as temperature and photoperiod (Watters and O’Dee 2000, Baker and Hornbach 2001, Galbraith and Vaughn 2009, Gascho Landis et al. 2012). 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 availability and abundance of potential hosts (Schwalb et al. 2011), the success of transformation (Roberts and Barnhart 1999), and phenological synchrony of glochidial release with host fish occurrence (Pandolfo et al. 2012, Schneider et al. 2018). Thus, mussel-dependent ecosystem services and population resiliency are tightly linked to the natural thermal regime (Spooner and Vaughn 2008, Gates et al. 2015), making them a model system from which to gain a better understanding of how riverine thermal regimes shape population and community structure for aquatic biota (Allen et al. 2013).

Thermal studies for mussels have largely focused on glochidia 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 (Dimock and Wright 1993, Pandolfo et al. 2010, Archambault et al. 2014, Ganser et al. 2015, Martin 2016), and these thermal studies have been carried out in geographic regions (i.e., the midwestern, southeastern, and western United States) with stream types that are likely not representative of those occurring in the southwestern United States or other arid/semiarid 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 ESA (USFWS 2009, 2011, 2016). For these species, there has been little consideration of how elevated water temperatures will impact 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 due to 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 state’s mussel fauna, 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 my study was to identify thermal tolerance limits for mussels from the southwestern United States using glochidia of 3 endemic and 7 widespread species representing 4 tribes of the Unionidae Family (Graf and Cummings 2007). The specific objectives of my study were to: (1) assess the effects of a range of water temperatures on glochidial survival, (2) use the resulting data to compare upper thermal limits between species and populations, and (3) discuss how these results likely relate to the decline of these species, as well as the management implications and potential solutions for minimizing these threats. The resulting data from my study will likely help inform overall management and conservation efforts for mussels within Texas and expand current understanding of how thermal stress affects mussels, particularly species occurring in arid/semiarid regions such as the southwestern United States.

Methods

Study area

My study was conducted at 8 sites in 7 rivers across the Colorado, Guadalupe, San Antonio, and Neches river basins of central and east Texas (Fig. 2.1). Within Central Texas, the westernmost sites were in the San Saba and Llano rivers of the Colorado River drainage. The San Saba and Llano rivers are major tributaries of the middle Colorado River and are located within the Edwards Plateau Woodland of the Edwards Plateau ecoregion (Griffith et al. 2007). Baseflows within the San Saba and

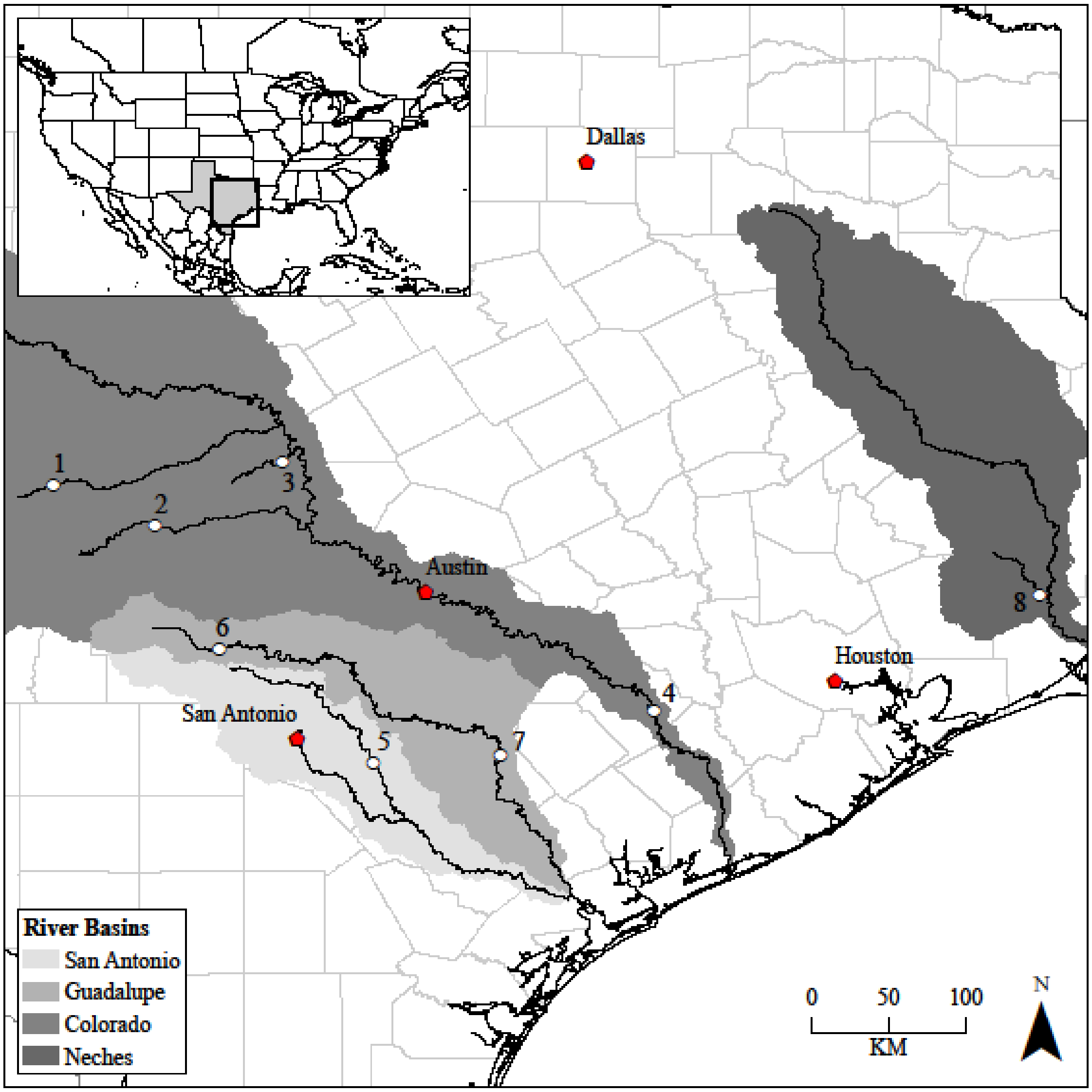

Figure 2.1. Collection sites (white circles) for gravid freshwater mussel species on the (1) San Saba River, (2) Llano River, (3) Cherokee Creek, (4) Colorado River, (5) Cibolo Creek, (6) Upper Guadalupe River, (7) Lower Guadalupe River, and (8) Village Creek.

Llano rivers are derived primarily from spring inflows from the Edwards-Trinity Plateau Aquifer (Wolaver et al. 2012). The third site, Cherokee Creek, is located near both these rivers, but in contrast is a minor tributary of the middle Colorado River. Similar to the baseflows within the Llano and San Saba, baseflow within Cherokee Creek is sourced primarily from groundwater input and springflows, though from the Ellenburger-San Saba and Marble Falls aquifers. The flow regime across all 3 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 (Brune 1975, Blum et al. 1994). Water temperatures in the San Saba and Llano rivers average 28 °C and 29 °C, respectively, in July and 7 °C and 9 °C in January, respectively (SWQM 2017). For Cherokee Creek, water temperature data are sparse, but averages 29 °C in July and 14 °C in February (SWQM 2017).

The fourth site in Central Texas is located in the mainstem 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 to the upper reaches of the drainage (Wolaver et al. 2012), and is primarily derived from surface runoff and releases from 6 reservoirs (Lake Buchanan, Inks Lake, Lake LBJ, Lake Marble Falls, Lake Travis and Lake Austin), though periods of drought and low flow are common (Blum and Valastro, Jr. 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 are situated within the upper and lower reaches of the Guadalupe River, respectively. Specfically, the upstream site is located within the upper reach, which flows through the Balcones Canyonlands of the Edwards Plateau ecoregion, while the downstream site is 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 sourced from a combination of spring-fed tributaries, local groundwater inputs, upstream dam releases, and surface runoff. The flow regime within the upper reaches of the Guadalupe River is not managed, but flow within the lower reaches is modified by 7 mainstem impoundments, including Canyon Lake reservoir, a deep storage bottom release reservoir (Perkin and 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, though there is significant spatiotemporal variability between the 2 reaches (SWQM 2017).

The seventh and final site within Central Texas is 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 runoff, and spring flow, although historically, it was derived from spring inflows from the Edwards-Trinity and Trinity Aquifers. The flow regime within the Cibolo Creek is now characterized by short, high pulse events during heavy rainfall followed by elevated baseflows (SARA 2017). Water temperatures in Cibolo Creek average 29 °C in July and 12 °C in January (SWQM 2017).

In east Texas, the eighth and final site is 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 runoff. The flow regime within this system is characterized by sluggish flow followed by prolonged periods of flooding during heavy rainfall events. Daily water temperatures for July and January average 29 °C and 11 °C, respectively (SWQM 2017).

Species

I examined thermal tolerances for glochidia of 8 species representing 4 tribes (Amblemini, Lampsilini, Pleurobemini, and Quadrulini) in the Family Unionidae (Graf and Cummings 2007): *Amblema plicata*, *Fusconaia mitchelli*, *Lampsilis bracteata*, *Lampsilis hydiana*, *Lampsilis satura*, *Lampsilis teres*, *Obovaria arkansasensis*, and *Cyclonaias petrina* (Table 2.1). Three of these species (*A. plicata*, *L. hydiana*, and *L. teres*) are considered stable with broad distributions (Williams et al. 2008, NatureServe 2017), while *F. mitchelli*, *L. bracteata*, and *C. petrina* are state-listed Texas endemics (TPWD 2010). Additionally, both *C. petrina* and *L. bracteata* are candidates for federal

Table 2.1 List of species whose glochidia were tested (*Amblema plicata*, *Fusconaia mitchelli*, *Lampsilis bracteata*, *Lampsilis hydiana*, *Lampsilis satura*, *Lampsilis teres*, *Obovaria arkansasensis*, and *Cyclonaias petrina*), collection date and river from which gravid mussels were collected, and glochidia viability at onset of the thermal trials.

Tribe	**Species**	**Collection Date**	**Population**	***N* gravid females**	**Viability (%)**
Amblemini	*A. plicata*	28-Jun-17	Lower Guadalupe River	1	94.2
Lampsilini	*L. bracteata*	25-May-17	Llano River	3	64.3
	L. bracteata	18-Jul-17	San Saba River	3	79.6
	L. bracteata	10-Apr-18	Cherokee Creek	2	91.7
	L. bracteata	10-Apr-18	Upper Guadalupe River	3	89.4
	L. hydiana	25-May-17	Lower Guadalupe River	2	79.6
	L. hydiana	11-Apr-18	Cibolo Creek	3	92.4
	L. satura	19-Apr-17	Village Creek	3	94.5
	L. teres	9-Jun-17	Colorado River	3	85.2
	O. arkansasensis	19-Apr-17	Village Creek	3	95.4
Pleurobemini	*F. mitchelli*	5-Apr-17	Lower Guadalupe River	2	83.7
Quadrulini	*C. petrina*	5-Apr-17	Lower Guadalupe River	1	70.5
	C. petrina	28-Jun-17	Lower Guadalupe River	1	84.5

protection under the 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.

Thermal tolerance testing

I collected gravid female mussels from the Guadalupe, San Antonio, Colorado, and Neches river basins from April 2017 to April 2018 (Table 2.1). Following collection, I transported mussels in insulated coolers to the Texas A&M AgriLife Extension and Research Center in Dallas, Texas. Upon arrival in the laboratory, mussels were held at the same water temperature as that of the collection site (19–23 °C), which was gradually adjusted to the holding temperature (<2.5 °C /d) to 20 ± 1 °C. I fed mussels 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, California).

For Lampsilini species (*L. bracteata*, *L. hydiana*, *L. satura*, *L. teres*, and *O. arkansasensis*), I flushed glochidia from the gills of gravid females by puncturing the ventral margin of the marsupium with a hypodermic syringe. For all other species, I isolated individual mussels in plastic cups and checked daily for the presence of glochidia or conglutinates, which were freed from their conglutinate casing prior to testing (ASTM 2006*b*). All glochidia were <24-h old at the start of each experimental trial, and I used all gravid mussels within 1 to 14-d after collection from the wild. Viability of glochidia was determined by introducing a saturated NaCl solution to a subsample to observe the closure of valves (Zale and Neves 1982, ASTM 2006*b*). Only glochidia with ≥70% viability were thermally tested, except *L. bracteata* from the Llano River (mean = 64.3, *SE* = 5%; Table 2.1).

Glochidia were acclimated to 27 °C and thermal tolerances were tested at a series of 4 temperatures ranging from 30–39 °C (Fig. 2.2) alongside a non-acclimated control (20 °C) (Pandolfo et al. 2010). The 27 °C acclimation temperature was determined based on aggregated mean summer water temperatures from the 5 study basins (27.1–29.8 °C) over a 10-year period (2007–2016) using data from the Texas Commission of Environmental Quality (TCEQ) and Surface Water Quality Monitoring Information System (SWQMIS). At the start of each trial, glochidia were acclimated from the holding temperature (20 °C) to the acclimation temperature (27 °C) by 1 °C/h increments; an additional 2-h acclimation period was used once the acclimation temperature was reached. Experimental tests were conducted in 100-mL beakers for 24-h in non-aerated, reconstituted hard water, as outlined by the ASTM (2006*a*,*b*)guidelines for glochidia with 3 replicates of each temperature (including the control). Specifically, beakers were placed in fiberglass water baths containing ~5 L of water and maintained at 1 of 4 experimental temperatures (Fig. 2.2) or a 20 °C control with a 300-watt titanium heater (Finnex, Countryside, Illinois) attached to a temperature controller (Aqua Logic, San Diego, California). Temperature in the water baths was verified using a YSI ProODO (YSI Inc., Yellow Springs, Ohio). At 24-h (i.e., conclusion of the test) viability was assessed for a subsample of ~50–250 glochidia by exposing them to a saturated

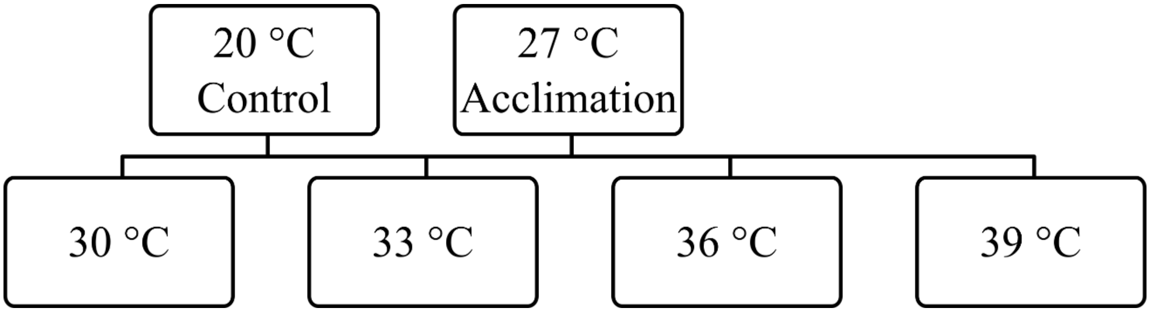

Figure 2.2. Experimental design following Pandolfo et al. (2010) showing acclimation and experimental temperatures. Experimental temperatures were assessed alongside a non-acclimated 20°C control.

NaCl solution and then examining their responses under an Olympus SZ51 microscope (Olympus America, Center Valley, Pennsylvania). Glochidia that exhibited a shell-closure in response to the NaCl solution were considered viable while those that did not close their shell were considered non-viable (ASTM 2006*b*). For a subset of 4 populations (2 species), I also assessed viability at 12-h in addition to the 24-h subsample.

Statistical analyses

LT50/05 (lethal temperatures resulting in 50% or 5% mortality) and their 95% confidence intervals (CIs) were determined by a 2-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. Statistical comparisons of LT50/05 values across and within species were conducted using the confidence interval ratio test (Wheeler et al. 2006). This method compares the ratios of 2 LT50/05s (or any other ratio of lethality by temperature) to 1 or the log (L50 ratio) to 0. The 95% confidence intervals are then constructed, and if the CI does not contain 1 (or 0 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).

Results

Across species

Paired comparisons of 24-h LT50s and LT05s revealed significant differences in thermal tolerances across species (Tables 2.2 and 2.3; Fig. 2.3A). *Cyclonaias petrina* collected during the spring had the highest 24-h LT50 (36.4 ± 0.4 °C; LT50 ± 95% CI), while *A. plicata* had one of the lowest 24-h LT50 (28.3 ± 1.0 °C). Among the *Lampsilini* species, *L. bracteata* collected during the summer from the San Saba River had the highest 24-h LT50 (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 also was collected during the spring from Village Creek; and, finally, *L. teres* (31.1 ± 0.5 °C) which also was collected during the summer from the lower Colorado River. The 12-h LT50s also varied across species for which it was calculated. *Fusconaia mitchelli*, collected during the spring from the Lower Guadalupe, had the highest 12-h LT50 (36.0 ± 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). However, these differences were generally not significant (Fig. 2.3B), and for *L. bracteata* and *L. hydiana*, there were no differences in the corresponding LT50 estimates (Fig. 2.3B).

The 24-h LT05 differed across species, generally mirroring the results for LT50 estimates, but rank order from highest to lowest by LT05 was different. *Lampsilis*

Table 2.2. Matrix of confidence interval ratios of 24-h 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. Asterisk indicates 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 and are abbreviated as follows: AP = *A. plicata*, LB = *L. bracteata*, LH = *L. hydiana*, LS = *L. satura*, LT = *L. teres*, OA = *O. arkansasensis*, and CP = *C. petrina*. 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**
AP	Amblemini	AP (1)	Lower Guadalupe	Summer	28.3 ± 1.0	-	4.11*	6.47*	4.87*	5.51*
LB1	Lampsilini	LB (3)	Llano	Summer	32.4 ± 0.3	0.84–0.91	-	2.36*	0.76*	1.40*
LB2	Lampsilini	LB (3)	San Saba	Summer	34.7 ± 0.3	0.78–0.84	0.92–0.94	-	1.60*	0.96*
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
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	-
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
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
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
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
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
CP1	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
CP2	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

Table 2.2. Continued.

Trial	Tribe	Species (*n*)	Population	Season	LT50	LH1	LH2	LS	LT	OA
AP	Amblemini	AP (1)	Lower Guadalupe	Summer	28.3 ± 1.0	6.11*	5.42*	4.18*	2.83*	4.97*
LB1	Lampsilini	LB (3)	Llano	Summer	32.4 ± 0.3	2.00*	1.31*	0.06	1.28*	0.85*
LB2	Lampsilini	LB (3)	San Saba	Summer	34.7 ± 0.3	0.35	1.05*	2.29*	3.63*	1.50*
LB3	Lampsilini	LB (2)	Upper Guadalupe	Spring	33.1 ± 0.3	1.24*	0.55	0.70*	2.04*	0.09
LB4	Lampsilini	LB (3)	Cherokee	Spring	33.8 ± 0.2	0.60*	0.09	1.33*	2.68*	0.54*
LH1	Lampsilini	LH (2)	Lower Guadalupe	Summer	34.4 ± 0.3	-	0.69	1.94*	3.28*	1.15*
LH2	Lampsilini	LH (3)	Cibolo	Spring	33.7 ± 0.2	0.99–1.03	-	1.24*	2.59*	0.45
LS	Lampsilini	LS (3)	Village	Spring	32.5 ± 0.5	1.04–1.08	1.02–1.06	-	1.34*	0.79*
LT	Lampsilini	LT (3)	Lower Colorado	Summer	31.1 ± 0.5	1.09–1.12	1.06–1.10	1.02–1.07	-	2.13*
OA	Lampsilini	OA (3)	Village	Spring	33.2 ± 0.5	1.02–1.05	1.00–1.03	0.96–1.00	0.92–0.96	-
CP1	Quadrulini	CP (1)	Lower Guadalupe	Spring	36.4 ± 0.4	0.93–0.96	0.91–0.94	0.87–0.91	0.84–0.87	0.90–0.93
CP2	Quadrulini	CP (1)	Lower Guadalupe	Summer	26.9 ± 1.4	1.21–1.34	1.18–1.32	1.14–1.27	1.09–1.22	1.17–1.30

Table 2.2. Continued.

Trial	**Tribe**	**Species (*n*)**	**Population**	**Season**	**LT50**	**CP1**	**CP2**
AP	Amblemini	AP (1)	Lower Guadalupe	Summer	28.3 ± 1.0	8.14*	1.33
LB1	Lampsilini	LB (3)	Llano	Summer	32.4 ± 0.3	4.03*	5.44*
LB3	Lampsilini	LB (3)	San Saba	Summer	34.7 ± 0.3	1.67*	7.80*
LB4	Lampsilini	LB (2)	Upper Guadalupe	Spring	33.1 ± 0.3	3.27*	6.20*
LB5	Lampsilini	LB (3)	Cherokee	Spring	33.8 ± 0.2	2.63*	6.84*
LH1	Lampsilini	LH (2)	Lower Guadalupe	Summer	34.4 ± 0.3	2.03*	7.44*
LH2	Lampsilini	LH (3)	Cibolo	Spring	33.7 ± 0.2	2.72*	6.75*
LS	Lampsilini	LS (3)	Village	Spring	32.5 ± 0.5	3.96*	5.50*
LT	Lampsilini	LT (3)	Lower Colorado	Summer	31.1 ± 0.5	5.31*	4.16*
OA	Lampsilini	OA (3)	Village	Spring	33.2 ± 0.5	3.17*	6.29*
CP1	Quadrulini	CP (1)	Lower Guadalupe	Spring	36.4 ± 0.4	-	9.47*
CP2	Quadrulini	CP (1)	Lower Guadalupe	Summer	26.9 ± 1.4	1.28–1.42	-

Table 2.3 Matrix of confidence interval ratios of 24-h LT05 estimates for paired species comparisons acclimated to 27 °C. The confidence intervals are given below the diagonal, and the difference between LT05 estimates is given above the diagonal. Asterisk indicates the confidence interval of the ratio did not contain 1, and thus LT05 comparisons were significantly different. Species (n) provides the number of gravid females from which glochidia were collected for each species and are abbreviated as follows: AP = *A. plicata*, LB = *L. bracteata*, LH = *L. hydiana*, LS = *L. satura*, LT = *L. teres*, OA = *O. arkansasensis*, and CP = *C. petrina*. 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**	**LT05**	**AP**	**LB1**	**LB2**	**LB3**	**LB4**
AP	Amblemini	AP (1)	Lower Guadalupe	Summer	22.2 ± 2.0	-	3.37*	5.69*	5.00*	7.55*
LB1	Lampsilini	LB (3)	Llano	Summer	25.5 ± 0.8	0.78–0.95	-	2.32*	1.63*	4.17*
LB2	Lampsilini	LB (3)	San Saba	Summer	27.9 ± 0.4	0.72–0.87	0.88–0.95	-	0.69	1.86*
LB3	Lampsilini	LB (2)	Upper Guadalupe	Spring	27.2 ± 0.7	0.74–0.89	0.90–0.98	0.99–1.07	-	2.55*
LB4	Lampsilini	LB (3)	Cherokee	Spring	29.7 ± 0.4	0.68–0.81	0.83–0.89	0.91–0.97	0.88–0.94	-
LH1	Lampsilini	LH (2)	Lower Guadalupe	Summer	29.1 ± 0.6	0.69–0.83	0.84–0.91	0.93–0.99	0.90–0.97	1.00–1.05
LH2	Lampsilini	LH (3)	Cibolo	Spring	28.7 ± 0.6	0.70–0.84	0.86–0.92	0.94–1.00	0.91–0.98	1.01–1.06
LS	Lampsilini	LS (3)	Village	Spring	24.2 ± 1.1	0.82–1.01	1.00–1.12	1.09–1.21	1.06–1.18	1.17–1.29
LT	Lampsilini	LT (3)	Lower Colorado	Summer	22.1 ± 1.2	0.90–1.11	1.08–1.22	1.19–1.33	1.15–1.30	1.27–1.42
OA	Lampsilini	OA (3)	Village	Spring	24.2 ± 1.1	0.82–1.01	0.99–1.11	1.09–1.21	1.06–1.18	1.17–1.29
CP1	Quadrulini	QP (1)	Lower Guadalupe	Spring	27.4 ± 0.9	0.73–0.89	0.89–0.97	0.98–1.06	0.95–1.03	1.05–1.12

Table 2.3. Continued.

Trial	**Tribe**	**Species (*n*)**	**Population**	**Season**	**LT05**	**LH1**	**LH2**	**LS**	**LT**	**OA**
AP	Amblemini	AP (1)	Lower Guadalupe	Summer	22.2 ± 2.0	6.95*	6.51*	2.01	0.03	2.08
LB1	Lampsilini	LB (3)	Llano	Summer	25.5 ± 0.8	3.58*	3.13*	1.36	3.41*	1.29
LB2	Lampsilini	LB (3)	San Saba	Summer	27.9 ± 0.4	1.26*	0.82	3.68*	5.72*	3.61*
LB3	Lampsilini	LB (2)	Upper Guadalupe	Spring	27.2 ± 0.7	1.95*	1.50*	2.99*	5.03*	2.92*
LB4	Lampsilini	LB (3)	Cherokee	Spring	29.7 ± 0.4	0.60	1.04*	5.54*	7.58*	5.47*
LH1	Lampsilini	LH (2)	Lower Guadalupe	Summer	29.1 ± 0.6	-	0.44	4.94*	6.98*	4.87*
LH2	Lampsilini	LH (3)	Cibolo	Spring	28.7 ± 0.6	0.99–1.04	-	4.49*	6.54*	4.43*
LS	Lampsilini	LS (3)	Village	Spring	24.2 ± 1.1	1.14–1.26	1.13–1.25	-	2.04*	0.07
LT	Lampsilini	LT (3)	Lower Colorado	Summer	22.1 ± 1.2	1.24–1.39	1.22–1.37	1.02–1.17	-	2.11*
OA	Lampsilini	OA (3)	Village	Spring	24.2 ± 1.1	1.14–1.26	1.12–1.24	0.93–1.06	0.85–0.98	-
CP1	Quadrulini	QP (1)	Lower Guadalupe	Spring	27.4 ± 0.9	1.02–1.10	1.01–1.09	0.83–0.93	0.76–0.86	0.83–0.94

Table 2.3. Continued.

Trial	**Tribe**	**Species (*n*)**	**Population**	**Season**	**LT05**	**CP1**
AP	Amblemini	AP (1)	Lower Guadalupe	Summer	22.2 ± 2.0	5.23*
LB1	Lampsilini	LB (3)	Llano	Summer	25.5 ± 0.8	1.85*
LB2	Lampsilini	LB (3)	San Saba	Summer	27.9 ± 0.4	0.46
LB3	Lampsilini	LB (2)	Upper Guadalupe	Spring	27.2 ± 0.7	0.23
LB4	Lampsilini	LB (3)	Cherokee	Spring	29.7 ± 0.4	2.32*
LH1	Lampsilini	LH (2)	Lower Guadalupe	Summer	29.1 ± 0.6	1.72*
LH2	Lampsilini	LH (3)	Cibolo	Spring	28.7 ± 0.6	1.28*
LS	Lampsilini	LS (3)	Village	Spring	24.2 ± 1.1	3.22*
LT	Lampsilini	LT (3)	Lower Colorado	Summer	22.1 ± 1.2	5.26*
OA	Lampsilini	OA (3)	Village	Spring	24.2 ± 1.1	3.15*
CP1	Quadrulini	QP (1)	Lower Guadalupe	Spring	27.4 ± 0.9	-

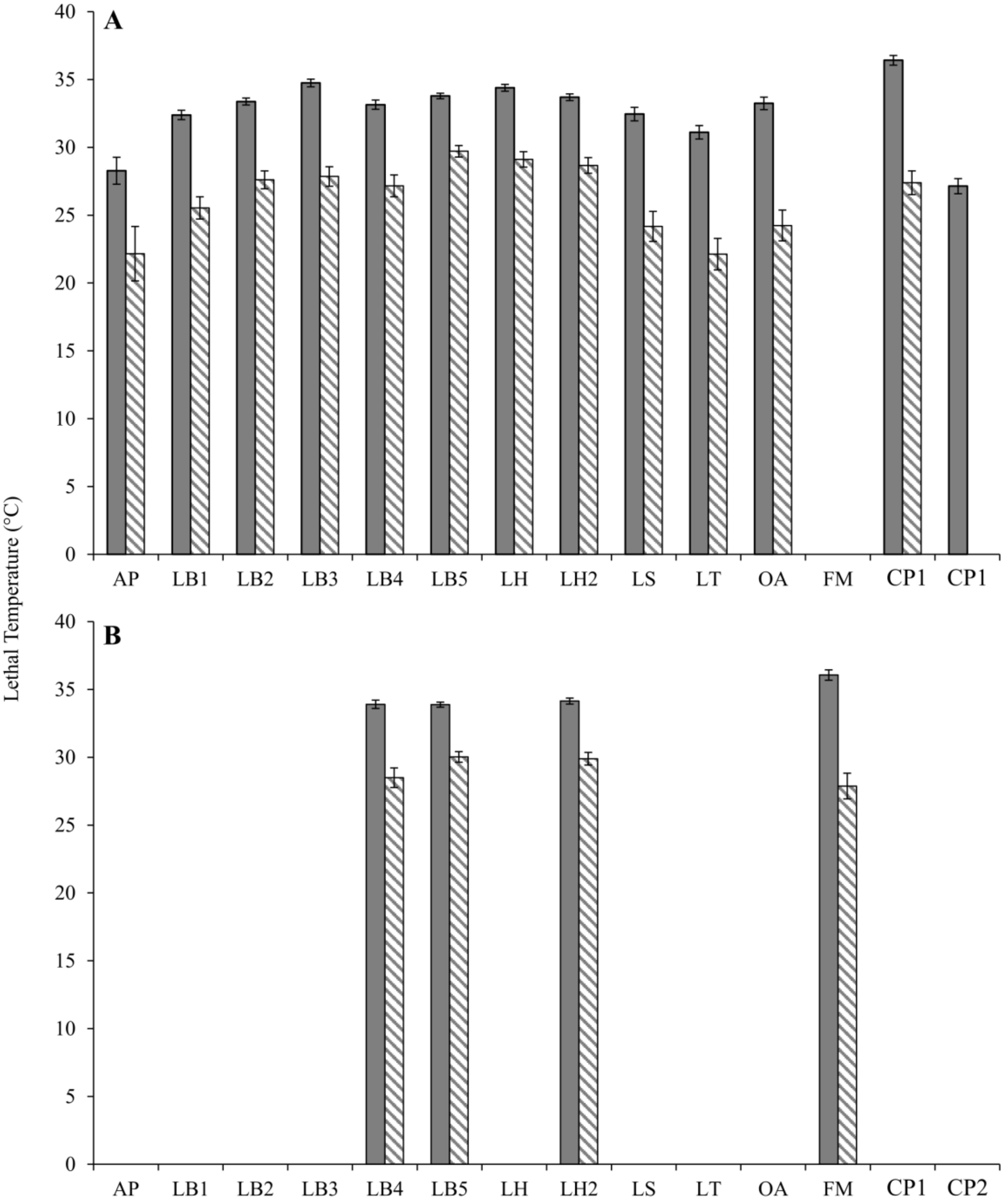

Figure 2.3. Comparison of (A) 24-h and (B) 12-h LT50 (solid bar) and LT05 (hatched bar) estimates at 27 °C acclimation. Error bars denote 95% confidence intervals.

bracteata collected during the spring from Cherokee Creek had the highest LT05 value (29.7 ± 0.4 °C), while *A. plicata* (22.2 ± 2.0 °C) and *L. teres* (22.1 ± 1.2 °C) had the lowest LT05. *Cyclonaias petrina*, which had the highest estimated LT50, had an LT05 of 27.4 (±0.9) °C. The 12-h LT05s were similar to the 12-h LT50 estimates in that they varied by species, although this difference was generally not significant nor was it significantly different from the corresponding 24-hr LT05 estimates. The 12-h LT05s also mirrored the results of the 24-h LT05s in that there was a reversal in rank order between Pleurobemini or Quadrulini and Lampsilini species as it relates 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.

Within species

Comparing LT50/LT05 values within species, I found that for *L. bracteata*, the LT50s 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 (34.7 ± 0.3 °C); followed by Cherokee Creek (33.8 ± 0.2 °C); followed by the Upper Guadalupe (33.1 ± 0.3 °C); and finally the Llano River (32.4 ± 0.4 °C). The 24-h LT05 across the same populations followed a similar pattern, although onset of mortality occurred much sooner, between 25.5 (Llano population) and 29.7 °C (Cherokee Creek population). Comparing the effect of seasonality on LT50/LT05 estimates, I found that for *C. petrina*, there were significant differences between spring and summer. Specifically, the 24-h LT50 for *C. petrina* collected during the spring was

36.4 ± 0.4 °C, while the same population sampled during the summer had a 24-h LT50 of 26.9 ± 1.4 °C.

Discussion

My study is the first to report lethal thermal tolerances for early life stages of mussels occurring in the southwestern United States. The results of my study along with others in recent years (e.g., Pandolfo et al. 2010, Ganser et al. 2013, Archambault et al. 2014, Martin 2016) show that upper thermal limits differ among species, but overall, that LT50 and LT05 values are similar across regions, with 24-h LT50s averaging 31.6 °C (21.4 – 37.2 °C) and 24-h LT05s averaging 27.9 °C (15.6 – 36.7 °C; Pandolfo et al. 2010, Archambault et al. 2014). These results suggests that while there may be some limited regional 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 and Graf 2009). Similar observations have been made for marine mollusks (Bayne 1965, 1976, Zippay and Hofmann 2010). I also found the difference between LT50s and LT05s by species was narrow, ranging 3.9 to 9.0 °C, which suggests that a relatively small increase in water temperature can result in mortality, which mirrors other glochidia thermal tolerance studies (Pandolfo et al. 2010, Archambault et al. 2014). Prior to onset of mortality, or within the window between LT05 and LT50 estimates, it is likely that sub-lethal effects also are occurring, which could reduce fitness in later life stages. For example, the larval stages of marine invertebrates subjected to sub-lethal thermal stress have been shown to have decreased juvenile survival and growth, with these effects often extending into adulthood (Delorme and Sewell 2014, Kessel and Phillips 2018). Finally, I also found reversals in rank order between Pleurobemini or Quadrulini and Lampsilini species as it relates to extreme LT50 and LT05 estimates, which suggests that onset of lethal and sub-lethal 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 LT05s may confer protection to gravid females and their brood.

Within-species variation in thermal tolerance has been documented for many marine and freshwater taxa (Urban 1994, Sorte et al. 2011), although it has not been well studied in unionid mussels (but see Doucet-Beaupré et al. 2010, Galbraith et al. 2012). In my study, I found that *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, initial viability from the Llano population was low (64.4%) compared to that of the San Saba River (79.6%), and although I adjusted survival data to correct for <100% viability, reduced glochidial fitness could have affected the calculated median lethal temperature. This difference in thermal tolerance could be the result of where these sites are located (i.e., stream position) within these rivers 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 their genetic structure (Kentaro Inoue, pers.

comm), morphology of their mantle lures (Howells et al. 2011), and timing of brooding and spawning (Seagroves 2017).

Seasonality in water temperature likely plays an important role in regulating mussel metabolic rates (Baker and 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 my study, I found within-species differences for glochidial LT50s and LT05s based on the time of year gravid females were collected. Specifically, for *C. petrina*, both the LT50s and LT05s 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 to 29 °C in the summer. This difference suggests that thermal tolerance of glochidia varies seasonally, at least for this species, and likely stems from energetic trade-offs between survival, growth and reproduction to maximize fitness (Cody 1966, Macarthur and Levins 1967). Thus, for brooding female mussels, energy normally allocated to offsetting the effects of elevated water temperature is tied up in reproduction, resulting in lower upper thermal limits during summer months (Pörtner et al. 2006). Similarly, among marine mollusks thermal stress can have transgenerational effects whereby sub-lethal environmental factors impacting the parental generation can impact the survival or growth of offspring negatively (Kessel and Phillips 2018).

While I was able to identify upper thermal tolerances for a suite of mussel species in the southwestern United States, some of which are highly imperiled, there were a few shortcomings in my approach, which I adapted from other published studies and guidelines (e.g., ASTM 2006*b*, Pandolfo et al. 2010, Archambault et al. 2014). First, the length of time 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 species (e.g., Zimmerman and Neves 2002, Ingersoll et al. 2006, Akiyama and Iwakuma 2007); however, for toxicity tests, a duration of 24-h is recommended (ASTM 2006*b*, Cope et al. 2008). Based on my 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). For example, I found the viability of *F. mitchelli* was >80% at 12-h, but dropped to <40% by 24-h, which precluded accurate estimation of 24-h median lethal temperatures. Second, species within Pleurobemini and Quadrulini produce conglutinates, which are aggregated glochidia surrounded by a membrane that are then ingested by a fish host (Barnhart et al. 2008). Viability of glochidia within a conglutinate is typically higher than the viability of free glochidia, which suggests that conglutinate membranes could confer protection (Fritts et al. 2014). For example, conglutinates of *Ptychobranchus jonesi* maintained >90% viability for 3-d and only dropped below 75% by day 10 (McLeod et al. 2017). Thus, thermal tolerance testing of free glochidia for species that produce conglutinates may not be entirely accurate, suggesting that thermal testing should include both conglutinates and free glochidia, not just one or the other.

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 (Stillman

2003, Deutsch et al. 2008), due in part to their inability to acclimate to rapidly changing temperature regimes. The results of my study show that several of the focal species may already be experiencing temperatures near their upper thermal limits. In central Texas rivers, where most of my study sites were located, summer maximum water temperature ranges from 30–34 °C, which are close to or exceed 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 catastrophic, which is likely 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 (TWDB 2016). Thus, future increases in population growth coupled with climate change, which is predicted to be severe for the southwestern United States (2–5 °C increase by 2100; Jiang and Yang 2012), will likely 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 semiarid regions will likely be further imperiled as human demand for water continues to increase.

The results of my 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 et al. 2014). However, thermal tolerance data for mussels, and their host-fish, has yet to be fully considered within this framework. Thus, it is likely that current environmental flow standards are not protective of mussels, particularly during subsistence flows when water depth and velocity are at their lowest. Thermal tolerance data from studies such as this one 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. In turn, this information could provide guidance on flows required by mussels to maintain and protect population performance (i.e., growth, survivorship, and reproduction) to ensure long-term persistence. Additionally, 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 to evaluate potential consequences of climate and land use changes on mussel populations or critical habitat.

CHAPTER III

UPPER THERMAL LIMITS OF ADULT FRESHWATER MUSSELS FROM THE GUADALUPE RIVER

Introduction

Water temperature is an important abiotic driver of aquatic ecosystems and directly impacts metabolic rates, physiology, and life history traits of ectotherms (Vannote and 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 and 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 (Helmuth et al. 2002, Brown et al. 2004).

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 and Naiman 2010, Somero 2010). Knowledge of sublethal and lethal effects from changes in water temperature are unknown for a number of aquatic species, however, 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 southwestern United States.

Freshwater mussels are one of the most imperiled aquatic faunas, due in part, to changes in water temperatures (Pandolfo et al. 2010, Galbraith et al. 2012). 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 and Warren, Jr. 1998) and limited mobility as adults (Amyot and Downing 1997). Elevated water temperature can directly affect mussels by causing mortality or result in sub-lethal effects such as altering oxygen consumption and metabolic demands (Rodland et al. 2008, Spooner and Vaughn 2008, Pandolfo et al. 2009), which, in turn, can diminish the ecosystem services provided by mussels (e.g., nutrient cycling; Vaughn and Hakenkamp 2001, Vaughn 2008). Elevated water temperature also can negatively impact growth and reproduction such as gamete development, glochidial release, and host-fish interactions (Watters and O'Dee 2000, Baker and Hornbach 2001, Galbraith and Vaughn 2009, Gascho Landis et al. 2012), which over time can lead to population declines.

Despite the importance of water temperature on mussels, quantitative information on lethal temperatures has been limited to 15 species and none of these species are from arid or semi-arid regions such as Texas (Dimock and Wright 1993, Pandolfo et al. 2010, Archambault et al. 2014, Ganser et al. 2015, Martin 2016). The species that have been tested are from the midwest or southeastern United States where flow and water temperature regimes differ from those in arid and semi-arid regions. Because of this, the portability of those results to other species or populations of the same species, but in different regions is unknown. This concern is likely well supported given that recent studies have shown that mussel sensitivity to water temperature and waterborne contaminants can vary widely across species (Wang et al. 2007, Gillis et al. 2008), which would suggest thermal sensitivity of mussel species from arid and semi-arid regions may not be well predicted by the sensitivity of mussel species from other geographic regions.

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 my study was to estimate the upper thermal limits of 3 mussel species, one of which is a species of high conservation concern, representing 3 tribes of the Unionidae Family (Graf and Cummings 2007), and therefore 3 potentially different evolutionary and physiological adaptions to thermal stress, from the Guadalupe River of central Texas. The specific objectives of my 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 my 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.

Methods

Study area

My study was conducted in the lower Guadalupe River within the Floodplains and Low Terraces of the Western Gulf Coastal Plain ecoregion (Griffith et al. 2007; Fig. 3.1). This portion of the Guadalupe River can experience high rates of precipation, resulting in short, high-magnitude flow events interspersed with longer periods of low flow (Perkin and 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 7 mainstem impoundments, including Canyon Lake reservoir, a deep storage bottom release reservoir (Perkin and Bonner 2011). As a consequence, the natural flow regime in this reach, to include temperature, has been altered (Phillips 2012). Average water temperatures in the lower Guadalupe are approximately 30 °C in July and 13 °C in January, though temperatures can reach up to 38 °C during summer months (Young et al. 1972, Phillips 2012).

Study animals

I examined thermal tolerances for adults of 3 species representing 3 tribes (Amblemini, Pleurobemini, and Quadrulini) in the Family Unionidae (Williams et al. 2017): *Amblema plicata, Cyclonaias petrina*, and *Fusconaia mitchelli* (Table 3.1). *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

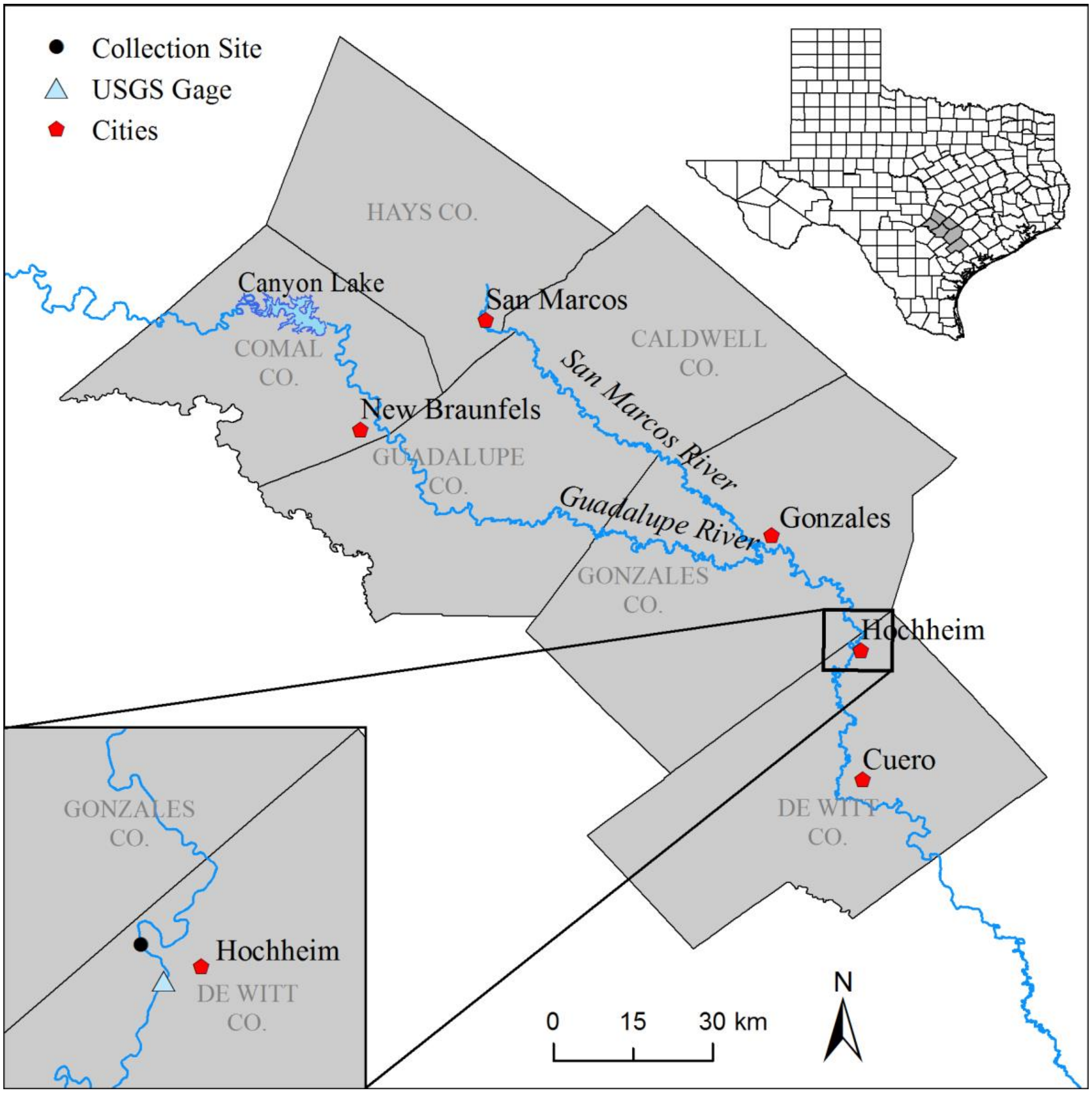

Figure 3.1. Map of study site, temperature logger location, and USGS gaging station

Table 3.1. Summary of test species (*Amblema plicata*, *Cyclonaias petrina*, and *Fusconaia mitchelli*) and water quality parameters during 10-d trials. Test temperatures are the acclimation temperatures under which each species was tested. Collection date is the date mussels were collected from the field. The *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 treatment and acclimation temperatures. Length and wet weight are mean ± *SE* for all organisms in each thermal trial. Water quality parameters (DO, ammonia, hardness, alkalinity, and pH) are averaged (mean ± *SE*) across all test chambers.

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. petrina	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

Mississippi River drainage (Mulvey et al. 1997). Previous studies in Oklahoma have found *A. plicata* to be thermally tolerant (Spooner and Vaughn 2008). *Cyclonaias petrina* and *F. mitchelli* are Texas endemics with narrow distributions. Both are currently state-listed and are being considered for listing under the Endangered Species Act (ESA; TPWD 2010, USFWS 2011). *Cyclonaias petrina* is known historically from the Colorado and Guadalupe River drainages of central Texas (Howells 2002, 2010), while *F. mitchelli* historically occurred in the Brazos, Colorado, and Guadalupe River drainages (Pfeiffer et al. 2016).

I collected adult mussels from the lower Guadalupe River (near Hochheim, Texas) in September–December 2016 and 2017, except for *F. mitchelli*, which I collected in April 2017 (Table 3.1). *Amblema plicata* were collected twice: once during a winter acclimated, non-reproductive period and a second collection following the end of their reproductive season. Following collection, I transported adult mussels to the Texas A&M AgriLife Extension and Research Center in Dallas, Texas in insulated coolers. Upon arrival in the laboratory, I held mussels at a holding temperature 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; Campbell, California).

Experimental design

For the winter collected *A. plicata*, I randomly assigned adult mussels to 1 of 3 different acclimation treatments (23°C, 27°C, or 30°C), each consisting of 5 experimental temperatures increased by 3°C increments (Fig. 3.2) and assessed

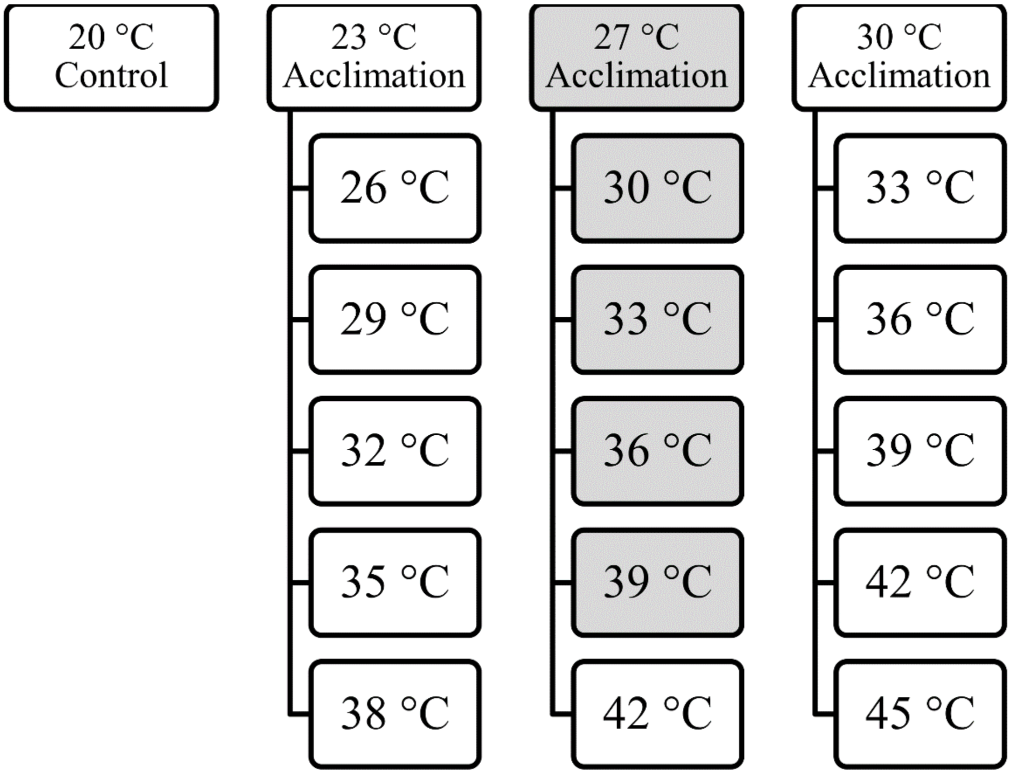

Figure 3.2. Experimental design showing initial acclimation and experimental temperatures based on Pandolfo et al. (2010) to include final trials (shaded gray). All experimental temperatures were assessed alongside a non-acclimated 20°C control.

alongside a non-acclimated control (20 °C) (Pandolfo et al. 2010). Different acclimation temperatures were examined to determine whether or not it affected thermal tolerance. Analyses of upper thermal limits across the 3 acclimation treatments showed no significant differences. As a result, I concluded that acclimation temperature does not affect the thermal tolerance of adult mussels, which mirrors results for glochidia and juvenile life stages (Pandolfo et al. 2010, Archambault et al. 2014). Based on those results, I chose an acclimation temperature of 27°C for all subsequent trials with a series of 4 temperatures ranging from 30-39°C (Fig. 3.2). The rationale for these temperatures is they correspond to the actual breadth of temperatures encountered by mussels in the Guadalupe River during the warmest months (range: 24.4–33.4 °C, based on 10 years [2007–2016] of Texas Commission of Environmental Quality (TCEQ) data from the Surface Water Quality Monitoring Information System (SWQMIS)). Adults were acclimated at a rate of <3 °C/d to the acclimation temperature of 27°C, once at that temperature individuals were then held for 96-h prior to testing. Mussels were then randomly assigned to a specific test temperature (Fig. 3.2).

During testing, mussels were placed in fiberglass tanks (91 × 61 × 46 cm) each partitioned into 3 water-tight chambers by 0.56-cm acrylic sheets. Thus, each tank represented 1 test temperature replicated up to the 3 times. Experimental chambers were filled with ~38 L reconstituted hard water, which was maintained at a given experimental temperature with a 300-watt titanium heater (Finnex; Countryside, Illinois) using a temperature controller (Aqua Logic; San Diego, California). Within each tank, individuals were placed in separate 946-mL food storage containers fitted with 6-mm plastic netting (*n* = 5 per chamber for winter collected *A. plicata* and *n* = 4 for all others; Table 3.1) to allow water circulation. Tests were 10-d in duration and aerated, using air stones to ensure the saturation of dissolved oxygen (DO) remained above 6 mg/L, with 50 % water renewal at 96 and 168 h, including the control. Water temperature and DO were monitored daily with a YSI ProODO (YSI Inc.; Yellow Springs, Ohio). Mussel survival was assessed at 3-h intervals for the first 24-h and at 12-h intervals for the remainder of the experiment. Mussels showing gaped behavior or those unresponsive to gentle probing to elicit foot retraction or valve closure were considered moribund.

In situ water temperature monitoring

To evaluate whether water temperatures were in exceedance of estimated upper thermal tolerances, water temperatures at the collection site were monitored using iButton temperature loggers (DS1922L, Maxim Integrated; San Jose, CA). iButtons were water proofed following Lautz et al. (2010) and then attached to a composite stake, which was then inserted into the river bottom at the collection site. Loggers were deployed from April 2016 to March 2018, which encompassed stream temperatures for 2 summers, and recorded temperature at 20 minute intervals to 0.5 °C resolution. In addition, I monitored flow (depth) at the collection site using a Hobo level logger (Onset Computer Corporation, Bourne, MA). Water level loggers were housed in a perforated PVC pipe secured to a rebar stake, which was then inserted into the river bottom. The water level logger was deployed from November 2016 to March 2018 and recorded at 15-minute intervals to a 0.05-m resolution once calibrated.

Statistical analyses

LT50/05 (lethal temperatures resulting in 50% or 5% mortality) and their 95% confidence intervals (CIs) were determined by fitting a 2-parameter logistic regression curves to survival data. Statistical comparisons of LT50/05 values across and within species were conducted using the confidence interval ratio test (Wheeler et al. 2006). This method compares the ratios of 2 LT50/05s (or any other ratio of lethality by temperature) to 1 or the log of the L50 ratio to 0. The 95% confidence intervals are constructed, and if the CI does not contain 1 (or 0 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 are in exceedance of estimated upper thermal limits, I performed a frequency and duration analysis following Castelli et al. (2012) using the LT50/05 and *in situ* water temperature data. 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., LT50/05 values). The resulting data can then be categorized into either “persistent” or “catastrophic” periods based on the frequency, duration and magnitude of high temperatures events. For the UCAT approach, persistent events are those that occur frequently (e.g., 2 to 3 times per year), but their duration and magnitude is less severe than catastrophic events, which are infrequent, but long in duration and magnitude. In my study, I converted 2 years (April 2016–March 2018) of sub-daily (every 20 min) water temperature data to daily mean data. From this data, I plotted the cumulative duration of continuous events that were above estimated LT50 and LT05 values determined from the acute (96-h) and chronic (10-d) trials. The resulting data was then plotted by calculating the cumulative frequency of the proportion events of the same duration (i.e., number of days) that exceeded a given threshold on the *x*-axis and the duration of a continuous event (i.e., number of days) on the *y*-axis. Based on the shape of the resulting plots inflection points were then used to identify persistent and catastrophic durations. To determine the range of discharge (CMS) for either the persistent or catastrophic conditions, I related temperature data from the site to discharge/water depth data from a U.S. Geological Survey gaging station located approximately 1.6 river kilometers downstream (08174700 near Hochheim, Texas). To do this, I related sub-daily (every 15 min) measures of water depth made at the collection site using level loggers to discharge/water depth from the down downstream USGS gage for the same period. I then calculated associated daily flow statistics (mean, median, and range) for each persistent and catastrophic duration event identified in the UCAT analysis.

Results

Lethal temperature exposures

The 24, 48, 96-h and 10-d LT50 and LT05 values and their 95% confidences limits for all species are summarized in Table 3.2. Water quality data were within ranges recommended by ASTM (2006*b*) and were similar among temperature treatments and between tests (see Table 3.1), and control survival was 100% across all trials on day 10.

Table 3.2. LT50 and LT05 values and their associated 95% confidence intervals (where given by logistic regression) for all test species (winter- and summer-collected *A. plicata*, *C. petrina*, and *F. mitchelli*) and acclimation temperatures (23, 27, and 30 °C) at 24, 48, 96, and 10-d (240-h) time intervals. LT50/LT05 = Lethal Temperatures at 50% and 5% mortality, respectively. Asterisk denotes confidence limits provided by consecutive lower temperatures (100% survival) and upper temperatures (100% mortality) where at time intervals where no partial mortality occurred.

Species	**Acclimation Temperature**	**LT50**			
		24-h	**48-h**	**96-h**	**10-d**
Winter *A. plicata*	23	36.5 (35.0–38.0)*	36.5 (35.0–38.0)*	36.5 (35.0–38.0)*	36.5 (35.0–38.0)*
	27	37.5 (36.0–39.0)*	37.5 (36.0–39.0)*	36.6 (33.6–39.7)	36.3 (34.5–38.0)*
	30	38.7 (36.8–40.6)	37.5 (36.0–39.0)*	37.5 (36.0–39.0)*	37.5 (36.0–39.0)*
Summer *A. plicata*	27	37.5 (36.0–39.0)*	37.5 (36.0–39.0)*	37.5 (36.0–39.0)*	36.6 (33.4–39.7)
C. petrina	27	37.5 (36.0–39.0)*	37.5 (36.0–39.0)*	36.4 (33.8–39.1)	36.2 (34.5–37.9)
F. mitchelli	27	36.2 (34.6–37.8)	34.5 (33.0–36.0)*	33.7 (32.6–34.7)	32.4 (31.1–33.6)

Table 3.2. Continued.

Species	**Acclimation Temperature**	**LT05†**			
		24-h	**48-h**	**96-h**	**10-d**
Winter *A. plicata*	23	36.1 (35.0–38.0)*	36.1 (35.0–38.0)*	36.1 (35.0–38.0)*	36.1 (35.0–38.0)*
	27	37.2 (36.0–39.0)*	37.2 (36.0–39.0)*	35.9 (35.3–36.5)	35.5 (32.3–38.7)*
	30	37.9 (30.8–45.1)	37.2 (36.0–39.0)*	37.2 (36.0–39.0)*	37.2 (36.0–39.0)*
Summer *A. plicata*	27	37.2 (36.0–39.0)*	37.2 (36.0–39.0)*	37.2 (36.0–39.0)*	35.9 (35.0–36.7)
C. petrina	27	37.2 (36.0–39.0)*	37.2 (36.0–39.0)*	35.7 (33.5–37.9)	35.4 (30.0–40.9)
F. mitchelli	27	35.4 (30.4–40.5)	34.2 (33.0–36.0)*	30.5 (28.6–32.4)	28.4 (26.0–30.9)

Acute 96-h 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-d 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.

Fusconaia mitchelli had significantly lower acute (96-h) and chronic (10-d) LT50 and LT05 than *A. plicata* or *C. petrina* (Tables 3.3 and 3.4; Fig. 3.3). Additionally, both acute and chronic LT50s and LT05s declined with time and were lower on day 10 for *F. mitchelli*, though not significantly. The 96-h and 10-d LT50/05s did not differ significantly between *A. plicata* collected during either of the 2 seasons or *C. petrina* (Tables 3.3 and 3.4). Very little mortality was recorded for *A. plicata* and *C. petrina* at 36 °C, and no mortality occurred in the 30 or 33 °C experimental temperature treatments, regardless of treatment. However, for *F. mitchelli* the 36 °C treatment resulted in 100% mortality within 48-h, and by 10 days, low to moderate mortality had occurred for the 30 and 33 °C (25 and 42%, respectively). The mean difference between LT50 and LT05 values within species was 0.9 °C and ranged from 0.2 to 3.9 °C.

UCAT analysis

At the collection site, mean annual water temperature was 23.0 °C and daily means ranged from 8.8 to 32.5 °C during the 2-year period of record. Monthly mean summer temperatures (June–August, 2016–2017) ranged from 26.6–31.8 °C and highest mean daily water temperatures occurred in July and August of 2017, averaging 31.8 and 31.4 °C, respectively. The highest absolute value recorded was 33.7 °C, which occurred in July of 2017. Mean daily temperatures did not exceed the LT50 threshold

Table 3.3. Matrix of confidence interval ratios of 96-h LT50 estimates for all trials. The confidence intervals of the ratio are given below the diagonal and the difference between LT50 estimates is given above the diagonal. Asterisk indicates the confidence interval of the ratio did not contain 1, thus LT50 comparisons were significantly different. Species are abbreviated as follows: AP (W) = winter collected *A. plicata*, AP (S) = summer collected *A. plicata*, CP = *C. petrina*, and FM = *F. mitchelli*. Acclimation is the temperature (°C) to which mussels were acclimated. Duration is the time interval (hours) for which LT50s were estimated.

Duration	**Species**	**Acclimation**	**LT50 (95% CI)†**	**96-h**						**240-h (10-d)**	
				AP (W)	**AP (W)**	**AP (W)**	**AP (S)**	**CP**	**FM**	**AP (W)**	**AP (W)**
				23	**27**	**30**	**27**	**27**	**27**	**23**	**27**
96	**AP (W)**	**23**	36.5 (35.0–38.0)†	-	0.16	1.00	1.01	0.06	2.79	0.00	0.20
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
96	**AP (W)**	**30**	37.5 (36.0–39.0)†	-7.30–9.25	-7.30–9.25	-	0.01	1.07	3.79	1.00	1.20
96	**AP (S)**	**27**	37.5 (36.0–39.0)†	-0.56–2.51	-0.34–2.30	-7.57–9.57	-	1.07	3.80	1.01	1.21
96	**CP**	**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
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*
240	**AP (W)**	**23**	36.5 (35.0–38.0)†	-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
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	-
240	**AP (W)**	**30**	37.5 (36.0–39.0)†	-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
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
240	**CP**	**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
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

Table 3.3. Continued.

				240-h (10-d)			
Duration	**Species**	**Acclimation**	**LT50 (95% CI)†**	**AP (W)**	**AP (S)**	**CP**	**FM**
				30	**27**	**27**	**27**
96	**AP (W)**	**23**	36.5 (35.0–38.0)†	1.00	0.11	0.29	4.11
96	**AP (W)**	**27**	36.6 (33.6–39.7)	0.85	0.04	0.45	4.26*
96	**AP (W)**	**30**	37.5 (36.0–39.0)†	0.00	0.89	1.30	5.11
96	**AP (S)**	**27**	37.5 (36.0–39.0)†	0.01	0.90	1.30	5.12
96	**CP**	**27**	36.4 (33.8–39.1)	1.07	0.17	0.23	4.05*
96	**FM**	**27**	33.7 (32.6–34.7)	3.79	2.90	2.49*	1.32
240	**AP (W)**	**23**	36.5 (35.0–38.0)†	1.00	0.11	0.29	4.11
240	**AP (W)**	**27**	36.3 (34.5–38.0)	1.20	0.31	0.09	3.91*
240	**AP (W)**	**30**	37.5 (36.0–39.0)†	-	0.89	1.30	5.11
240	**AP (S)**	**27**	36.6 (33.4–39.7)	-7.65–9.70	-	0.40	4.22*
240	**CP**	**27**	36.2 (34.5–37.9)	-7.73–9.80	0.91–1.11	-	3.81*
240	**FM**	**27**	32.4 (31.1–33.6)	-8.64–10.96	1.02–1.24	1.05–1.19	-

†Confidence intervals (95%) could not be calculated because no partial mortality occurred, and limits represent consecutive lower temperatures (100% survival) and upper temperatures (100% mortality).

Table 3.4. Matrix of confidence interval ratios of 96-h LT05 estimates for all trials. The confidence intervals are given below the diagonal and the difference between LT05 estimates is given above the diagonal. Asterisk indicates the confidence interval of the ratio did not contain 1, thus LT05 comparisons were significantly different. Species are abbreviated as follows: AP (W) = winter collected *A. plicata*, AP (S) = summer collected *A. plicata*, CP = *C. petrina*, and FM = *F. mitchelli*. Acclimation is the temperature to which mussels were acclimated. Duration is the time interval (hours) for which LT05s were estimated.

Duration	**Species**	**Acclimation**	**LT05 (95% CI)†**	**96-h**						**240-h (10-d)**	
				AP (W)	**AP (W)**	**AP (W)**	**AP (S)**	**CP**	**FM**	**AP (W)**	**AP (W)**
				23	**27**	**30**	**27**	**27**	**27**	**23**	**27**
96	**AP (W)**	**23**	36.1 (35.0–38.0)†	-	0.21	1.10	1.03	0.46	5.65	0.00	0.64
96	**AP (W)**	**27**	35.9 (35.3–36.5)	0.16–1.85	-	1.30	1.24	0.26	5.45*	0.21	0.43
96	**AP (W)**	**30**	37.2 (36.0–39.0)†	-7.45–9.39	-7.37–9.30	-	0.07	1.56	6.75	1.10	1.74
96	**AP (S)**	**27**	37.2 (36.0–39.0)†	-0.60–2.55	-0.38–2.31	-7.76–9.77	-	1.50	6.68	1.03	1.67
96	**CP**	**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*	0.46	0.17
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*
240	**AP (W)**	**23**	36.1 (35.0–38.0)†	-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
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	-
240	**AP (W)**	**30**	37.2 (36.0–39.0)†	-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
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
240	**CP**	**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
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

Table 3.4. Continued.

Duration	Species	Acclimation	LT05 (95% CI)	240-h (10-d)			
				AP (W)	**AP (S)**	**CP**	**FM**
				30	**27**	**27**	**27**
96	**AP (W)**	**23**	36.1 (35.0–38.0)[†]	1.10	0.26	0.69	7.70
96	**AP (W)**	**27**	35.9 (35.3–36.5)	1.30	0.06	0.48	7.50*
96	**AP (W)**	**30**	37.2 (36.0–39.0)[†]	0.00	1.36	1.79	8.80
96	**AP (S)**	**27**	37.2 (36.0–39.0)[†]	0.07	1.30	1.72	8.73
96	**CP**	**27**	35.7 (33.5–37.9)	1.56	0.20	0.22	7.24*
96	**FM**	**27**	30.5 (28.6–32.4)	6.75	5.39*	4.96	2.05
240	**AP (W)**	**23**	36.1 (35.0–38.0)[†]	1.10	0.26	0.69	7.70
240	**AP (W)**	**27**	35.5 (32.3–38.7)	1.74	0.37	0.05	7.06*
240	**AP (W)**	**30**	37.2 (36.0–39.0)[†]	-	1.36	1.79	8.80
240	**AP (S)**	**27**	35.9 (35.0–36.7)	-7.93–10.01	-	0.42	7.44*
240	**CP**	**27**	35.4 (30.0–40.9)	-8.03–10.13	0.85–1.17	-	7.01*
240	**FM**	**27**	28.4 (26.0–30.9)	-10.00–12.62	1.15–1.37	1.03–1.47	-

[†] Confidence intervals (95%) could not be calculated because no partial mortality occurred, and limits represent consecutive lower temperature (100% survival) and upper temperature (100% mortality).

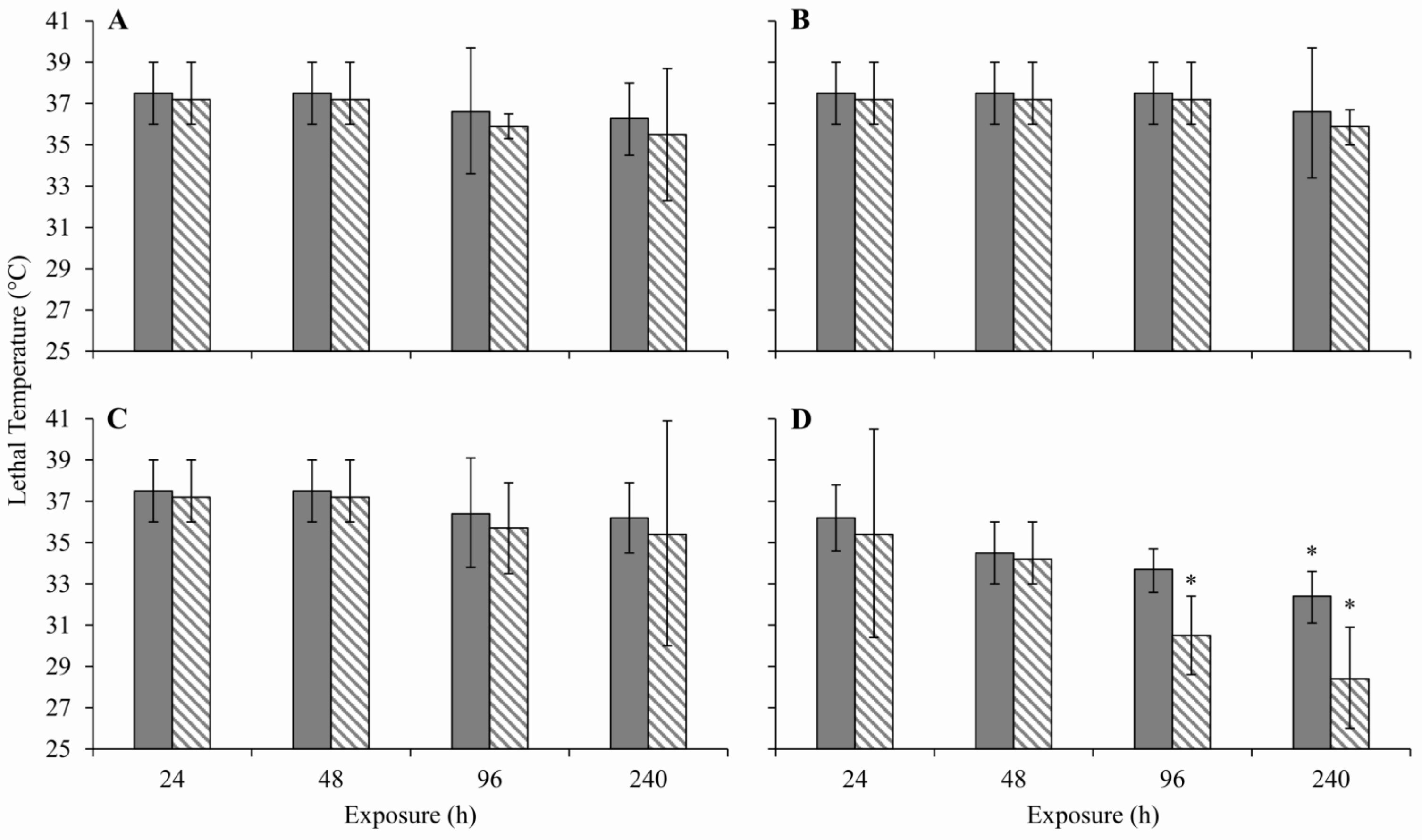

Figure 3.3. Comparison of LT50s (solid bar), LT05s (hatched bar), and their 95% confidence intervals (error bars) at 24, 48, 96, and 240-h time intervals shown for (A) *A. plicata* (winter), (B) *A. plicata* (summer), (C) *C. petrina*, and (D) *F. mitchelli* at 27 °C acclimation. Asterisk indicates a significant difference between LT50s or LT05s across time intervals based on the CI Ratio test ($p < 0.05$).

for any of the 3 species tested. However, mean daily temperatures were in exceedance of both the 96-h acute and 10-d chronic LT05 estimates for *F. mitchelli*. The 96-h acute threshold (31.2 °C) was exceeded in 4 separate events ranging from 3 to 30 days in duration, while the 10-d chronic threshold (28.4 °C) was exceeded in 3 separate events ranging from 32 to 85 days in duration (Fig. 3.4). Mean temperatures were similar for both persistent and catastrophic duration events (as defined by inflection points on the UCAT curves). However, catastrophic events had both a longer average duration and lower mean flows than persistent events (Table 3.5). Mean difference between catastrophic and persistent associated flows was 16.9 ± 4.8 $m^3 s^{-1}$ (mean ± *SE*).

Discussion

Mussels experience lethal and sub-lethal stress when exposed to elevated water temperatures and this response can vary widely across species (e.g., Spooner and Vaughn 2008, Pandolfo et al. 2010, Archambault et al. 2014, Martin 2016). However, thermal limits are not known for most mussel species, and factors affecting how thermal tolerance varies within species, between seasons and across reproductive periods is not well studied. Moreover, thermal limits of other mussel species may not translate to other species or locations. In my study, I am the first to report lethal thermal tolerances for adult mussels occurring in the southwestern United States and to demonstrate that geographical range and phylogeny may be important determinants of mussel thermal tolerance and as such should be considered when evaluating upper thermal limits for other mussel species.

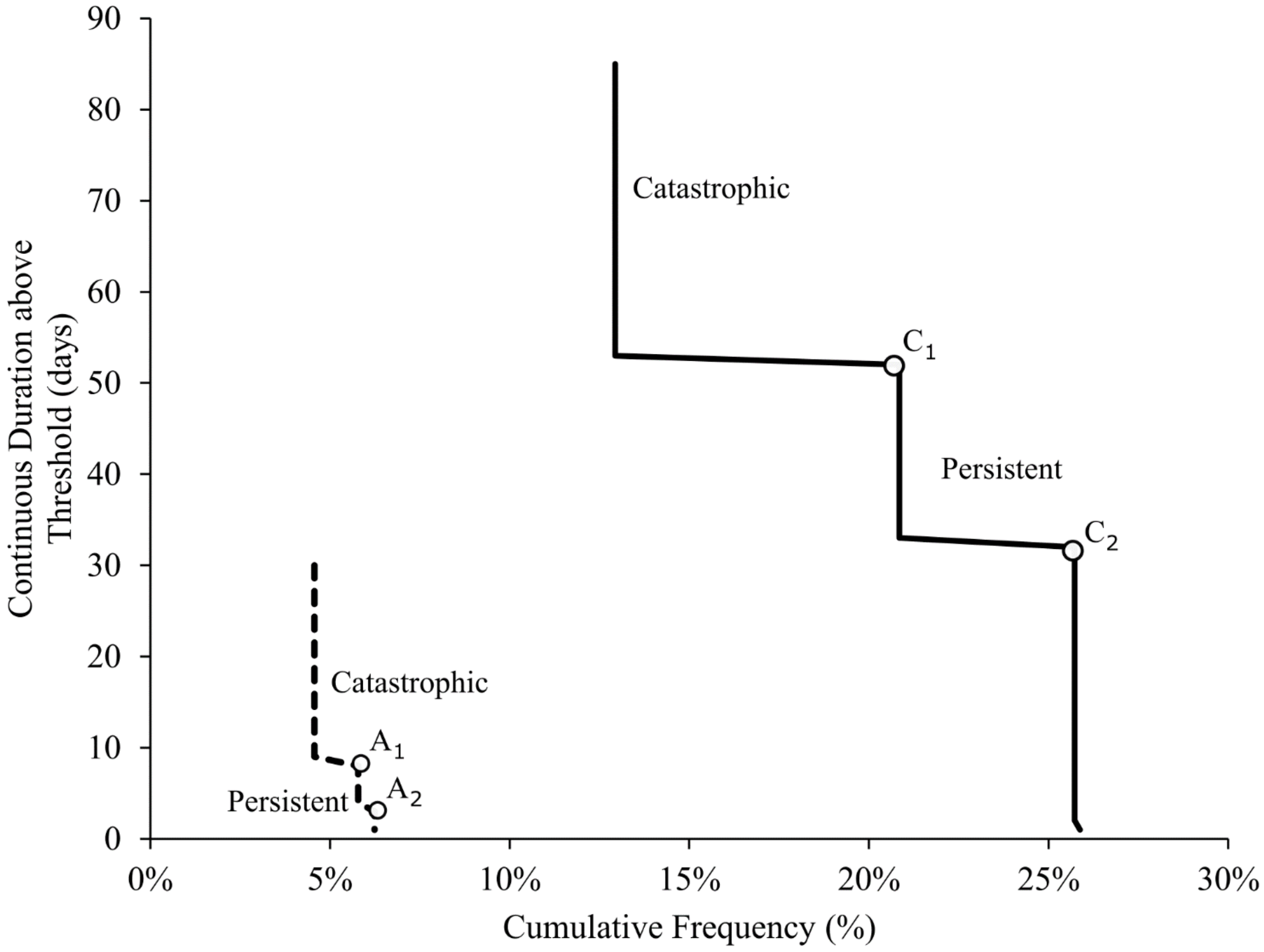

Figure 3.4. Cumulative frequency of events from April 2016–March 2018 in the Guadalupe River. UCAT curves represent acute (solid line) and chronic (dashed line) based on LT05 thermal thresholds for *F. mitchelli*.

Table 3.5. Mean daily statistics for water temperature and flow at the collection site in the lower Guadalupe River for persistent and catastrophic duration events using acute and chronic LT05 thresholds for *Fusconaia mitchelli*.

Threshold		**Duration**	**Temperature (°C)**			**Discharge (m^3 s^{-1})**		
			Mean	**Minimum**	**Maximum**	**Mean**	**Minimum**	**Maximum**
Chronic	Catastrophic	85	30.9	28.4	32.5	20.9	15.3	31.9
	Persistent	52	30.4	28.4	32.1	42.6	27.4	93.3
Acute	Catastrophic	30	31.9	31.2	32.5	18.2	15.3	21.6
	Persistent	8	31.7	31.4	32.1	30.3	27.4	31.8

My results show that freshwater mussel species can respond differently to thermal stress. In my study, I found adult *A. plicata* to be thermally tolerant with acute 96-h LT50s ranging from 36.3–37.5 °C across all acclimation and seasonal treatments, and the upper thermal limits I estimated mirror those of juveniles tested from the midwest (96-h LT50 = 36.4 °C at 27 °C acclimation; Archambault et al. 2014). This would suggest that mussel upper thermal limits may be fixed, regardless of geographic location or life stage, though size of geographical range could partially explain this result (see discussion on relationship between geographical range and thermal niche). I also found adult *C. petrina* to be thermally tolerant (96-h LT50 = 36.4 °C; 95% CI: 33.8–39.1 °C) and, as with *A. plicata*, my estimates are similar to those of glochidia (24-h LT50 = 36.4 °C at 27 °C acclimation; Jennifer Khan, Texas A&M University, unpublished data). This result provides further evidence that upper thermal limits of a mussel species are likely conserved across life stages, though in contrast to *A. plicata*, phylogeny and not geographical range may help explain overall thermal tolerance (see discussion on relationship between phylogeny and thermal niche). Finally, I found that *F. mitchelli* was the most thermally sensitive (96-h LT50 = 33.7 °C; 95% CI: 32.6–34.7 °C) of the species I tested and, compared to *A. plicata* and *C. petrina*, had the smallest overall geographic range.

Environmental temperatures play a pivotal role in determining species' distributions (e.g. Southward 1958, Merriam 1984), and an organism's thermal tolerance is a critical aspect of its physiological niche (Spicer & Gaston 1999). 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 not a consensus across taxa. For example, Calosi et al. (2008) found that a 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 et al. 2012), and for freshwater fish, temperature seems to be one of the main determinants of spatial distribution (e.g., Heino 2002). Among the study species, both *A. plicata* and *F. mitchelli* follow this pattern. *Amblema plicata* has a wide distribution across most of the midwest and southeast United States, while *Fusconaia mitchelli* is a Texas endemic with the narrowest range of the tested species.

Physiological limits, such as thermal tolerance, are often phylogenetically constrained (Chown et al. 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 *C. pustulosa* have found this species tolerant of short duration exposure to high water temperatures and emersion (Bartsch et al. 2000). Similarly, Spooner and Vaughn (2008), demonstrated that *C. pustulosa* clearance, biodeposition, and nutrient excretion rates 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. petrina. In my study for *C. petrina*, I estimated the 96-h LT50 was 36.4 °C (95% CI: 33.8–39.1 °C) and the 10-d LT50 was 36.2 °C (95% CI: 34.5–37.9 °C), which are similar, but slightly higher, than the temperature reported by Spooner and Vaughn (2008). This is not unexpected because their results reflect sub-lethal stress; whereas, my estimates are measuring acute and chronic lethality. Assuming Spooner and Vaughn's (2008) results are reflective of sub-lehtal thresholds for *C. petrina* this would then indicate the difference between sub-lethal and lethal stress is narrow, mirroring thermal studies of glochida and juveniles (Pandolfo et al. 2010). That is, relatively small increases in water temperature could quickly result in widespread mortality. Thermal tolerance also may be phylogenetically conserved for *Villosa delumbis* and *Villosa vibex*, based on the results of physiological biomarkers for thermal stress and acute LT50s (Pandolfo et al. 2010, Fritts et al. 2015). These results combined with my study provide initial evidence that overall physiological tolerances may be set at and conserved across major taxonomic groupings while specific limits are likely established through local adaptation (Somero 2010, Hoffmann et al. 2013). Thus, for species whose thermal tolerance has not been tested phylogeny could 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 I identified upper thermal limits for 3 mussel species, there were a few limitations in my study, which I adapted from other published studies and guidelines (e.g., ASTM 2006*b*, Pandolfo et al. 2010, Archambault et al. 2014). 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 my study, experimental units were aerated to control for dissolved oxygen, but because I used a static system water quality may not have been optimum and as a result biased my 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 (Ganser et al. 2015). Thus, in my 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 the case because I measured ammonia levels in the experiment and compared those results against published mussel ammonia toxicity studies (Augspurger et al. 2003, Newton et al. 2003) and renewed test water twice during the 10-d trial. That said, ammonia toxicity of mussels has not been studied for the species I studied, and so it could be that subtle increases in ammonia, even below published sub-lethal 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 I doubt this is an issue given the diet I used follows published studies and guidelines. Second, my threshold for sub-lethal 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 (Portner 2001). Thus, future studies should use more biologically based thresholds for sub-lethal effects such as respiration and/or metabolic activity, which would likely improve sub-lethal 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-d chronic duration of my study may not be sufficient for long-lived species such as mussels. Both *C. petrina* and *F. mitchelli* are moderately long-lived (> 10 years; J. Dudding, Texas A&M University, pers. comm.), while *A. plicata* can live upwards of 50 years (Haag and Rypel 2011), and so relative to their longevity the 10-d 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 Gatenby et al. 1996, Ganser et al. 2015). Finally, my 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.

Conservation implications

The analysis of thermal events in the lower Guadalupe River combined with the estimates of upper thermals demonstrate mussels are likely experiencing sub-lethal thermal stress at acute and chronic levels during summer months. Depending on when thermal stress occurs the resulting sub-lethal effects could affect population performance, and therefore long-term viability, by redirecting energy normally allocated to growth and reproduction towards maintenance (Portner 2001, 2002). Theoretically, these shifts in energy allocation could result in decreased fecundity and/or complete inhibition of reproduction as well as reduced and/or cessation of individual growth (Petes et al. 2007). Overtime, these effects would likely compound leading to population decline and if severe enough eventually extirpation and finally 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 I used follows that of Castelli et al. (2012), but other methods are available (see Maloney et al. 2012). Through the UCAT analysis I demonstrate that temperatures exceeding 31 °C (mean discharge: 20.9 $m^3\ s^{-1}$) 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. petrina*, upper thermal thresholds were higher (96-h LT05 = 35.9 and 35.7 °C, respectively), indicating thermal stress may not be a as frequent, but still likely an issue, especially with expected increases in population growth and climate change predictions for this region (Loaiciga et al. 2000). However, it is important to note that my results from the UCAT analysis may not be entirely accurate because they are derived from flow and water temperature data during 2 consecutive wet years. Prior to my study, the lower Guadalupe River experienced a severe 4-year drought that rivaled the drought of record (Verdon-Kidd et al. 2017). The results from my 2-year study period show that onset of catastrophic water temperature events (i.e., can negatively affect multiple generations) occur at flows around 20.9 $m^3\ s^{-1}$ and corresponded with the longest duration (85 consecutive days) of thermal exceedance during my study. In comparison, during 2011 (the most severe year of the drought) average discharge was 9.4 ± 0.2 m^3 s^-1, well below my estimated catastrophic threshold and flows during 2011 were at or below this threshold for 296 days. Based on these data, it is likely temperature threshold values during the drought (i.e., 2011–2015) were exceeded for all 3 species, which would indicate systemic and widespread thermal stress to all mussel species during this period, not just *F. mitchelli*.

The drought of 2011–2014 was extreme, although smaller droughts are common within this basin (Bowles and Arsuffi 1993). Overall, Texas experiences a mild drought 32% of the time, while moderate, severe, and extreme droughts occur 11% of the time (Verdon-Kidd et al. 2017). Since the turn of the 20th century, extreme hydrologic drought conditions have been recorded in Texas 6 times, with the most severe occurring in 2011. The data I collected provide information on mussel habitat in the Guadalupe basin during 2 non-drought years, which is important for baseline information. However, as this system is prone to droughts and to better manage the effects of these events on mussels, additional *in situ* water monitoring is needed to accurately assess thermal stress to mussels in this basin and to develop appropriate flow management conservation actions to help potentially mitigate this impact.

In Texas, there is a process set forth by Senate Bill 3 in 2007 for the development of flow standards that support a sound ecological environment by addressing water quantity and, to a lesser extent, quality issues. This process also is informed by technical guidance from the Texas Instream Flow Program (TIFP 2008) and the lower Guadalupe

River was included in this process with a study currently ongoing. However, thermal tolerances of mussels were not explicitly considered, so flow recommendations may not be sufficient to ensure long-term viability of the remaining populations. Based on my results, I have 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. However, before this information is used to prescribe flows it should first be linked to data on mussel habitat persistence (i.e., areas that remain stable during both low and high flows periods) to provide a holistic picture of mussel habitat that accounts for 2 broad ecological constraints: flows that are too high, resulting in bed scour, and those that are too low, resulting in thermal stress. Information on host fish behavior during high flow events and thermal tolerances during low flow could be integrated at a later date to further refine specific flow recommendations. Finally, long-term water temperature datasets at locations with significant mussel populations are scant, at least in places like Texas, and so to ensure environmental flow recommendations for mussels are accurate and to better understand how mussel population performance and persistence is influenced by water temperature long-term monitoring sites where continuous water temperatures is recorded is sorely needed.

CHAPTER IV

CONCLUSIONS

The results of my study indicate 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. Once specific thresholds have been identified, managers could then adopt a water management program specific to the river system in question. For rivers in arid and semi-arid regions of Texas, this approach would mean protecting the hydrologic regime from excessive extraction during periods of low flow and requiring environmental flows to maintain aquatic habitat.

However, thermal tolerance data for mussels has yet to be fully considered within this environmental flow framework. Thus, it is likely that current environmental flow standards are not protective of mussels, particularly during subsistence flows when water depth and velocity are at their lowest. Thermal tolerance data from my study 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. In turn, this information could provide guidance on flows required by mussels to maintain and protect population performance (i.e., growth, survivorship, and reproduction) to ensure long-term persistence.

In conclusion, the results of my study show some species are already living close to their upper thermal limits. The rivers and groundwater in central Texas and other arid or semi-arid regions of the Southwest face increased demands as rapid human population growth coupled with climate change will likely lead to more frequent and intense droughts and elevated stream temperatures in these systems. However, despite these conflicts, laboratory data on thermal tolerances may be used to generate target thermal thresholds, which will provide a useful management tool for maintaining stream temperature. The challenge lies in linking the experimental data of upper thermal limits to *in situ* water temperature data in a manner that realistically reflects the likely consequences of a change in thermal regime on aquatic biota. The UCAT analysis provides a quantitative metric for assessing potential risks to freshwater mussels and establishing protective thresholds. Additionally, thermal tolerance data combined with life-history information could provide insight into population viability under various types of flow management practices or as a way to evaluate potential consequences of climate and land use changes on mussel populations or critical habitat.

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