

## RESEARCH ARTICLE

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# Thermal tolerance exceedances of an endangered unionid mussel in the Rio Grande Basin, with implications for river management

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## Abstract

1. Freshwater mussels are globally imperilled, which is attributed to their sensitivity to changes in streamflow and temperature. The Black River in south-eastern New Mexico, USA, harbours a stronghold population of the federally endangered unionid *Popenaias popeii* (Texas hornshell). Decreasing discharge and elevated water temperatures are considered the primary factors responsible for the species' decline. However, the impact of these factors has not been quantified directly, which may hinder conservation efforts.
2. The upper thermal tolerances (LT<sub>05</sub> and LT<sub>50</sub>) of Black River *P. popeii* larvae (glochidia) and newly transformed juveniles were evaluated. Individuals were acclimated to 27°C and then immersed at five experimental temperatures (28, 30, 32, 34 and 36°C) for 24 h (glochidia) or 96 h (juveniles). The thermal tolerances of *P. popeii* and its presumed host fishes were then overlain onto *in situ* water temperature and discharge data to determine thermal exceedances in the past 15 years. Temperature was hindcast back to 2007 using machine learning (random forest model) and coupled with discharge.
3. For glochidia, LT<sub>05</sub> was exceeded frequently (41%) and LT<sub>50</sub> was exceeded occasionally (13%) during spring and summer periods, whereas juvenile thresholds were never exceeded. Upper continuous duration above threshold (UCAT) analysis revealed periods of catastrophic high temperature, which were used to determine discharge bottlenecks.
4. This study demonstrates how laboratory-derived physiological thresholds can be used in conjunction with environmental data to evaluate the hydrological needs of aquatic organisms, which is useful in efforts to maintain flow regimes that protect native ecosystems.

## KEYWORDS

climate change, conservation, environmental flow, hindcasting, machine learning, random forest, thermal tolerance, Unionidae, water management

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## 1 | INTRODUCTION

The natural flow regime, defined as the characteristic patterns of flow volume, timing, and variability of a river (Poff et al., 1997), plays a fundamental role in shaping the distribution and abundance of aquatic organisms. Human-mediated disruptions of the natural flow regime, combined with climate change, have led to changes in instream habitat, phenology, and interspecific relationships (Bunn & Arthington, 2002; Spooner et al., 2011; Krabbenhoft, Platania & Turner, 2014; Kjelland et al., 2015; Santos et al., 2017). These impacts, in turn, have resulted in species declines and changes in community composition (Dudgeon et al., 2006; Barnum, Weller & Williams, 2017; Perkin et al., 2017).

Changes in flow, such as the depletion of groundwater inputs or decreased discharge, can lead to shifts in water temperature that cause further impacts on aquatic species (Caissie, 2006; van Vliet et al., 2011; Kaandorp et al., 2019). In general, organisms possess thermal tolerances that determine growth, movement, and reproduction, making temperature an important determinant of a species' realized niche (Pörtner, Bock & Mark, 2017; Pörtner, 2021). For example, warming water temperatures have been shown to have adverse effects on the survival of larval dragonfly and shift the phenology of adult emergence (McCauley et al., 2015). In the Rocky Mountains, USA, the range of *Salvelinus confluentus* (bull trout) contracted owing to the fish abandoning warmer, low-altitude reaches more quickly than they colonized cooler, high-altitude reaches, as a result of water temperature increases induced by climate change (Eby et al., 2014). These types of range contractions are predicted to continue under future climate change (Comte et al., 2013; Comte & Grenouillet, 2013; Comte & Grenouillet, 2015; Rogers et al., 2020).

Freshwater mussels (Unionidae), hereafter unionids, are particularly sensitive to environmental change. Unionids are filter-feeding benthic ectotherms that require a host fish to reproduce (Haag, 2012). Owing to their relatively sedentary nature, mussels have a reduced ability to respond to changes in water quality, such as elevated temperatures above their thermal optima (Kappes & Haase, 2012). Unionids are therefore more vulnerable to environmental change than more mobile organisms. A study across European rivers of margaritiferid mussels, a sister family to unionids, found climate warming in the past 100 years has probably contributed to their decline by reducing suitable habitat, which is predicted to worsen under future climate change (Bolotov et al., 2018). In the southern USA, droughts have led to low flows and elevated water temperatures, causing substantial unionid die-offs (Gates, Vaughn & Julian, 2015). For example, species richness declined and community composition shifted to a lower proportion of thermally sensitive species and a higher proportion of thermally tolerant species in an Oklahoma, USA river during a period of drought and increased water extraction (Galbraith, Spooner & Vaughn, 2010).

Rivers in arid and semi-arid regions rely on precipitation events, which may become more infrequent with climate change, to maintain baseflows (Overpeck & Udall, 2020). This is exemplified by rivers in

the south-western USA, where stream flow has already declined owing to decreased precipitation and increased water extraction (Ficklin, Robeson & Knouft, 2016; Easterling et al., 2017). The Black River, a Pecos River tributary in the Rio Grande drainage of New Mexico and Texas, USA, has already experienced decreased discharge because of diminishing precipitation and increasing temperatures (Inoue et al., 2014). This trend is predicted to continue in the future, with mean annual precipitation decreasing by up to 6% and maximum surface temperatures increasing by up to 4.3°C by 2070 (Fick & Hijmans, 2017). The Black River contains nine rare aquatic taxa (Cowley & Sublette, 1987; Levine, Lang & Berg, 2012), including the federally endangered unionid *Popenaias popeii* (Texas hornshell). Increasing stream temperatures, combined with prolonged extreme low flows, are likely to have damaging consequences for these species.

A comparison of recently published thermal tolerance estimates for *P. popeii* from the Devils River, Texas (Rangaswami et al., 2023) with water temperatures in the Black River (reported in Inoue et al., 2014) indicates that exceedances of the species' thermal tolerances are likely to be happening in the Black River during periods of low flow. However, thermal tolerance can vary across populations (Fogelman et al., 2023), owing to intraspecific variation and local selection (Elderkin et al., 2007). Information on thermal tolerances and how they compare within and across populations is important for understanding population-level differences and developing evidence-based environmental flow recommendations (Olden & Naiman, 2010; Pyne & Poff, 2017; Zhao et al., 2018).

Given the differences in the thermal regime and genetic structure of *P. popeii* between the Devils and Black rivers, a follow-up study was conducted on the Black River population, including additional analyses of thermal tolerance exceedances. In this article, the vulnerability of *P. popeii* in the Black River, together with other populations in the Rio Grande, to elevated stream temperatures is assessed in the context of climate change and conservation. The primary objectives were to: (i) evaluate the upper thermal tolerances ( $LT_{05/50}$ ) of *P. popeii* glochidia and newly transformed juveniles; (ii) use *in situ* water temperature and flow data to determine thermal tolerance exceedances in the past 15 years; (iii) assess thermal exceedances across the entire range of *P. popeii*; and (iv) provide information that can aid conservationists and natural resource managers in protecting aquatic species that may be threatened by changes in flow or temperature.

## 2 | METHODS

### 2.1 | Study area

The Black River is a tributary of the Pecos River in New Mexico, USA, located within the Permian Basin. It resides above Capitan Aquifer and is underlain with gypsum and limestone bedrock (Bureau of Land Management, 1993). The river flows approximately 48 km before its confluence with the Pecos River, which drains into the Rio Grande. Mean daily discharge, recorded at the United States Geological

Survey (USGS) gauging station above Malaga (08405500), ranged from 0.0 to 339.8 m<sup>3</sup> s<sup>-1</sup> and averaged 0.3 m<sup>3</sup> s<sup>-1</sup> during the period 1947–2022. Reduced flows typically occur during the dry season in March, when the maximum volume of water is withdrawn from the river for agricultural use (Carman, 2007). High flows often occur from July to September, during the monsoon season (Carman, 2007).

## 2.2 | Study species

*Popenaias popeii* is a federally endangered freshwater mussel endemic to the Rio Grande Basin, with one population in New Mexico (Black River), two in Texas (Devils River and Pecos River – functionally extirpated), two in the Rio Grande along the Mexico/USA border (Lower Canyons of the Rio Grande and Lower Rio Grande near Laredo), and one in Coahuila, Mexico (Rio San Diego) (US Fish and Wildlife Service (USFWS), 2018). At present, it is thought to occur in only 21% of its presumptive historical range in the USA, with a predicted occupancy of 17% (Randklev et al., 2018). The decline of the species within the basin is thought to result in part from decreasing discharge and elevated temperatures (Inoue et al., 2014). *Popenaias popeii* occupies an approximately 14-km stretch of the Black River, confined naturally by the river running underground upstream of the reach and by extremely high salinity downstream (USFWS, 2016). Mussels reside mainly beneath undercut banks and behind boulders, which provide flow refuge (Lang, 2001). Approximately 34,390 individuals (95% CI: 14,068–48,458) occur in this stretch of the Black River (Berg & Hein, unpublished data). The lifespan of the mussels remains unknown, but two tagged individuals were recaptured in the Black River after a 15-year period (Inoue et al., 2014) and other species in the subfamily Ambleminae are known to live for more than 20 years (Carman, 2007). *Popenaias popeii* is considered an asynchronous short-term brooder, with its brooding period extending from approximately March until August (Lang, 2001). It has three primary host fishes in the Black River: *Carpiodes carpio* (river carpsucker), *Cyprinella lutrensis* (red shiner), and *Moxostoma congestum* (grey redhorse) (Levine, Lang & Berg, 2012). USFWS has attributed the imperilment of *P. popeii* partly to diminishing stream flow (USFWS, 2018).

## 2.3 | Collection and maintenance

Three gravid females were collected from one site (Barbara's) on the Black River, New Mexico, in May and June 2021 (Figure 1), and transported to the Texas A&M AgriLife Extension and Research Center in Dallas, Texas, in sealed plastic bags containing moist paper towels. Upon reaching the laboratory, each mussel was placed in a separate cup with 150-µm mesh at the bottom and window screen at the top, and held at room temperature (20 ± 3°C) within an aerated 20-gallon (91-L) aquarium until testing. They were fed twice per day with a 200-mL combination of Shellfish Diet 1800 and Nanno 3600 Instant Algae (Reed Mariculture, San Jose, CA, USA).

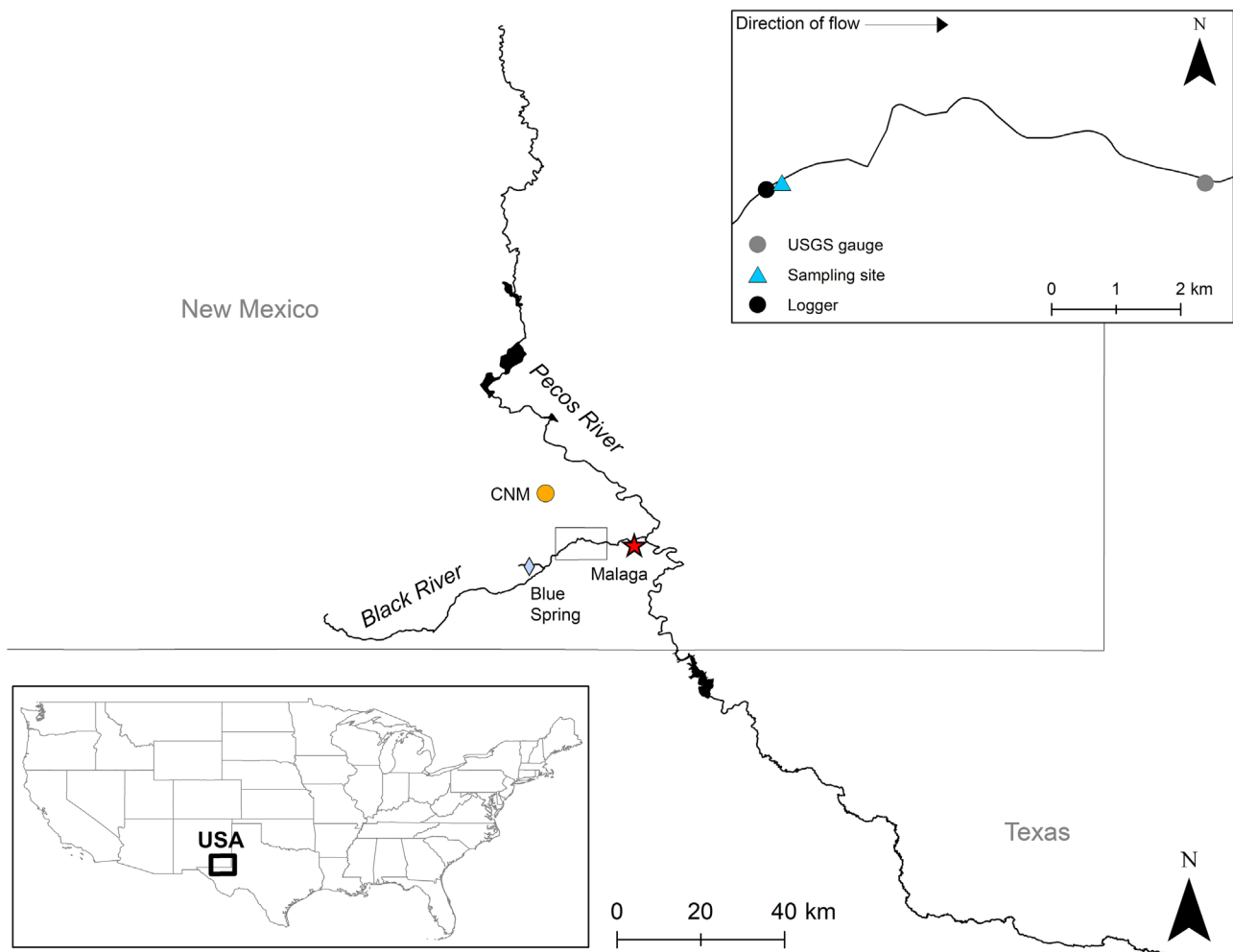
## 2.4 | Thermal tolerance testing

Glochidia were extracted from females by flushing their gills with water using an 18-gauge syringe. Glochidia were considered viable if they exhibited valve closure in response to a concentrated NaCl solution. Subsamples with viability of >80% were deemed ideal for thermal testing (ASTM International, 2006); however, some subsamples with <80% viability were used because of the limited number of individuals removed from the population, as *P. popeii* is endangered. The brood of one female was used for glochidial testing (initial viability, >80%) and the broods of two females were combined to infect fish to produce juveniles (initial viability, 40%).

Upper thermal tolerances (LT<sub>05/50</sub>) of glochidia and juveniles were determined following the methods described by Khan et al. (2019) and Khan et al. (2020). Trials were conducted by placing approximately 250 viable glochidia in a non-aerated 100-mL beaker containing 80 mL of reconstituted hard water with a salinity of 0.34 ppt (±0.05 ppt) (ASTM International, 2006). Glochidia were acclimated to 27°C from ambient temperature (20 ± 3°C) at a rate of 1°C per hour in a refrigerated incubator (Heratherm IMP 400; Thermo Scientific, Waltham, MA, USA), and were subsequently held at 27°C for 2 h prior to testing (ASTM International, 2006). Each beaker was placed in a fibreglass water bath containing approximately 40 L of water and held at one of five experimental temperatures (28, 30, 32, 34, and 36°C), a non-acclimated control (20°C), or an acclimated control that remained in the incubator (27°C) (Figure 2). Each test temperature and control contained three replicates. Temperatures within the water baths were maintained using a 300-W titanium heater connected to a temperature controller (ITC-308; Inkbird, Guangdong, China) and verified using Hobo pendant loggers (MMX2201 and MX2202; Onset Computer Corporation, Bourne, MA, USA). After 24 h, glochidial viability was reassessed by extracting a subsample of approximately 50 initially viable glochidia, which were suspended in solution, from each of the three replicates from each test temperature and observing valve closure in response to a saturated NaCl solution under a dissecting microscope (SZ51, Olympus America, Center Valley, PA, USA; ASTM International, 2006).

Untested glochidia were used to inoculate fish for juvenile production using the methods described by Johnson et al. (2012). *Campostoma anomalum* (central stoneroller) were added to a bucket containing hard water (0.34 ± 0.05 ppt) together with the glochidial solution to achieve a concentration of 4,000 glochidia per litre. The water was aerated and stirred constantly for 15 min, after which the fish were moved to individual aquatic habitat (AHAB) aquaria, which were maintained at 20°C. Filter cups with 150-µm mesh were placed below the outflow of each AHAB tank to capture sloughed glochidia and juveniles. The siphoned material was examined under a dissecting microscope (SZ51; Olympus America, Center Valley, PA, USA). Metamorphosed juveniles were distinguished based on their opaque shells and the presence of an active foot or adductor muscles (Blakeslee et al., 2013).

Newly transformed juveniles were placed in non-aerated 100-mL beakers containing 80 mL of hard water (0.34 ± 0.05 ppt) (ASTM



**FIGURE 1** Study area in the Black River, New Mexico, showing logger location (Life History site 2; black circle), collection site (Barbara's; blue triangle), USGS gauging station (08405500 Black River above Malaga, NM; grey circle), weather station (Cavern City Air Terminal, CNM; orange circle), and Blue Spring (light-blue diamond) along the Black River. Gravid *Popenaias popeii* (Texas hornshell) females from Barbara's were used for thermal tolerance experiments on glochidia and juveniles.

International, 2006). They were acclimated to 27°C from ambient temperature ( $20 \pm 3^\circ\text{C}$ ) at a rate of 1°C per hour in a refrigerated incubator (Heratherm IMP 400; Thermo Scientific, Waltham, MA, USA), and subsequently held at 27°C for 24 h prior to testing. Three beakers were placed into each temperature treatment (28, 30, 32, 34, and 36°C), a non-acclimated control (20°C), or an acclimated control that remained in the incubator (27°C). They were held in fibreglass water baths as described above. After 48 h, a 75% water change was performed (ASTM International, 2006). After 96 h, viability was assessed by visually checking for foot and adductor muscle movement under a dissecting microscope (SZ51; Olympus America, Center Valley, PA, USA; ASTM International, 2006).

## 2.5 | River measurements

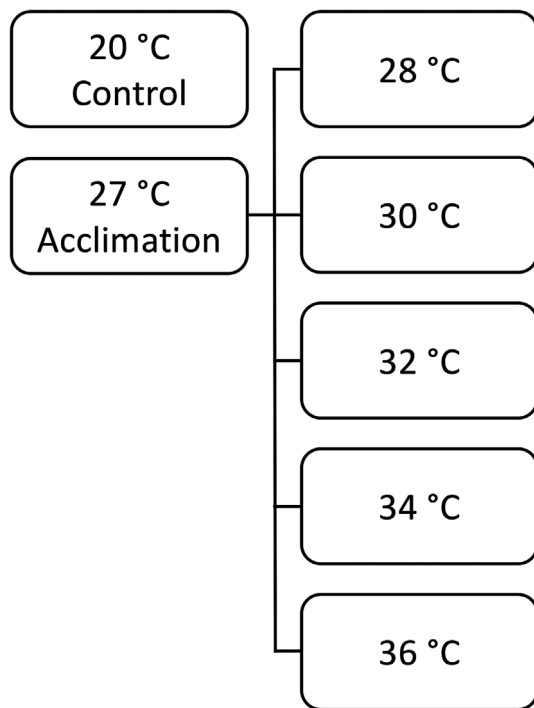
Hobo water level loggers (U20L; Onset Computer Corporation, Bourne, MA, USA) were deployed at Life History site 2 (LH2; Inoue et al., 2014) in the Black River (Figure 1). Loggers were enclosed in perforated polyvinyl chloride (PVC) pipes and anchored to the stream bed with a

piece of rebar. Loggers recorded the water temperature at 30-m intervals from 12 August 2020 to 6 October 2022. Discharge data dating back to 2007 were obtained from USGS gauging station 08405500 (Black River above Malaga, NM, USA), located approximately 5.8 km from LH2 and 5.6 km from the *P. popeii* collection site (Figure 1). The mean daily discharge from the gauge above Malaga was related to the maximum daily water temperature from the LH2 Hobo logger. Water temperature data for the Rio Grande near Laredo (from September 2021 to November 2022) and for Pecos River near Pandale (from August 2021 to August 2022) were obtained from Hobo water level loggers deployed at those sites (Figure S1).

## 2.6 | Statistical analysis

### 2.6.1 | Thermal tolerance

Lethal temperatures resulting in 5% and 50% mortality ( $LT_{05}$  and  $LT_{50}$ , respectively) were determined for glochidia by fitting mortality data to a regression model. As the initial glochidial viability was less than



**FIGURE 2** Experimental design, following Khan et al. (2019), showing acclimation and experimental temperatures plus non-acclimated (20°C) and acclimated (27°C) controls for *Popenaias popeii* glochidia (24 h) and juveniles (96 h) in the Black River. Each treatment and control contained three replicates.

100%, survival values were adjusted by dividing the treatment viability (assessed after 24 h) by the initial viability (obtained immediately after extracting glochidia from females). All statistics were performed in R 3.4.1 (R Core Team, 2017). To estimate  $LT_{05/50}$ , dose-response models were created using the `drm()` function in the 'drc' package (version 1.0-1). The best-fit model for each threshold was selected using a combination of log likelihood value, Akaike's information criterion (AIC), estimated residual standard error and *P*-value from a lack-of-fit test using the `mselect()` function in the 'drc' package, along with the standard error and 95% confidence intervals. To determine whether pairs of LT thresholds differed significantly, confidence interval ratio tests with a Bonferroni correction for multiple comparisons were performed using the `comped()` function in the 'drc' package (Wheeler, Park & Bailer, 2006). For the null hypothesis that population LTs are the same, the ratio should be equal to 1. A 95% confidence interval was constructed using the variance of each LT estimate, and the null hypothesis was rejected if this interval did not contain 1 (Wheeler, Park & Bailer, 2006). 95% CI ratio tests were also used to compare LT values between the Black River and Devils River populations.

## 2.6.2 | Random forest (RF) hindcasting

Water temperature was hindcast using discharge data from the USGS gauge on the Black River above Malaga (08405500) and temperature

data from Hobo water level loggers at LH2 in conjunction with air temperature data from the Cavern City Air Terminal weather station at Cavern City Air Terminal (CNM) in Carlsbad, New Mexico (Figure 1). Dew point, relative humidity, wind speed, precipitation, visibility, and air temperature were obtained from the weather station to match the period of record, because these factors have significant impacts on water temperature model estimates (Cluis, 1972; Mohseni & Stefan, 1999). Other weather condition variables were available but were excluded owing to incomplete availability for the period of record. Additional weather conditions (previous hour air temperature and change in prior air temperature) were also created because of their strong correlation with water temperature (Kothandaraman, 1971; Cluis, 1972). Hourly averages for all variables were then created for use in RF models (Cutler et al., 2007). Specifically, a 1,000-tree RF model was created using 70% of the available data for training and the remaining 30% for testing. Stream temperature was the response variable, and the flow parameters and weather conditions were used as predictor variables. Model variable splits were determined by preliminary testing to find the lowest mean of squared residuals ( $MSE_{OOB}$ ; Liaw & Wiener, 2002), with seven variable splits identified as the optimum value ( $MSE_{OOB} = 0.4515016$ ). The accuracy of the RF model was assessed with  $MSE_{OOB}$  and the  $R^2$  value. The percentage variance explained was also calculated (Liaw & Wiener, 2002). Once the model was trained and tested, it was used to predict stream temperature values for the period 2007–2022.

## 2.6.3 | Thermal exceedances

To evaluate the water temperature exceedances of *P. popeii* thermal tolerance, a uniform continuous above-threshold (UCAT) analysis was performed (Castelli, Parasiewicz & Rogers, 2012; Khan et al., 2020) using 24-h glochidial  $LT_{05/50}$  estimates, logger water temperature from 2020–2022, hindcast water temperature from 2007–2020, and discharge from the USGS gauging station from 2007–2022. In this analysis, the cumulative frequency of *in situ* water temperatures exceeding  $LT_{05/50}$  values was used to construct a curve with two inflection points, which represent temperature thresholds beyond which adverse impacts to mussels are expected to occur. Exceedances were calculated for the spring/summer bioperiod from March to August, when female *P. popeii* brood and release glochidia, which subsequently encyst on fish and develop into juveniles (Lang, 2001). To calculate exceedance frequencies, continuous temperature events above the  $LT_{05/50}$  threshold (a set of consecutive days upon which the threshold was exceeded by the maximum daily water temperature) were summed and divided by the duration of the period of interest. The cumulative frequencies (%) of temperature exceedance events were plotted on the *x*-axis against the duration of events (days) plotted on the *y*-axis, creating a UCAT curve (Castelli, Parasiewicz & Rogers, 2012). To identify inflection points on the UCAT curves, three-segment piecewise linear regressions were conducted using the `lm()` function in base R. Exceedance events above

the first inflection point occurring for long durations are considered 'catastrophic' and are thought to affect multiple generations. Events between the two inflection points that occur more frequently are considered 'persistent' and may affect one generation. Events below the second inflection point are considered 'typical' and are thought to be part of the natural variation of the system and have minimal impacts on the organisms (Castelli, Parasiewicz & Rogers, 2012).

## 2.7 | Population comparisons

The  $LT_{05}$  and  $LT_{50}$  values for *P. popeii* from the Black River and the Devils River (Grass Patch and Ruthies; values obtained from Rangaswami et al., 2023) were also overlain onto *in situ* water temperature data from the past year for two other populations of *P. popeii*: Rio Grande near Laredo and Pecos River near Pandale (Figure S1). Water temperature data were taken from loggers at sites where live individuals have been collected (Randklev et al., 2016). For the exceedance analyses, thermal tolerance estimates from the Devils River were used for the Laredo population and estimates from the Black River were used for the Pecos population, based on geographical proximities.

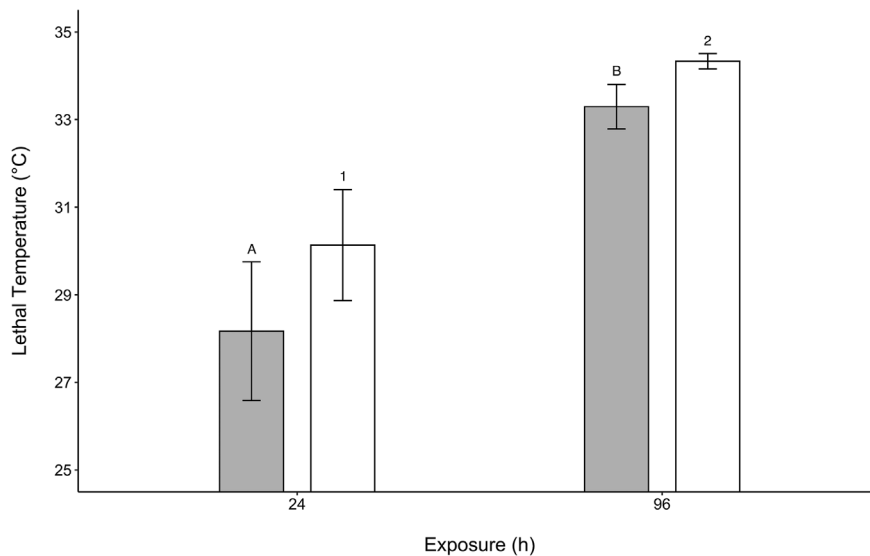
## 3 | RESULTS

### 3.1 | Thermal tolerance

Juveniles had significantly higher  $LT_{05}$  and  $LT_{50}$  values than glochidia, based on 95% CI ratio tests.  $LT_{05}$  was 28.2°C for 24-h glochidia versus 33.3°C for 96-h juveniles (Figure 3; Table 1).  $LT_{50}$  was 30.1°C for 24-h glochidia and 34.3°C for 96-h juveniles (Figure 3; Table 1). In addition, glochidial  $LT_{05/50}$  did not differ significantly when measured at 12 h compared with 24 h (Table S1).

### 3.2 | RF hindcasting

Results for the RF model developed for Black River stream temperature showed a high percentage of variance explained (98%) and a low  $MSE_{OOB}$  (0.04) based on testing data. Predicted values from the period 2007–2022 showed a strong seasonality component that matched well with the observed data collected from the period 2020–2022, further indicating good model performance. Predicted values ranged from 4.0 to 32.3°C, with an average of 18.7°C ( $\pm 7.3^\circ\text{C}$  SD).



**FIGURE 3** Comparison of lethal temperatures for 5% (grey bars,  $LT_{05}$ ) and 50% (white bars,  $LT_{50}$ ) of *Popenaias popeii* glochidia (24 h) and juveniles (96 h) from the Black River. Error bars indicate 95% confidence intervals. Values annotated with the same letter or number do not significantly differ, based on a 95% confidence interval ratio test ( $P < 0.05$ ).

**TABLE 1** Laboratory-derived lethal thresholds for *Popenaias popeii* glochidia and juveniles from the Black River, New Mexico (Barbara's) and two sites in the Devils River, Texas (Grass Patch and Ruthies; Rangaswami et al., 2023).

Population	24-h glochidia		96-h juvenile	
	$LT_{05}$	$LT_{50}$	$LT_{05}$	$LT_{50}$
Black River	28.2 ± 0.73 (26.6–29.8)	30.1 ± 0.59 (28.9–31.4)	33.3 ± 0.24 (32.8–33.8)	34.3 ± 0.08 (34.2–34.5)
Grass Patch	29.2 ± 0.20 (28.7–29.6)	31.6 ± 0.07 (31.4–31.7)	-	-
Ruthies	32.5 ± 0.03 (32.4–32.5)	32.7 ± 0.03 (32.7–32.8)	-	33.0 ± 0.31 (32.2–33.7)

Note: Mean ± SE (95% CI),  $N = 3$ .

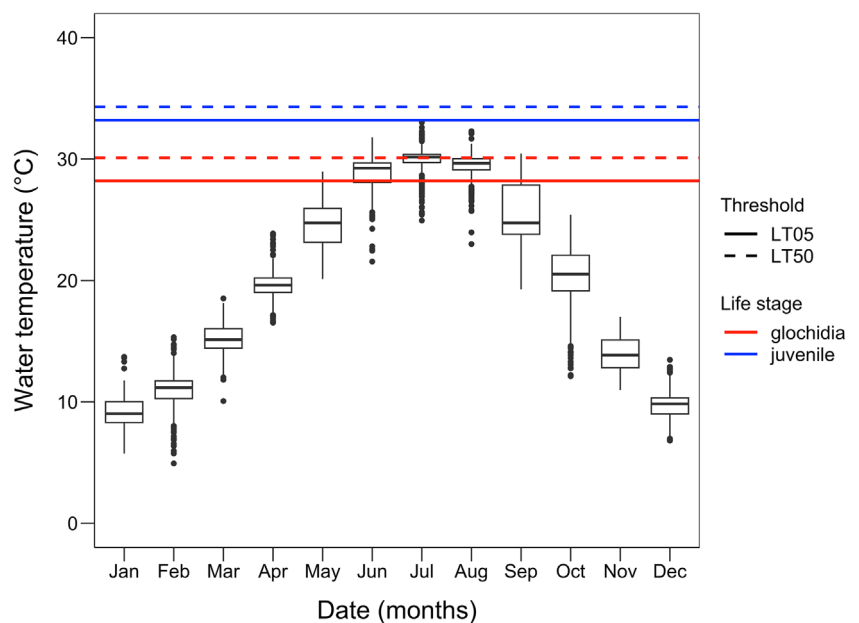
### 3.3 | Thermal exceedances

The maximum daily water temperature at LH2 from 1 October 2007 to 6 October 2022 ranged from 4.9 to 33.0°C, and was lowest in February 2021 and highest in July 2022 (Figure 4). The highest absolute water temperature occurred on 11 July 2022, when the mean daily discharge was  $0.10 \text{ m}^3 \text{ s}^{-1}$ . The mean daily discharge throughout the study period ranged from 0.00 to  $26.2 \text{ m}^3 \text{ s}^{-1}$ , reaching its lowest point in May 2021 and June–August 2022, and reaching its highest point in September 2013 (Figure 5).

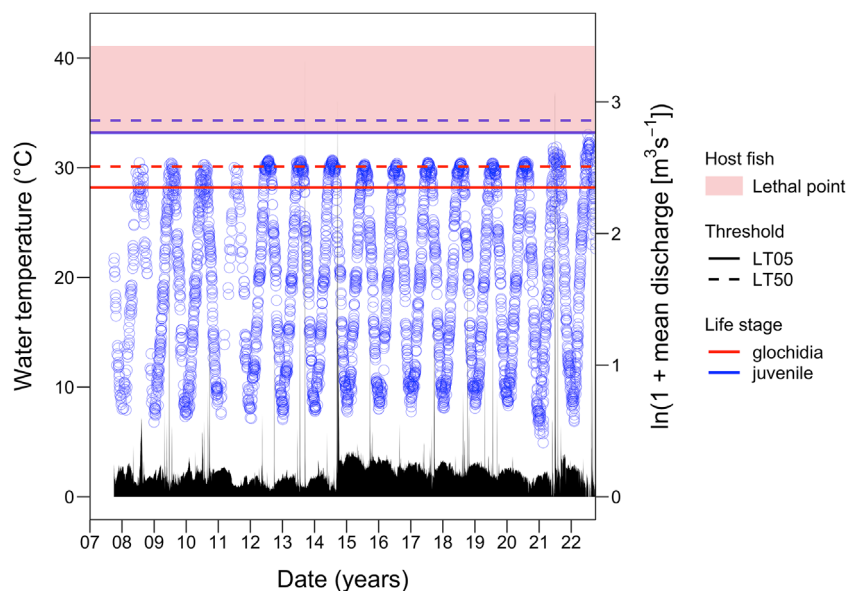
For glochidia, the 24-h  $LT_{05}$  was exceeded by the maximum daily water temperature during the spring/summer bioperiod 93 times, for a total of 1,010 days (range, 1–82 days; mean, 10.9 days; mean daily discharge,  $0.00\text{--}0.75 \text{ m}^3 \text{ s}^{-1}$ ; average daily

discharge,  $0.16 \text{ m}^3 \text{ s}^{-1}$ ; maximum daily temperature, 28.2–33.0°C; Figure 6a). The total exceedance frequency during the bioperiod was 41.2%. The longest event occurred from 11 June to 31 August 2012, a period that had an average mean daily discharge of  $0.15 \text{ m}^3 \text{ s}^{-1}$ . Catastrophic events lasted >63 consecutive days, persistent events were 38–63 days, and typical events lasted <38 days (Table 2).

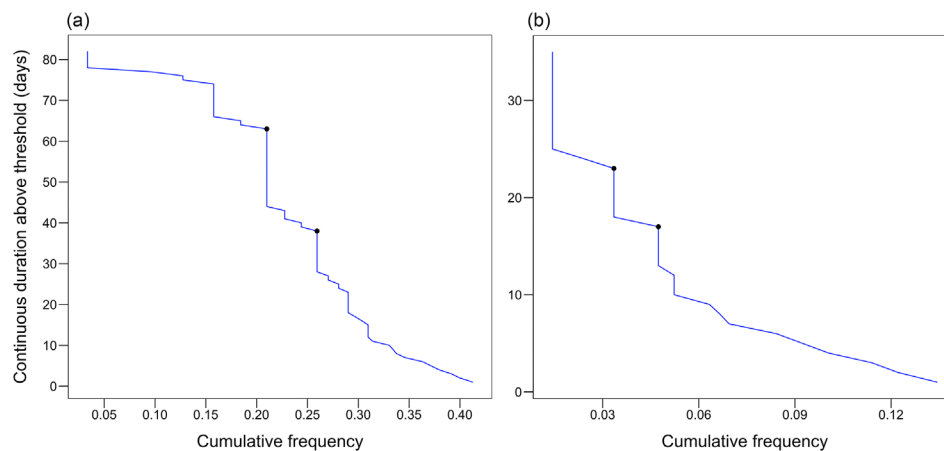
Glochidial 24-h  $LT_{50}$  was exceeded 77 times during the spring/summer bioperiod for a total of 329 days (range, 1–35 days; mean, 4.3 days; mean daily discharge,  $0.00\text{--}0.29 \text{ m}^3 \text{ s}^{-1}$ ; average daily discharge,  $0.13 \text{ m}^3 \text{ s}^{-1}$ ; maximum daily temperature, 30.1–33.0°C; Figure 6b). The total exceedance frequency during the bioperiod was 13.4%. The longest exceedance event occurred from 6 July to 9 August 2022, which had an average mean daily discharge of



**FIGURE 4** Maximum daily water temperature from October 2007 to October 2022 overlain with thermal tolerance thresholds for *Popenaias popeii* in the Black River, New Mexico at Life History site 2. Red lines, 24-h glochidia; blue lines, 96-h juveniles; solid lines,  $LT_{05}$ ; and dashed lines,  $LT_{50}$ .



**FIGURE 5** Maximum daily water temperature and mean daily discharge from October 2007 to October 2022 in the Black River overlain with thermal tolerance data for *Popenaias popeii* from Barbara's and its host fishes (obtained from Rangaswami et al., 2023). Blue circles, maximum daily water temperature; black data, mean daily discharge; red lines, thresholds for 24-h glochidia; blue lines, thresholds for 96-h juveniles; solid lines,  $LT_{05}$ ; dashed lines,  $LT_{50}$ ; and pink rectangle, lethal tolerance range for host fish.



**FIGURE 6** Upper continuous duration above threshold (UCAT) of maximum daily water temperature during the spring/summer bioperiod for mussels at two life stages in the Black River, New Mexico. The leftmost black circle indicates the inflection point between typical and persistent events and the rightmost black circle indicates the inflection point between persistent and catastrophic events, determined by piecewise linear regression. Axes are scaled differently for each UCAT curve to show the trends more clearly. (a) Glochidial 24-h  $LT_{05}$  threshold. (b) Glochidial 24-h  $LT_{50}$  threshold.

**TABLE 2** Water temperature and discharge data for catastrophic, persistent, and typical exceedance events for 24 h thermal tolerance ( $LT_{05/50}$ ) of *Popenaias popeii* glochidia in the Black River, New Mexico, from 2007–2022 during the spring/summer bioperiod.

Threshold	Event type	Duration(days)	Maximum daily temperature( $^{\circ}C$ )	Mean daily discharge( $m^3 s^{-1}$ )
$LT_{05}$	Catastrophic	>63	30.0 (28.2–33.0)	0.14 (0.00–0.38)
	Persistent	38–63	29.8 (28.2–30.7)	0.17 (0.08–0.53)
	Typical	<38	29.5 (28.2–31.9)	0.18 (0.00–0.75)
$LT_{50}$	Catastrophic	>23	31.2 (30.2–33.0)	0.07 (0.00–0.17)
	Persistent	17–23	30.4 (30.1–30.7)	0.11 (0.07–0.28)
	Typical	<17	30.4 (30.1–31.9)	0.15 (0.00–0.29)

Note: Means are given, with ranges in parentheses.

$0.05 m^3 s^{-1}$ . Catastrophic events lasted >23 days, persistent events lasted 17–23 days, and typical events lasted <17 days (Table 2). In contrast, the 96 h juvenile  $LT_{05}$  and  $LT_{50}$  values were never exceeded.

### 3.4 | Population comparisons

Comparing estimates for *P. popeii* from the Black River with those published for the Devils River (Rangaswami et al., 2023), the glochidial thermal tolerances were similar for both populations (Table 1). Specifically, there were no significant differences in the 24-h  $LT_{05}$  or  $LT_{50}$  values between the Black River and the Devils River–Grass Patch, but there were differences between the Black River and the Devils River–Ruthies. Thermal tolerance exceedances between the rivers were also compared. In the Black River, the 24-h  $LT_{05}$  was exceeded more than 20% of the time in the periods 2020–2022 and 2007–2022. In contrast, exceedances were near or below 6% for similar time periods in the Devils River (Rangaswami et al., 2023; Table 3). The exceedances for 24-h  $LT_{50}$  show a similar pattern, as the Black River  $LT_{50}$  was exceeded 7% of the time in the period 2020–2022 and 15% of the time in the period 2007–2022. In contrast,  $LT_{50}$

exceedances in the Devils River were near or below 3% (Rangaswami et al., 2023; Table 3). Comparing tolerances to water temperatures in the Pecos near Pandale from August 2021 to August 2022 indicates that the water temperature exceeded the Black River  $LT_{05}$  22% of the time and  $LT_{50}$  9% of the time (Table 3). The Rio Grande near Laredo exceeded the Devils River 24-h  $LT_{05/50}$  6%–34% of the time from September 2021 to November 2022 (Table 3).

## 4 | DISCUSSION

The upper thermal tolerances ( $LT_{05}$  and  $LT_{50}$ ) of *P. popeii* glochidia and newly transformed juveniles were successfully determined. *Popenaias popeii* is considered endangered, in part because of elevated water temperatures (USFWS, 2016). The Black River population, now restricted to a 14-km reach, is deemed by many to be at risk of extirpation and a harbinger of what is likely to happen to other populations (Inoue et al., 2014). Although at present there appears to be active recruitment in the Black River as well as in populations within the Rio Grande in Texas (Carman, 2007; Randklev et al., 2016), this could be impaired by elevated water temperatures.



**TABLE 3** Thermal exceedance frequencies for *Popenaias popeii* in the Black River, Devils River (Grass Patch and Ruthies; Rangaswami et al., 2023), Rio Grande near Laredo, and Pecos River near Pandale using glochidial 24-h  $LT_{05/50}$  thresholds from the Black and Devils populations overlain onto *in situ* water temperature data from each river.

Population	Threshold	Exceedance frequency (%)	Period of record
Black River	BR $LT_{05}$	26.3	2020–2022 (logger)
	BR $LT_{50}$	14.5	
	BR $LT_{05}$	23.1	2007–2022 (hindcast)
	BR $LT_{50}$	7.0	
Grass Patch	GP $LT_{05}$	6.1	2018–2021
	GP $LT_{50}$	3.3	
Ruthies	RU $LT_{05}$	2.2	2015–2021
	RU $LT_{50}$	1.5	
Rio Grande near Laredo	GP $LT_{05}$	33.5	2021–2022
	GP $LT_{50}$	12.6	
	RU $LT_{05}$	6.0	
	RU $LT_{50}$	5.6	
Pecos River near Pandale	BR $LT_{05}$	22.2	2021–2022
	BR $LT_{50}$	8.5	

Abbreviations: BR, Black River; GP, Grass Patch; RU, Ruthies.

Based on thermal tolerance estimates and water temperature data, it appears that glochidial thermal limits were exceeded frequently in the Black River within the past 15 years. Specifically, the 24-h  $LT_{05}$  was exceeded >40% of the time and the  $LT_{50}$  was exceeded >13% of the time during the spring/summer bioperiod, when female *P. popeii* brood and release their glochidia (Table 2). This suggests that *P. popeii* glochidia in the Black River are routinely experiencing thermal stress, which could severely limit recruitment. The unexpectedly high number of catastrophic exceedance events underscores this point. In general, catastrophic events have an adverse impact on multiple generations owing to their magnitude and duration, and as such should be infrequent (Castelli, Parasiewicz & Rogers, 2012). In this study, there were eight catastrophic events (six for  $LT_{05} > 63$  days and two for  $LT_{50} > 23$  days) over the past 15 years (2007–2022) during the spring/summer bioperiod. The mean daily discharge during these events ranged from 0 to  $0.38 \text{ m}^3 \text{ s}^{-1}$ , and averaged  $0.13 \text{ m}^3 \text{ s}^{-1}$ , which is extremely low. Low discharge can exacerbate the effects of high air temperature as smaller volumes of water heat more rapidly (Olden & Naiman, 2010). Thermal tolerance exceedances, particularly catastrophic events, are likely to have had adverse impacts on *P. popeii* in the Black River and could jeopardize the long-term viability of the population (Taeubert, El-Nobi & Geist, 2014; Sangsawang, Kovitvadhi & Kovitvadhi, 2019).

Thermal stress is influenced by human activities and climate (Olden & Naiman, 2010), and given its suspected role in the decline of *P. popeii* (USFWS, 2016), the risk of this impact on other populations was evaluated by comparing thermal tolerance estimates between populations. Although the Devils River–Ruthies population had higher tolerances than the Devils River–Grass Patch or Black River populations, these differences were small (within  $2.6^\circ\text{C}$ ). Similar variation in tolerance has been found for other Texas unionids, including *Lampsilis bracteata* (Texas fatmucket), which had variations of up to  $3^\circ\text{C}$  in the 24-h glochidial  $LT_{50}$  values across populations (Khan

et al., 2019; Goldsmith et al., 2021). The present study suggests that the thermal tolerance of *P. popeii* is conserved across populations, although with some minor variation. This finding is noteworthy because the Black River population diverged and became genetically isolated from the rest of the Rio Grande populations approximately 80,200 years ago, followed by a bottleneck event 32,600 years ago (Inoue, Lang & Berg, 2015). Despite the two rivers occurring in slightly different climatic zones, with the Black River running through an area that is more arid, with colder winters, there does not appear to be local selection for acute thermal tolerance, as tested in this study.

Future studies should focus on within-population variability to evaluate adaptive capacity for populations where thermal stress is a concern. We were unable to do so in our study as glochidia were extracted from only one female for thermal testing, because of the limited number of gravid females available at the time of sampling and restrictions on collecting a US federally endangered species. The females used in this study were collected from a healthy mussel bed in good physiological condition and responded sufficiently to external stimuli. Female freshwater mussels often show a high degree of multiple paternity, making it more likely that glochidia in this study were representative of the population sampled (Christian et al., 2007; Ferguson et al., 2013; Wacker et al., 2018). In follow-up studies, using a greater number of females would allow researchers to assess variability within the population as well as make more robust comparisons between populations.

The thermal tolerances of *P. popeii* from different populations appear to follow a similar pattern to salinity tolerances from two geographically separated populations on the Rio Grande (Hart, Miller & Randklev, 2019). Conservation of thermal and salinity tolerances for *P. popeii* across populations means that estimates from tested populations can be used with confidence to draw inferences for untested populations. Upper thermal limits for early life stages of other Texas unionids across various drainages were found to be

similar among species, probably because mussels are only exposed to the maximum daily water temperature for a short period of time and multiple species generally occur in the same habitats within river systems (Khan et al., 2019). In other parts of North America and Europe, some studies have found substantial differences in tolerance among species (Rodland et al., 2009; Pandolfo et al., 2010), while others have found tolerance to be similar across species (Perepelizin & Boltovskoy, 2011; Galbraith, Blakeslee & Lellis, 2012; Archambault, Cope & Kwak, 2014a; Archambault, Cope & Kwak, 2014b). It is likely that climate drives differences in thermal tolerance among species in different regions. Malish & Woolnough (2019) suggested that latitudinal gradients may be responsible for differences in tolerance between unionid populations (Malish & Woolnough, 2019). In addition, a study of the thermal limits of aquatic insects from mid-latitude temperate streams and low-latitude tropical streams found that lowland insects had narrower thermal breadths than their midland counterparts (Shah et al., 2017).

Upper thermal tolerance exceedances were much more frequent in the Black River than in the Devils River, suggesting that *P. popeii* is living near its upper thermal limit in the Black River and substantiating claims that thermal stress is a risk to this population (USFWS, 2016). The differences in thermal exceedances between the Black and Devils rivers can probably be explained by human activity. The Devils River is a spring-fed system with limited human impacts: in fact, it has often been described as 'pristine' (Randklev et al., 2016; Green et al., 2019; Robertson et al., 2019; Caldwell et al., 2020). In contrast, the Black River is heavily used by the agricultural and oil and gas industries, which extract both groundwater and surface water for activities such as irrigation and the fracking of fossil fuels (Bren School of Environmental Science & Management, 2014). In addition, the Black River is impounded by several low-head dams (Lang, 2001), which may block fish passage and impair mussel reproduction (Watters, 1996). Dams may also reduce the suitable habitat for mussels by changing lotic habitat into lentic habitat within impounded areas (Randklev et al., 2015). In the Black River, riffles contain much higher mussel density than pools (Inoue et al., 2014). Groundwater pumping and water withdrawals, combined with changes in flow resulting from river impoundment, can serve as catalysts for thermal stress by reducing discharge, which is exacerbated during periods of drought, a common phenomenon in the Black River (Carman, 2007; Olden & Naiman, 2010).

Given the similarities in thermal tolerance between populations in the Black and Devils rivers, the  $LT_{05}$  and  $LT_{50}$  values from these populations were overlain onto *in situ* water temperature data from the Rio Grande, near Laredo, and the Pecos River, near Pandale. Exceedances indicate that the Laredo population is likely to be experiencing thermal stress (Table 3). There were also frequent exceedances in the Pecos River, near Pandale (Table 3). Although other water quality issues, such as salinization, have been indicted as the main cause of extirpation of *P. popeii* in the Pecos River (Randklev et al., 2016; USFWS, 2016; Hart, Miller & Randklev, 2019), overlaying the thermal tolerances from the Black River onto water temperature data from the Pecos River suggests that elevated temperatures may also have contributed to its decline. These comparisons, as well as

those between the Black and Devils rivers, suggest that thermal stress is a concern for all remaining *P. popeii* populations.

#### 4.1 | Conservation implications

This study demonstrates that placing physiological tolerance in the context of environmental data can be used to identify ecological 'bottlenecks', which can aid in conservation management. In future studies, it would be useful to test the thermal tolerance of other *P. popeii* populations to validate our biological overlays. Thermal tolerance estimates could also be refined by incorporating diel swings in water temperature to mimic natural variation (Martin, 2016). In addition, it is important to consider the relationship between *P. popeii* and its host fishes. To date, only one study has identified the mussel's hosts, which were confirmed in the Black River (Levine, Lang & Berg, 2012). Further studies are needed to identify the hosts in other parts of its range. Moreover, the  $LT_{05/50}$  values of the host fish species also remain unknown, and lethal thermal tolerance tests for these species have not been conducted within the Rio Grande drainage, nor has the impact of elevated water temperature on the parasitic relationship been studied directly. It would be useful to carry out a study similar to that reported by Taeubert, El-Nobi & Geist (2014), in which fish were infected with mussel glochidia at several water temperatures and host mortality and transformation success was monitored over several weeks.

The Black River *P. popeii* population is already declining, with changing discharge and temperature likely to be playing a major role. In the period 1997–2012, there was an approximately 3% decline in survivorship that corresponded with an approximately  $0.2 \text{ cm}^3 \text{ s}^{-1}$  reduction in mean monthly discharge (Inoue et al., 2014). Finite population growth ( $\lambda$ ) also declined during this time and was  $<1$  when the study concluded (Inoue et al., 2014). Estimates of population size in 2011–2012 versus 2017–2018 showed a decline of  $>25\%$  (Berg & Hein, unpublished data). This study demonstrates that in addition to decreased discharge, elevated stream temperatures may also be responsible for the population's decline. In 2017, the USFWS entered into a Candidate Conservation Agreement (CCA) and a CCA with Assurances (CCAA) with stakeholders that is administered by the Center of Excellence (CEHMM), a nonprofit consulting firm. These agreements are designed to encourage collaboration among water users, commercial industries, municipalities, and other stakeholders to protect *P. popeii* and other aquatic fauna in the Black River. The CCA and CCAA stipulate a minimum flow requirement of 9.3 cfs (approx.  $0.26 \text{ cm}^3 \text{ s}^{-1}$ ) at the USGS Black River above Malaga gauge (USFWS & CEHMM, 2017). This is higher than the average discharge levels observed during catastrophic events ( $0.13 \text{ cm}^3 \text{ s}^{-1}$ ). However, catastrophic exceedances occurred while discharge was as high as  $0.38 \text{ cm}^3 \text{ s}^{-1}$ , which is slightly above the average historical (1947–2022) mean daily discharge of  $0.34 \text{ cm}^3 \text{ s}^{-1}$ . This suggests that the negotiated minimum flow will still result in significant thermal stress to *P. popeii* if such minimum flows occur when

surface temperatures are high. The results of this study underscore the importance of protecting instream flows for *P. popeii* in the Black River, especially during the hot summer months when the majority of thermal exceedances occur. Furthermore, maintaining these flows is likely to protect the entire biological community of the Black River, one of the few remaining surface flows in the northern Chihuahuan Desert. Such an approach to water management is necessary in many arid regions of North America and throughout the world.

## AUTHOR CONTRIBUTIONS

**Xenia L. Rangaswami:** Idea development (equal); data collection (equal); data analysis (lead); figure and table preparation (lead); writing—original draft (lead). **Alexander H. Kiser:** Data analysis (equal); writing—reviewing and editing (equal). **Matthew Ramey:** Writing—reviewing and editing (equal). **James A. Stoeckel:** Writing—reviewing and editing (equal). **David J. Berg:** Data collection (equal); writing—reviewing and editing (equal). **Roel R. Lopez:** Writing—reviewing and editing (equal). **Charles R. Randklev:** Idea development (equal); data collection (equal); data analysis; writing—original draft (supporting); writing—reviewing and editing (lead).

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest associated with this work.

## DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author, upon reasonable request.

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## SUPPORTING INFORMATION

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