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Salinity tolerance of a rare and endangered unionid mussel, *Popenaias popeii* (Texas Hornshell) and its implications for conservation and water management

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ABSTRACT

Unionid mussels are considered sensitive to salinity and there is growing concern in arid and semi-arid regions that declining flows coupled with anthropogenic impacts are amplifying natural salinity levels. In this study, we tested the effects of varying salinity concentrations (3.0, 4.0, 5.0, 6.0, 7.0 and 10.0 ppt NaCl) on survival of adult *Popenaias popeii*, (Texas Hornshell). This species occurs in the Rio Grande basin of Texas and northern Mexico, an arid to semi-arid stream plagued by salinization, and was recently listed as Endangered under the U.S. Endangered Species Act. We performed 2, 4, and 10-day toxicity tests on individuals from two disjunct populations: Laredo, TX, and the Lower Canyons of the Rio Grande near Big Bend National Park. We found no significant differences in LC50 estimates between populations at 96-hrs or 10-days but significant differences in TUD50s at 5 ppt between populations, which indicates that tolerance does not vary but sensitivity may between these populations. Overlaying LC50 estimates at 10-days for both populations on plots of salinity (ppt) measured over time, we show parts of the Rio Grande periodically approach or exceed 4.0 ppt, indicating these reaches are becoming unsuitable for *P. popeii* and populations within them at risk.

1. Introduction

Salinization caused by anthropogenic activities (i.e., secondary salinization) is an ongoing and under-researched problem impacting most, if not all, arid and semi-arid regions around the world (Bailey et al., 2006; Canedo-Arguelles et al., 2013; Williams, 1987). Anthropogenic activities such as groundwater pumping, irrigation, impoundments, and the production of brine from oil and gas operations can increase saline conditions directly through additional salt contributions or indirectly by reducing instream flow (Bailey et al., 2006). Reductions in instream flow is particularly problematic for rivers in these regions because they can have naturally elevated salinity levels due to natural salt seeps and deposits, and so the loss of freshwater contributions can amplify salinization in these systems (Bailey et al., 2006; Canedo-Arguelles et al., 2013; Williams, 1987). The effects of salinization on freshwater biota vary spatiotemporally but can lead to periods in which salt concentrations exceed critical thresholds, resulting in impacts to growth, reproduction, and survivorship (Ercan and Tarkan, 2014). Over time, these impacts can result in changes to species distributions through extirpation and replacement with more tolerant organisms and, if they are severe enough, can lead to extinction (Hoagstrom, 2003; Miyazono et al., 2015; Porter-Goff et al., 2013).

The Rio Grande basin, located in the semi-arid region of the southwestern United States, drains a total of 870,236 km^2 within Colorado, New Mexico, and Texas and the states of Chihuahua, Coahuila, Nuevo Leon, and Tamaulipas in northern Mexico (Benke and Cushing, 2005; Kammerer, 1990). The mainstem and its tributaries serve as a major water supply for communities that exist throughout the basin, and this demand for water, coupled with changing climate, has led to reductions in instream flow, which have exacerbated natural salinity inputs (Miyazono et al., 2015; URGBBEST, 2012). For example, in the Pecos River, recent studies have shown that salinity has increased from historical levels and now ranges from 6.0 to 12.0 ppt but can exceed 30.0 ppt depending on location. The salinization of this river has led to golden algae blooms, resulting in several large fish kills that have impacted fish community structure such that 13 of the 44 fish species native to this system are now extirpated (Hoagstrom, 2009). Similar impacts have been seen in aquatic macroinvertebrates, such as insect larvae and ostracods (Davis, 1980).

Among the unionid species impacted is *Popenaias popeii* (Texas

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hornshell), which is endemic to the Rio Grande River basin in New Mexico, Texas, and the northern part of Mexico. This species has recently been listed as Endangered under the ESA (Endangered Species Act) by the U.S. Fish & Wildlife Service (USFWS, 2018), and only four significant populations are currently reported in the U.S., which include the following: Black River, NM; Devils River, TX; Lower Canyons area of the Rio Grande Wild and Scenic River, TX; and Rio Grande near Laredo, TX (Randklev et al., 2018). A fifth population is known to occur in the lower Pecos River (Randklev et al., 2016), but elevated salinity in this river may preclude its long-term survival. Overall, several factors have been implicated in the decline of *P. popeii*, including the degradation of water quality due to changes in land use, river impoundment, ground water pumping, and salinization; however, none of these stressors have been explicitly tested (Randklev et al., 2018).

Unionid mussels (hereafter mussels) are considered to be sensitive to salinity because of their unique ecology, life history, and physiology. Mussels are primarily sessile and are reliant on fish for dispersal, as they have a parasitic larval stage, which means that their ability to cope with environmental impacts, such as salinization, is limited primarily to physiological mechanisms. As a group, mussels maintain the lowest internal salt concentrations of any animal because their body fluids are nearly isosmotic with the surrounding medium (Cummings and Graf, 2010). Under normal osmotic conditions, mussels can modify their body fluid solute concentration to match the ambient conditions using passive transport mechanisms (Cummings and Graf, 2010). However, mussels lack effective active mechanisms to create/maintain hypotonic body fluids if the ambient environment becomes more saline. This means when mussels are exposed to a hypertonic environment they experience a loss of cellular moisture which leads to dehydration, and, if prolonged exposure, mortality (Gainey and Greenberg, 1977). Recent research has demonstrated that mussels are sensitive to elevated salinity levels. For example, one study found that for *Elliptio complanata* from Pine Creek, PA, salt concentrations as low as 4.0 ppt NaCl resulted in an LC50 at 96 h. Sublethal effects (lower O2 consumption) were observed much sooner at concentrations as low as 2.0 ppt NaCl (Blakeslee et al., 2013). Another study assessing juvenile survival of the Northern Riffleshell (*Epioblasma torulosa rangiana*) below a brine discharge in the Allegheny River, PA, reported significant mortality at sites where salinity exceeded 2.0 ppt (Patnode et al., 2015).

In this paper, we examine the effects of salinity on a critically endangered mussel species to determine whether this factor has contributed to its imperilment within the Rio Grande. Our study also provides additional information on the salinity tolerances of mussel species, which have not been well researched, particularly in arid and semi-arid regions such as the southwestern United States. Our study has two objectives: first, we assess the salinity tolerance of two disjunct populations to determine whether salinity tolerance varies based on geographic location; second, we then use the resulting data along with water quality information to determine whether salinization could be a threat to the long-term persistence of this species.

2. Materials and methods

2.1. Study area

Adults of *P. popeii* were collected from two locations in the mainstem of the Rio Grande: 1) the middle Rio Grande basin just above Laredo, TX (Site 1); and 2) the upper Rio Grande basin in the Lower Canyons area downstream of Big Bend National Park (Site 2) (Fig. 1). We chose these locations because they are separated by a large geographic distance (~400 km) and differ in salinity regimes, with the Laredo site rarely if ever exceeding 0.37 ppt and the Lower Canyons generally higher and periodically reaching or exceeding 4.0 ppt (TCEQ and IBWC, 2013). Thus, this difference could presumably result in varying salinity tolerances between the two populations. The Laredo population of the Rio Grande is located within the Rio Grande Floodplain and Terraces of the Southern Texas Plains ecoregion (Griffith et al., 2007). Flow in this portion of the river is influenced by Lake Amistad near Del Rio, TX, and water withdrawals for agricultural, commercial and residential purposes (URGBBEST, 2012), resulting in substantial daily variation in stream discharge and water depth. This portion of the Rio Grande is also urbanized relative to the other collection site, and this land use along with agricultural and industrial activities have degraded the water quality (Griffith et al., 2007; TCEQ and IBWC, 2013). The population of the Lower Canyons (upstream of Lake Amistad), is located in the Low Mountains and Bajada province of the Chihuahuan Desert ecoregion (Griffith et al., 2007). Flow within this portion of the Rio Grande is derived primarily from the Rio Conchos and spring inflows from the Edward-Trinity Plateau Aquifer (URGBBEST, 2012), which could also influence local salinity concentrations. Water infrastructure projects in the Rio Conchos and upper Rio Grande have reduced flow, leading to declines in mean and peak stream discharge.

2.2. Collection and holding

Adult *P. popeii* of similar size (mean ± 1 SE; 72 mm ± 0.9) were collected from the mainstem of the Rio Grande near Laredo, TX, and the Lower Canyons of the Rio Grande Wild and Scenic River near Big Bend National Park in the summer, fall, and winter of 2017. Mussels were transported following previously described methods (Tsakiris and Randklev, 2014) and then shipped by air (overnight) or driven back to the Texas AgriLife Applied Aquatic Research Facility in Dallas, TX. Upon arrival, the temperature of the mussels was measured using an indirect infrared thermometer, and the resulting data were then used to set the water temperature in our holding system. Mussels remained in their shipping container (approx. 1–2 h.) until the temperature of the holding system and the temperature of the mussels were approximately the same (within 1–2 °C). Mussels were then placed in the holding system with reconstituted water (hereafter Artificial Freshwater) similar to that at their collection site. Hardness, alkalinity, and salinity were matched using the 5 salt recipe (Smith et al., 1997). Water temperature in the holding system was increased at a rate of 1 °C/day to reach the experimental temperature (approximately 18–20 °C). To ensure acclimation, mussels were held for approximately 7–10 days at this temperature prior to running the assay. Mussels were fed daily with a solution of Shellfish Diet 1800 and Nanno 3600 at a 1:2 ratio (Reed Mariculture) to reach a cell concentration of 2,250,000 cells/mL in the holding system (Wang et al., 2007).

2.3. Toxicity assay

Once mussels were sufficiently acclimated (no mortality in the holding system for 7–10 days), individuals were randomly assigned to one of ten 65.0-L glass aquaria. Each aquarium had its own filter and was filled with 65.0-L of reconstituted Artificial Freshwater (AFW), which was designed to match hardness and alkalinity levels in the Rio Grande where animals were collected. Morton Professional Choice Pool Salt was then added all at once to achieve the desired salinity (ppt NaCl) level for each individual treatment. The treatments for the Laredo population included 5 salinity treatments (4.0, 5.0, 6.0, 7.0, and 10.0 ppt NaCl) and a control (0.34 ppt); the latter is the resulting concentration from matching the hardness and alkalinity profiles for both study sites. Each treatment consisted of 10 individuals and was replicated three times for a total of 150 mussels not including control treatments. The treatments for the Lower Canyons population included three salinity treatments (3.0, 4.0, and 5.0 ppt NaCl) and a low salinity control (0.34 ppt), with each treatment replicated three times and five animals per replicate for a total of 45 animals not including the control. Our rationale for using a different treatment regime between the two populations was: 1) due to availability of animals, they were less abundant at the Lower Canyons site; 2) local spring inputs in the Lower

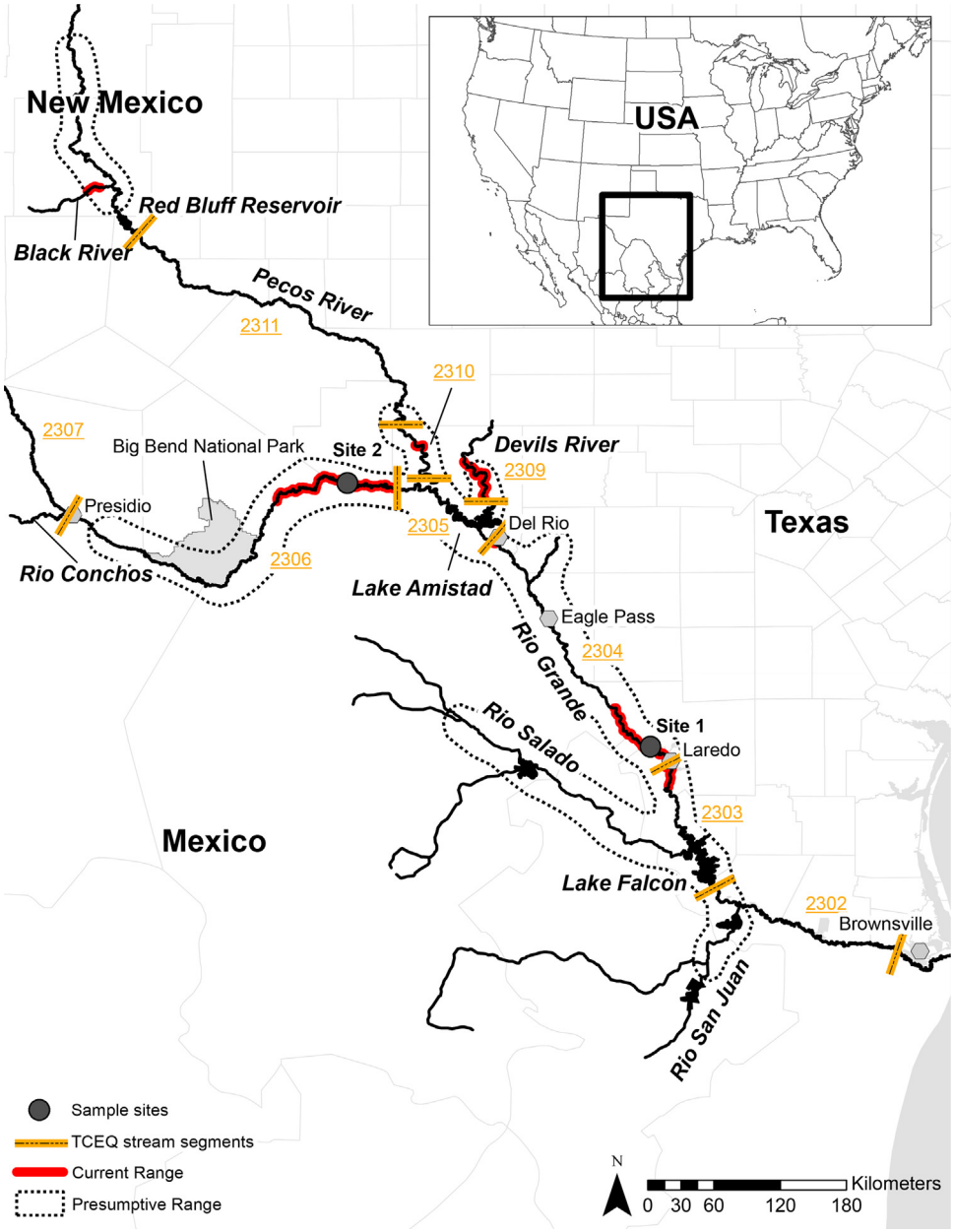

Fig. 1. Map of the study area showing sampling sites and TCEQ segment numbers. Mean, minimum, and maximum salinities (ppt) for these segments are provided in Table 1.

Canyons may be offsetting elevated salinity levels, which presumably would effect LCs and TUDs for this population; and 3) we were also attempting to identify sublethal effects of salinity exposure.

Prior to testing, mussels were marked with a numerical code using a Dremel tool to indicate their treatment and replicate. The mussels were then individually placed into 266 mL plastic cups filled halfway with rinsed sand and oriented with their anterior end down so that the posterior end could be viewed from outside of the tank to aid in assessing mortality and condition. The 266 mL cups were then placed into a 65.0-L glass aquaria containing one of the experimental salt-water concentrations. During the assay, water temperature was maintained at the ambient room temperature (20 ± 2 °C), and aeration was provided by hang-on-back filters. The dissolved oxygen ranged from 6 to 8 mg/L throughout the trial. The tanks were not flow-through, so water quality was monitored daily using Tetra EasyStrips, to ensure ammonia, nitrite, or nitrate levels never fell outside desired safe ranges (ammonia < 0 ppm, nitrite < 0 ppm, and nitrate < 40 ppm), which did not occur for any treatment. Salinity levels were monitored using an ExTech EC170 Salinity meter, accurate to 0.1 ppt, and if salinity rose or fell outside of the desired treatment range RO/DI water, or additional salt was added as appropriate.

Mussels were fed every other day with the same Reed Mariculture solution used during acclimation to maintain the water quality and clarity to allow visual monitoring. The mussels were exposed to experimental conditions for 10 days and monitored for signs of mortality 2–4 times a day. Mussels were considered healthy if they were actively filtering and exhibited valves that were slightly agape; they were considered moribund if they were open but did not reduce their gape when touched and were removed from the experiment.

2.4. Statistical analyses

LC50 (lethal concentrations (ppt) resulting in 50% mortality) and TUD50 (exposure time (days) for a given salinity to cause 50%

Table 1
Mean (± 1 standard error), minimum and maximum salinities (ppt) by TCEQ segment within the study area (Fig. 1). Dates of minimum and maximum values are provided in parenthesis. Period of record and number of observations within that period are also provided. Bold TCEQ segment numbers indicate that live individuals have been reported (2012 to present) in those segments.

TCEQ Segment	Location	Period of Record	n	Mean Salinity (ppt)	Min Salinity (ppt)	Max Salinity (ppt)
2302	Rio Grande – downstream of Lake Falcon	1968 – 2017	2769	0.32 (0.00)	0.03 (1978)	37.55 (2014)
2303	Rio Grande – Laredo to Lake Falcon	1974 – 2017	577	0.21 (0.00)	0.07 (1983)	0.64 (1990)
2304	Rio Grande – Del Rio to Laredo	1968 – 2017	2168	0.21 (0.00)	0.00 (2015)	0.37 (1993)
2305	Rio Grande – Lake Amistad	1974 – 2017	506	0.16 (0.01)	0.01 (1983)	2.40 (2015)
2306	Rio Grande – Presidio to Lake Amistad	1968 – 2017	1383	0.39 (0.01)	0.01 (1979)	4.04 (1993)
2307	Rio Grande – upstream of Presidio	1977 – 2017	944	0.74 (0.02)	0.00 (2002)	3.16 (1991)
2309	Devils River	1989 – 2017	73	0.03 (0.00)	0.02 (1989)	0.19 (2017)
2310	Pecos River – Independence Creek to confluence with Rio Grande	1986 – 2017	143	2.51 (0.08)	0.00 (1986)	5.45 (2017)
2311	Pecos River – Independence Creek to Red Bluff Reservoir	1968 – 2017	1136	7.51 (0.11)	0.00 (1968)	29.78 (2017)
N/A	Black River	2009 – 2011	6	0.85 (0.04)	0.75 (2010)	0.90 (2011)

mortality) were determined using probit analysis with a binomial distribution. We used a general additive model (GAM) approach to assess changes in salinity per TCEQ (Texas Commission on Environmental Quality) segment number (obtained from the TCEQ Surface Water Quality Web Reporting Tool; Table 1) by plotting salinity against time. We chose this approach because GAMs are robust to assumptions regarding independence and multicollinearity. Statistical comparisons of LC50 and TUD50 values across treatments were conducted using the confidence interval ratio test (Wheeler et al., 2006). This method compares the ratios of two LC50s (or any other ratio of lethality by toxicant) to 1 or the log (LC50 ratio) to 0. Confidence intervals were then constructed, and if the CI did not contain 1 (or 0 if the log was used), then the hypothesis that population LC or TUDs are the same was rejected (Wheeler et al., 2006). Probit models and the confidence interval test were implemented using the drc package in the R program (R Core Team 2017) (Ritz, 2016), and GAMs were analyzed using the MGCV package.

3. Results

3.1. Laredo population

For individuals from the Laredo population, the 6.0, 7.0, and 10.0 ppt NaCl treatments resulted in 100% mortality after 10-days. The 5.0 ppt treatment was lethal to all but one individual (97% mortality), whereas no individuals within the 4.0 ppt and control treatments perished (0% mortality). The LC50 values calculated for the 2, 4, and 10-day trial periods were 7.61, 5.72, and 4.78 ppt, respectively, and these values were significantly different from one another (Tables 2 and 3, Fig. 2). For the TUD, the median mortality values decreased with concentration such that TUD50 for the 5.0, 6.0, 7.0, and 10.0 ppt NaCl treatments were 4.97, 3.95, 2.66, and 1.41 days, respectively, and were significantly different from one another (Tables 2 and 3, Fig. 2); we could not calculate TUD50 for 4.0 ppt due to insufficient mortality.

3.2. Lower Canyons population

For individuals from the Lower Canyons population, the 5.0 ppt NaCl treatment resulted in 77% mortality after 10 days. The 4.0 ppt treatment was lethal to 20% of the individuals tested, and the 3.0 ppt and control treatments showed no mortality to any individuals. LC50 values for the 96 h and 10-day trial periods were 5.38 and 4.52 ppt, respectively, and these values were significantly different (Tables 2 and 3, Fig. 2). The LC50 for the 2-day trial period could not be calculated due to insufficient mortality. For TUD, the TUD50 was 7.90 days for 5.0 ppt, but we could not calculate TUD50 for 4.0 ppt due to insufficient mortality.

3.3. Laredo population vs. Lower Canyons population

We found no significant difference in LC50 estimates between populations at 96-hrs or 10-days (Tables 2 and 3, Fig. 2). In contrast, TUD for the Laredo and Lower Canyons populations differed significantly (Tables 2 and 3, Fig. 2). For the Laredo population, the TUD50 value at

Table 2
Lethal salinity concentrations (ppt) causing 50% (LC50) mortality in adult individuals of *Popenaias popeii* (Texas Hornshell) during 10-day, 96-hr and 48-hr time periods. Exposure time (days) to death for a given salinity causing 50% (TUD50) mortality for the same individuals is also presented. 95% confidence intervals are listed in parentheses. ND denotes that a value could not be determined because of low mortality, which precluded accurate estimation of LC50 or TUD50 values.

Site	Location	Concentration to death		Exposure time to death	
		Duration	LC50	Concentration	TUD50
1	Rio Grande – near Laredo	10-day	4.78 (4.72 – 4.84)	4	ND
		96-hr	5.72 (5.49 – 5.95)	5	4.97 (4.60 – 5.35)
		48-hr	7.61 (7.13 – 8.08)	6	3.95 (3.72 – 4.18)
				7	2.66 (2.45 – 2.87)
				10	1.41 (1.38 – 1.44)
2	Rio Grande – Lower Canyons	10-day	4.52 (4.24 – 4.79)	3	ND
		96-hr	5.38 (5.22 – 5.54)	4	ND
		48-hr	ND	5	7.90 (6.90 – 8.91)

Table 3
Confidence interval overlap ratio test of lethal concentrations (ppt) causing 50% (LC50) mortality in adult individuals of *Popenaias popeii* (Texas Hornshell) during 10-day, 96-hr and 48-hr time periods. Confidence interval overlap ratio test of exposure time (days) to death for a given salinity concentration causing 50% (TUD50) mortality for the same individuals. LC and TU values are not statistically different when the confidence interval for the ratio includes zero.

Comparison	Ratio	SE	LL	UL	Significant
Lethal concentrations (LC50)					
$Laredo_{50–10}$ vs. $Lower\ Canyons_{50–10}$	0.95	0.03	0.89	1.0	N
$Laredo_{50–96}$ vs. $Lower\ Canyons_{50–96}$	0.94	0.02	0.89	1.01	N
$Lower\ Canyons_{50–10}$ vs. $Lower\ Canyons_{50–96}$	0.84	0.03	0.78	0.90	Y
$Laredo_{50–10}$ vs. $Laredo_{50–96}$	0.84	0.02	0.80	0.87	Y
$Laredo_{50–10}$ vs. $Lower\ Canyons_{50–96}$	0.89	0.01	0.86	0.92	Y
$Laredo_{50–10}$ vs. $Laredo_{50–48}$	0.63	0.02	0.59	0.67	Y
$Lower\ Canyons_{50–10}$ vs. $Laredo_{50–96}$	0.79	0.03	0.73	0.85	Y
$Lower\ Canyons_{50–10}$ vs. $Laredo_{50–48}$	0.89	0.03	0.54	0.64	Y
$Laredo_{50–96}$ vs. $Laredo_{50–48}$	0.75	0.03	0.70	0.81	Y
$Lower\ Canyons_{50–96}$ vs. $Laredo_{50–48}$	0.71	0.02	0.66	0.76	Y
Time until death (TUD50)					
$Laredo_{50–5}$ vs. $Laredo_{50–6}$	0.79	0.04	0.72	0.87	Y
$Laredo_{50–5}$ vs. $Laredo_{50–7}$	0.53	0.03	0.48	0.59	Y
$Laredo_{50–5}$ vs. $Laredo_{50–10}$	0.28	0.01	0.26	0.31	Y
$Laredo_{50–5}$ vs. $Lower\ Canyons_{50–5}$	0.63	0.05	0.54	0.72	Y
$Laredo_{50–6}$ vs. $Laredo_{50–7}$	0.67	0.03	0.61	0.74	Y
$Laredo_{50–6}$ vs. $Laredo_{50–10}$	0.36	0.01	0.33	0.38	Y
$Laredo_{50–6}$ vs. $Lower\ Canyons_{50–5}$	0.50	0.04	0.43	0.57	Y
$Laredo_{50–7}$ vs. $Laredo_{50–10}$	0.53	0.02	0.48	0.58	Y
$Laredo_{50–7}$ vs. $Lower\ Canyons_{50–5}$	0.34	0.02	0.29	0.39	Y
$Laredo_{50–10}$ vs. $Lower\ Canyons_{50–5}$	0.18	0.01	0.15	0.20	Y

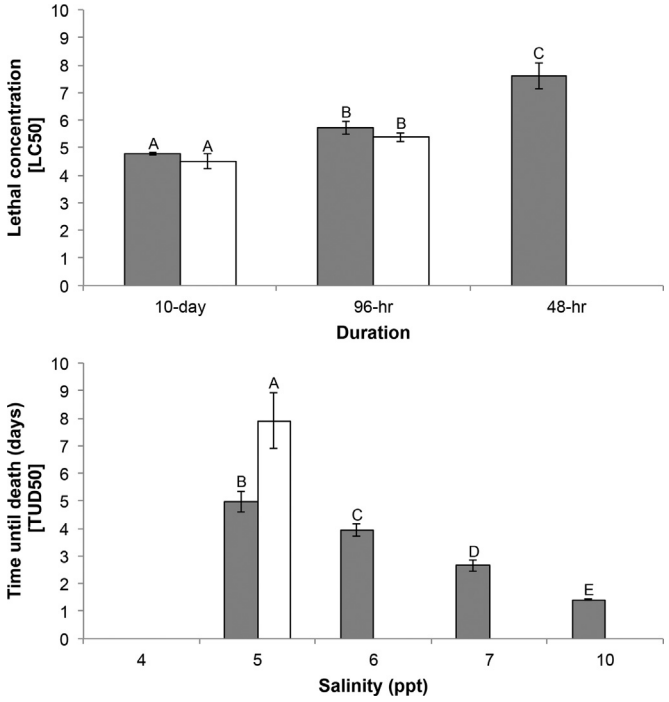

Fig. 2. Top graph represents lethal concentrations (ppt NaCl) causing 50% (LC50) mortality in adult individuals of *Popenaias popeii* (Texas Hornshell) during 10-day, 96-hr and 48-hr time periods. Bottom graph represents exposure time (days) to death for a given salinity (ppt NaCl) causing 50% (TUD50) mortality for the same individuals. Solid gray bars represent LC50 and TUD50 values for the Laredo population (Site 1; see Fig. 1). Solid white bars represent LC50 and TUD50 values for the Lower Canyons population (Site 2; see Fig. 1). Error bars denote 95% confidence intervals. LC or TUD values with the same uppercase letters are not significantly different based on the confidence interval overlap ratio test.

5.0 ppt was 4.97 days, which was much earlier than those for the Lower Canyons population, for which the TUD50 value was 7.90 days (Table 2, Fig. 2). At 4 ppt we could not do a comparison between populations because we were unable to calculate TUD50 values for either population due to insufficient mortality.

3.4. Salinities by TCEQ segment

Salinity varied spatially and temporally across the various TCEQ segments and showed a general pattern of increase over time depending on the segment (Figs. 1 and 3). A majority of the GAMs were characterized by significant smoothing functions, and these models had correlation coefficients ranging from 0.00 to 0.21 (Table 4); the exceptions were segment 2302 (Rio Grande downstream of Lake Falcon) and 2309 (Devils River). When comparing salinity across segments, we found that TCEQ segments 2305 (Lake Amistad and its interface with the Rio Grande), 2306 (Rio Grande from Presidio to Lake Amistad), 2307 (Rio Grande upstream of Presidio, TX), 2310 and 2311(Pecos River) had elevated salinities relative to other segments within the study area (Figs. 1 and 3). Mean salinities for these segments were less than 1.0 ppt, except for those on the Pecos River, which ranged from 2.51 to 7.51 ppt (Table 1). However, the maximum reported salinities within these segments were elevated, ranging from 2.4 to 4.04 ppt (Table 1). Overlaying the LC50 values for both the Laredo and Lower Canyon populations on these graphs shows that the salinities were occasionally (segments 2304 [Rio Grande from Del Rio to Laredo], 2305, 2306) or consistently (segment 2307) near the salinity limits where 50% mortality begins, and these limits were exceeded in some cases (segments 2306, 2310, 2311). Populations of *P. popeii* are known to occur in TCEQ segments 2304, 2309 (Devils River), 2306, and 2310 (Fig. 1).

4. Discussion

The effects of salinity can have negative consequences for freshwater ecosystems in arid and semi-arid regions because they often harbor endemic species with small population sizes and narrow distributions (Carlson and Muth, 1989). Salinity and other hydrological alterations can eliminate native species, which are often more sensitive to these types of impacts, allowing more tolerant cosmopolitan species to invade or displace native species (Julian Olden et al., 2004; Julien Olden and Poff, 2004; Julien Olden and Rooney, 2006; Rahel, 2010). Historically, at least 15 species of unionids occurred in the Rio Grande in Texas, but many of these species are in decline. Our findings empirically show that this decline may be related, in part, to salinization. Specifically, we found that salinities as low as 4.52 ppt NaCl resulted in mortality of *P. popeii* after 10 days of chronic exposure. LC50 values at 48-hr or 96-hr were much higher than this value, but are likely less ecologically relevant, except in cases of salinity slugs. TUD50 values showed that 50% mortality occurred at 5 ppt NaCl in 4.97 and 7.90 days for the Laredo and Lower Canyons populations, respectively. This indicates that while the 10-day LC50 may be the same between the two populations, the sensitivity at these concentrations varies, such that the Laredo population succumbs to mortality much sooner at 5 ppt than the Lower Canyons. The similarity in LC50s between populations is interesting because it suggests that local spring inputs within the Lower Canyons may be offsetting elevated salinity concentrations in the Lower Canyons, and therefore indirectly influencing tolerance for this population. The incongruence in TUD50 values at 5 ppt is equally interesting because it suggests there are population differences in salinity sensitivity, which for the Lower Canyons appears to be an adaptation to periodic increases in salinity that may not be ameliorated by spring inputs.

Over the last 100 years, stream flow in the Rio Grande has been reduced by ground water pumping, water withdrawals for irrigation and oil and gas activities, and the creation of reservoirs for water

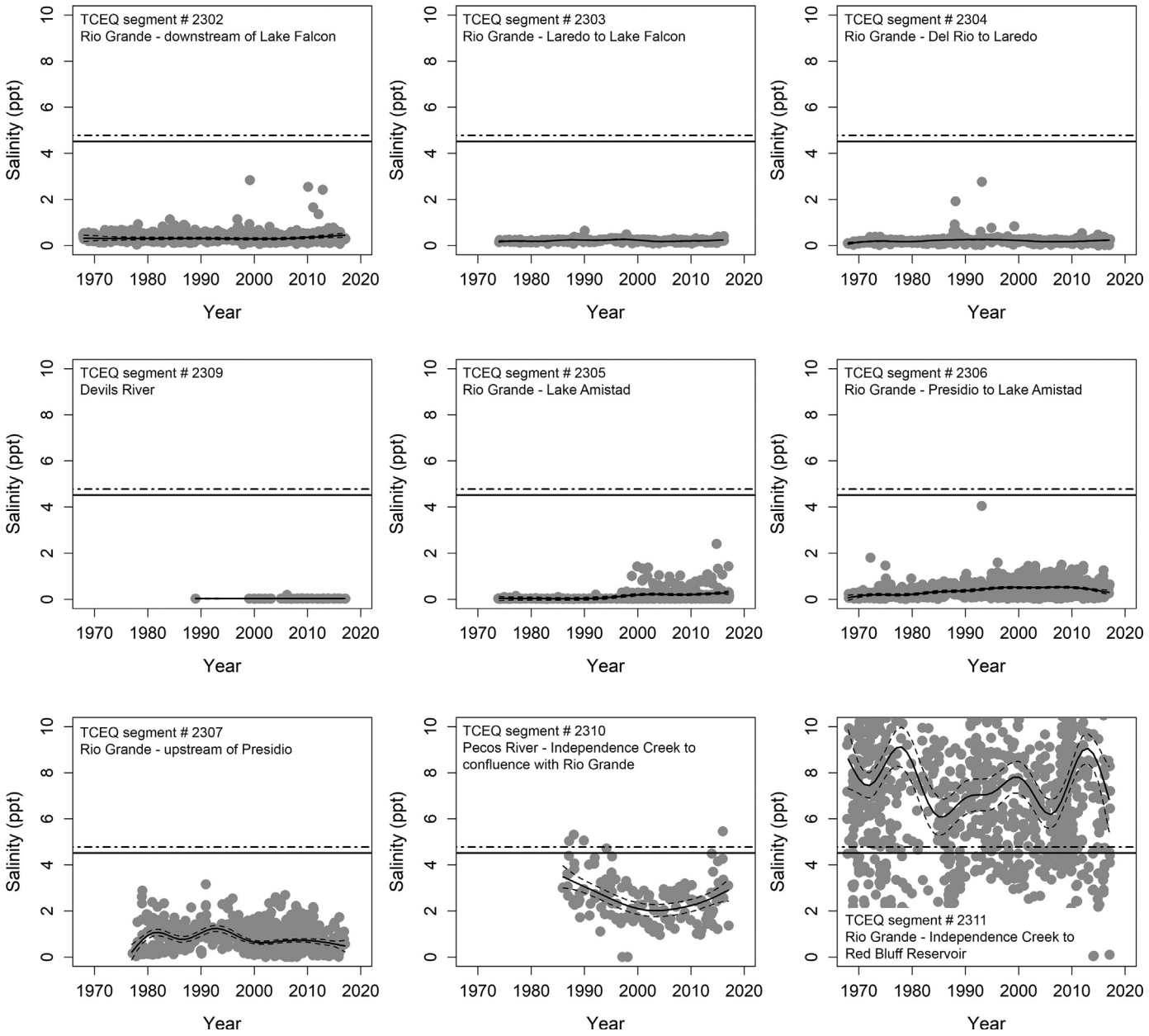

Fig. 3. Generalized additive models for salinity (ppt) measured over time by TCEQ segment within the study area (see Fig. 1). Fitted models (solid lines) and 95% confidence intervals (dashed lines) are plotted on observations (gray points). The 10-day LC50 values for the Laredo (dashed line) and the Lower Canyons populations (dash-dotted lines) are included in each plot to show whether the measured salinity within these segments is near to or exceeds these lethal concentration values. Mean, minimum, and maximum salinities (ppt) for these segments are provided in Table 1.

Table 4
Generalized additive modeling results for salinity (ppt) over time for TCEQ (Texas Commission on Environmental Quality segments within the Rio Grande. See Table 1 for period of record and summary statistics for each segment. The Black River is not included because long-term salinity data are not available for the reach where *P. popeii* still persists. Bold TCEQ segment numbers indicate that live individuals have been reported (2012 to present) in those segments.

TCEQ Segment	Location	Adjusted *r*2	Deviance explained	Estimated *df*	*F*-value	*p*-value
2302	RioGrande – downstream of Lake Falcon	0.00	0.38	3.3	0.08	0.08
2303	Rio Grande – Laredo to Lake Falcon	0.21	22.3	8.8	17.75	< 0.001
2304	Rio Grande – Del Rio to Laredo	0.12	12.0	8.7	32.68	< 0.001
2305	Rio Grande – Lake Amistad	0.13	13.4	5.4	11.41	< 0.001
2306	Rio Grande – Presidio to Lake Amistad	0.09	17.1	7.9	32.08	< 0.001
2307	Rio Grande – upstream of Presidio	0.09	9.53	8.7	10.33	< 0.001
2309	Devils River	− 0.01	< 0.01	1.0	0.00	0.993
2310	Pecos River – Independence Creek to confluence with Rio Grande	0.19	21.0	2.9	9.22	< 0.001
2311	Pecos River – Independence Creek to Red Bluff Reservoir	0.04	4.83	8.73	5.96	< 0.001

storage (URGBBEST, 2012). These issues have likely amplified the naturally elevated salinity in the basin. Our findings show temporal changes in salinity within portions of the mainstem of the Rio Grande and at least one of its tributaries. In our analysis, we found that salinity has increased within five of the nine TCEQ segments assessed, regularly reaching levels that exceed 1.0–2.0 ppt, which is approaching concentrations at which 50% of mortality occurs for *P. popeii*. These concentrations are half of what we report for 10-day LC50s, but because mussels are nearly isosmotic with the surrounding medium, sublethal effects at these lower concentrations presumably would be occurring. Sublethal effects are as problematic as outright mortality because population performance (i.e., growth, survivorship, and reproduction) is likely diminished as individuals shift energetic resources from growth and reproduction to maintenance (R. Levins and R.H. MacArthur's principle of allocation; Cody, 1966). Such shifts to stave off mortality hinder later growth and reproduction, producing no net gain in population performance. For example, laboratory studies have shown in fish that physiological effects, such as decreased oxygen consumption rates, can occur in response to elevated salinity, and this response can be immediate (i.e., within 24 h) (Morgan et al., 1997). Similar observations have been made for mussels, wherein changes in water quality leads to decreases in oxygen consumption rates, which presumably would affect metabolic processes, and if prolonged likely impact growth and reproduction as well as the ecosystem services provided by mussels (Cummings and Graf, 2010).

Our results reveal that populations of *P. popeii* in parts of the Rio Grande basin are being impacted by salinization. In particular, our findings for the Pecos River should serve as a cautionary tale for other regions and rivers where salinization is now becoming an issue, even those with robust mussel populations. Currently, salinity levels in the Pecos River exceed the 10-day LC50 tolerance limits we identified for *P. popeii*, although historically this likely wasn't the case. As late as ~1968, portions of the lower Pecos River contained a robust population of *P. popeii*, similar in abundance to current stronghold populations within the Lower Canyons area of the Rio Grande (Randklev et al., 2018). Recent surveys in the lower Pecos at locations where *P. popeii* may have been historically abundant have found only 3 live individuals, although numerous long-dead shells have been collected (Randklev et al., 2018). This implies that a population on par with current stronghold populations for this species has all but collapsed within 45 years and that this extirpation event appears to coincide with increases in salinity levels and concomitant decreases in flow.

Detailed knowledge on salinity tolerances for mussels is limited to less than 5% of the species known to occur in North America, and little is known about the salinity tolerances of mussel species occurring within the southwestern United States. The few studies that have examined this topic (Table S1) were performed in regions where the background salinity from natural sources is low and water quantity is rarely an issue (Beggel and Geist, 2015; Blakeslee et al., 2013; Ercan and Tarkan, 2014; Gillis, 2011; Patnode et al., 2015). This is completely different from what is occurring in rivers within the southwestern United States, such as the Rio Grande (Beggel and Geist, 2015; Blakeslee et al., 2013; Ercan and Tarkan, 2014; Gillis, 2011; Johnson et al., 2018; Patnode et al., 2015; Roy et al., 2015). In comparing our results to these other studies, salinity tolerances for adult mussels range from 4.0 to 12.0 ppt for adults depending on exposure time (Beggel and Geist, 2015; Blakeslee et al., 2013; Ercan and Tarkan, 2014; Gillis, 2011; Johnson et al., 2018; Patnode et al., 2015; Roy et al., 2015). Thus, our findings for *P. popeii* combined with these other studies indicate that adult mussels can survive elevated salinity concentrations, however, these studies also indicate there is a wide range of salinity tolerance for adults. This means the sensitivity of adult mussels to salinity within arid and semi-arid systems, or any other region, may not be well predicted by the salinity tolerances of other mussel species. Our results also indicate that tolerances determined from a single site may not account for variation within species. Although we found no differences in LC50s between the two populations, we did find significant differences in TUD values at 5 ppt, which would indicate intraspecific variation in salinity sensitivity.

In this study, we were able to successfully identify the salinity tolerances of a federally endangered mussel species from the southwestern United States, which, to our knowledge, is a novel finding. Based on these results we provide the following recommendations for additional research on salinity tolerance for *P. popeii*. First, conservationists and natural resource personnel should begin managing river systems to prevent mass lethality for wildlife populations, rather than to just stave off mortality. Because of this, sublethal endpoints such as biochemical (i.e., glycogen, lipids, and proteins) and physiological markers (i.e., respiration and nitrogenous waste excretion) to salinity toxicity should be identified (Baker and Hornbach, 2000; Barber et al., 1988; Haag et al., 1993; Patterson et al., 1997, 1999). These sublethal endpoints in turn could then be used as management benchmarks, wherein conservation and management activities are initiated when salinity concentrations reach or exceed these benchmarks to reduce impacts to reproduction and growth and to prevent mass mortality. Second, we tested only adult mussels of *P. popeii* in order to quickly provide information on its salinity tolerance prior to listing. Now that this species has been listed and efforts are underway to develop a species-specific conservation and recovery plan, future studies should test glochidia and juvenile stages. Previous studies have shown that glochidia and juveniles are more sensitive to chemical and thermal contaminates than adults, and one would therefore presume that this would also be the case for salinity tolerance (Blakeslee et al., 2013; Bringolf et al., 2007). Thus, future studies examining these life stages in conjunction with biochemical and physiological markers would be highly informative for conservation and management decisions. Third, recent studies have shown that host attachment and larval transformation can be negatively affected by salinities ranging from 0.5 to 3.0 ppt (Beggel and Geist, 2015; Blakeslee et al., 2013), which are already occurring in portions of the Rio Grande. Thus, future studies should also examine how elevated salinity impacts host attachment and larval transformation for *P. popeii*. Finally, seasonal variation in salinity tolerances, which presumably could occur, should also be examined as behavior, maintenance and reproductive processes vary based on water temperature, flow, and reproduction, among other factors (Cummings and Graf, 2010).

5. Conclusions

Our study increases the understanding of salinity tolerances of unionids, a critically imperiled fauna, and has important implications for water and land use management, particularly in the southwestern United States. Our results show that salinity tolerances between two disjunct populations of *P. popeii* are the same, however, the sensitivity to salinity, in terms of time until death, appears to differ, which could reflect local adaptation. This difference appears to be related to local spring inputs within the Lower Canyons, which highlights the importance of springs and groundwater input for this species. Our study also demonstrates that the decline of *P. popeii* within the Rio Grande basin, particularly in the Pecos River, is likely linked to elevated salinity and foreshadows what could be expected in other river basins where secondary salinization, direct or indirect, is increasing. We also show that *P. popeii* is living close to its salinity limit within other reaches of the Rio Grande, which should be cause for alarm among decision makers, resource managers and conservationists. It is well known that species experiencing sublethal levels of stress have less net energy to allocate to dealing with other stressors, both chemical and nonchemical (De Coen and Janssen, 1997; Sokolova et al., 2012). Thus, for *P. popeii* populations in these reaches, salinity should be evaluated as a potential limiting factor, and recovery efforts should be developed following guidelines made to mitigate thermal and chemical stress, whereby the cumulative effects of multiple stressors and salinity are considered together when developing watershed and/or site-specific water quality

criteria. Finally, based on our data and recent mussel modeling efforts within the basin (Randklev et al., 2018), habitat restoration combined with science-based flow recommendations must be undertaken in order to protect and potentially restore *P. popeii* within the Rio Grande basin.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2018.11.031.

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