FINAL PERFORMANCE REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-132-R-2

Endangered and Threatened Species Conservation

Survey of Texas Hornshell Populations in Texas

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25 August 2014

FINAL REPORT

STATE: Texas **GRANT NUMBER:** E – 132-R-2

GRANT TITLE: Survey of Texas Hornshell Populations in Texas, Yr 2&3

REPORTING PERIOD: 1 Sep 11 to 31 Aug 14

OBJECTIVE(S):

To assess the current distribution of *P. popeii* in Texas; evaluate long-term changes in distribution range; locate and describe existing populations, and determine species' habitat requirements.

Segment Objectives:

1. Assess the current distribution of *Popenaias popeii* in Texas;
2. Evaluate long-term changes in distribution range;
3. Locate and describe existing populations, and (4) determine species' habitat requirements.

Significant Deviation: None.

Summary Of Progress: Please see Attachment A.

Location: Terrell, Maverick, Webb, and Val Verde counties, TX

Cost: Costs were not available at time of this report.

Prepared by: Craig Farquhar **Date:** 25 Aug 2014

Approved by: **Date:** 25 Aug 2014
C. Craig Farquhar

ATTACHMENT A

TEXAS PARKS AND WILDLIFE DEPARTMENT

TRADITIONAL SECTION 6

Joint Project with New Mexico Department of Game and Fish

FINAL PERFORMANCE REPORT

State: Texas **Project Number:** 419446

Project Title: "Survey of Texas Hornshell Populations in Texas"

Time period: February 3, 2012 - August 31, 2014

Full Contract Period: 3 February 2012 **To:** 31 August 2014 (with requested 12-month no-cost extension)

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This joint Section 6 project is collaboration between the U.S. Fish and Wildlife Service (USFWS), Texas Parks and Wildlife Department (TPWD), SUNY Buffalo State (BS) and New Mexico Department of Game and Fish (NMDGF). It is coordinated between PIs Lyubov Burlakova and Alexander Karatayev (BS), agency biologists Brian Lang (NMDGF) and Marsha May (TPWD). Drs. David Berg and Kentaro Inoue (Miami University, Oxford, OH) performed comparative genetic analysis of *P. popeii* from Texas and New Mexico.

Abstract

Although almost all endangered bivalve molluscs belong to the freshwater order Unionoida, sufficient data are lacking for the majority of these species. As a result, a species may become rare, endangered, and even extinct before the first population assessment is conducted. This is especially true for endemic species, particularly those limited to remote regions with difficult access. In 2010-2014 we studied the current distribution and population densities of the endemic Rio Grande unionid *Popenaias popeii* in Texas, located and described existing populations and mussel growth rate, determined species' habitat requirements, and developed a method to reconstruct species' historical range and population size to evaluate changes in the population's size and distribution range over the last 100 years. Sampling over 250 sites in four rivers that constitute the entire historical range of *P. popeii* in Texas, we found that the species has been extirpated from two rivers, a 76% decrease in the combined total length of the rivers populated by the mussel. Using our estimations of *P. popeii* density we found an 86% overall decline in the population size of *P. popeii* in the state. The remaining population of this species in the Rio Grande is fragmented, with only one 190 km stretch between Laredo and Eagle Pass still supporting a high density of *P. popeii* in Texas. Based on growth increments we calculated that the maximum longevity of mussels larger than 85 mm in the Rio Grande may be up to 14 years. According to our preliminary analysis of mark-recapture data, *P. popeii* population in the Rio Grande experiences large fluctuations in density and high migration rates. High *P. popeii* dispersal rates along with smaller individual size and shorter live span in the Rio Grande compared to *P. popeii* population parameters in the Black River may be at least partially explained by larger fluctuations in the discharge rate and less stable environment in this large river. Genetic studies revealed a high degree of structure in the *P. popeii* populations at both a regional scale among Rio Grande drainages, and also a more local scale in the Black River. Species database with abundance and habitat data was submitted to TPWD Natural Diversity Database, making all data readily available for conservation, monitoring and decision making. Conservation plans for *P. popeii* should be aimed at promoting river management, including prevention of water over-extraction, damming, pollution, and maintaining flow regime in response to the species needs.

Introduction

Freshwater ecosystems provide many important goods and services such as food, clean water, and flood and erosion control, estimated to have a value of trillions of dollars annually (Millennium Ecosystem Assessment, 2005). Indeed, with ninety percent of the world's population living near fresh water, these unique ecosystems are hotspots of human activity (Kummy *et al*., 2011). At the same time, existing as semi-isolated islands in a terrestrially-dominated landscape, freshwater systems have garnered a disproportionally high number of endemic species compared to marine and terrestrial environments (Strayer and Dudgeon, 2010). Due to steeply rising human demands for water over the past century (Naiman and Turner, 2000; Jackson *et al*., 2001), these ecosystems have suffered a large global decline in biodiversity; many freshwater species are now extremely threatened, possibly more so than species in marine and terrestrial systems (Dudgeon *et al*., 2006; Strayer and Dudgeon, 2010).

Globally, molluscs (both Bivalvia and Gastropoda) represent 44% of all extinct animals and 24% of those critically endangered (IUCN, 2013). In North America, three quarters of all mollusc species are considered imperiled or extinct, exceeding the imperilment levels of fish (39%) and crayfish (48%) (Williams *et al.*, 1993; Johnson *et al.*, 2013). Nevertheless, at a global scale, only 8% of molluscs have been evaluated for extinction risks, compared to 100% of mammals and birds, 91% of amphibians, and 35% of fish (IUCN, 2013). This lack of attention is ironic not only because the vanishing mollusc species constitute an integral part of the biological diversity in threatened freshwater ecosystems, but also for the reason that they provide important ecological functions and services (Aldridge *et al.*, 2007; Vaughn, 2010).

Among mollusc species, the most threatened and the most data deficient are in the order Unionoida (IUCN, 2013); simultaneously, the lack of information on essential parameters for these species, such as distribution range and population size, greatly hampers the assessment of their conservation status. Often, a species may become rare, endangered, and even extinct before the first population assessment is conducted. This is especially true for endemic species which have a limited range restricted to remote regions with difficult access. However, in contrast to most other invertebrates, dead molluscs (particularly unionid bivalves) leave large calcareous shells that may remain in sediments for decades, providing evidence of former populations and helping to reconstruct their historical range.

Among the 48 species known in Texas (Burlakova *et al.*, 2011a), over 60% are rare or very rare (Burlakova *et al.*, 2011b), and 15 are listed as threatened by Texas Parks and Wildlife Department (TPWD) (Texas Register 2010). The Rio Grande endemic *Popenaias popeii* (Texas hornshell) was first described by Lea (1857) as *Unio popeii* from the Devils River in Texas and the Río Salado in Mexico. The distribution range of this species is restricted to the Rio Grande drainage in Texas (Singley, 1893; Taylor, 1967; Burlakova *et al.*, 2011a, b; Karatayev *et al.*, 2012, Appendix 2), New Mexico (Lang, 2001; Carman, 2007), and several Mexican tributaries (Simpson, 1914; Johnson, 1999; Strenth *et al.*, 2004). Singley (1893) described it as a "rare shell", Strecker (1931) reported that the species "seems to be rather scarce", Stansbery (1971) defined it as "rare and endangered", and Neck (1984) included *P. popeii* in his list of restricted and declining species of Texas. From the mid-1970s until the beginning of our study, no live *P. popeii* have been found in the Rio Grande drainage in Texas (Howells, 2001; Karatayev *et al.*, 2012). Because of this dramatic decline, *P. popeii* has been added to the Texas and New Mexico state's list of threatened species (Texas Register 35, 2010; NMDGF 2006), considered as New Mexico state endangered (NMDGF 2010), as critically endangered by IUCN (www.iucnredlist.org/details/17992/0), and is currently a candidate for listing (priority 8) in both states under the federal Endangered Species Act (Federal Register 2008, 2013).

Genetic studies show that New Mexico and Texas populations of *P. popeii* exhibit low within- and among-population variation at allozyme and mitochondrial DNA loci (Hoeh *et al.* 1999, 2009; Chapman *et al.*, 2008). These low levels of genetic variation could limit the evolutionary flexibility of the species to successfully meet future abiotic and biotic challenges, which poses imminent concerns for the long-term survival throughout its historic range.

Eleven live *P. popeii* were found in Texas in 2008, in the Rio Grande River in Terrell and Webb counties, and in the Devils River (Burlakova and Karatayev, 2008), however, not the size nor the boundaries or viability of these populations where known. The ecology and habitat requirements of this species in Texas were unknown as well. As a result, there is no estimation of existing threats, and no recovery plan for *P. popeii* in Texas. Only one other population in the United States outside Texas is in the Black River, Eddy County, New Mexico (Lang, 2001). Factors threatening extant populations of *P. popeii* in New Mexico include habitat modification, sedimentation, ground- and surface-water contamination, drought, floods, and aquatic invasive species (Lang, 2009, NMDGF, 2010). Based on the study of *P. popeii* in New Mexico, our colleagues developed a state recovery plan (Carman, 2007) and suggested numerous management strategies.

The most efficient means to secure the viability of existing subpopulations is by applying the knowledge of their distribution and habitat needs towards reducing and preventing threats through existing regulatory mechanisms, habitat restoration programs, and partnerships with various stakeholders (USFWS Endangered Species Recovery Program, 2009). Therefore, the knowledge about the species' distribution, existing populations, and habitat requirements is the first and the most essential step for their subsequent recovery plans. Due to the rarity of this species, a quantitative assessment of the *P. popeii* population in Texas has never been conducted, restricting our ability to monitor changes in its historical distribution and protect this species. This study aimed to determine the current distribution, long-term changes in distribution range, and habitat requirements of *P. popeii* in Texas. Our 2010-2014 studies provided information essential for the development of their recovery plans: located existing populations in need of protection, assessed their current status, determined existing threats and management options, and established sites to monitor these populations in the future.

Program Narrative Objectives

1. Assess the current distribution of *Popenaias popeii* in Texas;
2. Evaluate long-term changes in distribution range;
3. Locate and describe existing populations, and
4. Determine species' habitat requirements.

Approach

During the three-year study we will: (1) assess the current distribution of Texas Hornshell, *P. popeii*, in Texas, and compare it with the historical data to evaluate long-term changes in distribution range, locate existing populations, determine habitat requirements, and identify potential fish hosts; (2) establish population monitoring using mark-and-recapture methods; (3) use the microsatellite genetic tools developed by our New Mexico partners to enable further understanding of population processes; (4) evaluate growth, survivorship and population viability; (5) develop the recovery plan for *P. popeii* in Texas and recommend management actions necessary to protect the species. These objectives will provide necessary information to guide the implementation of effective conservation strategies.

Methods

Location

We studied the Rio Grande and its tributaries where *P. popeii* historically had been documented, including the Pecos and Devils rivers, and Las Moras Creek. The Rio Grande (total length: 2,830 km, including 1,470 km in Texas) is the one of the longest rivers in North America, which flows across seven physiographic provinces, from mountain forests and high mountain deserts to desert shrub and grassland (Dahm *et al.*, 2005). In the state of Texas, the Rio Grande forms the border between the United States and Mexico and has been intensively used by both countries during the last century for irrigation, industrial and domestic water consumption (Dahm *et al.*, 2005; Wong *et al.*, 2007). The Rio Grande suffers from water over-extraction, persistent drought, an increase in border populations, and subsequent increase in the water pollution and waste water discharge (Dahm *et al.*, 2005; Wong *et al.*, 2007; Douglas, 2009). The river flow is regulated by Amistad Dam (completed in 1969) and Falcon Dam (completed in 1953) that impound the Rio Grande along the border for irrigation and flood control along with several additional low water dams or weirs.

The Pecos River (total length 1,490 km, including 679 km in Texas) is the largest Rio Grande tributary in the United States. It flows from Mora County, New Mexico, southeast through Texas where it joins the Rio Grande. The river flows through flat, semi-arid lands along the upper section, and through canyons and desert terrain in the lower riches. Red Bluff Reservoir, a small lake established for hydroelectric power, is located near the Texas - New Mexico border. Below Red Bluff Reservoir, the Pecos River contains only a very limited flow of water, and passes through an arid region of West Texas where rainfall is sporadic and minimal. In the middle of 19th century the river was fast moving and deep, with slightly saline taste (Pope, 1854 cited from Hoagstrom, 2003). Several hydrological changes, initiated in the 1880s, contributed to streamflow salinization, including diminished streamflow due to aquifer depletion, reduced floods, groundwater overdraft, and increased prevalence of natural, high-salinity groundwater (Hoagstrom, 2003; 2009). Along with water overdraft and increasing salinity (Campbell, 1959; Davis, 1987; Gregory and Hatler, 2008), many other factors including contaminated runoff, oil field pollutants and blooms of toxic algae caused deterioration of the water quality (TPWD, 1974) as most of the industrial and agricultural activities in this area solely depend upon the Pecos River.

In contrast to the Pecos River, the Devils River is considered one of the cleanest, naturally flowing streams remaining in Texas due to its remote location and hostile environment (TPWD, 1974). It begins in Sutton County, flows southwest for 151 km through Val Verde County and empties into the northeastern shore of the Amistad Reservoir. The Devils River is intermittent in its upper reaches (from FM 189 to the southernmost crossing of State Highway 163 or Baker's Crossing) due to the arid nature of the region, and in some stretches, the river goes completely underground. The normal volume of water in the river progressively increases downstream with median daily discharge ~14 m^3s^{-1}, and water levels are subject to extreme fluctuations ranging from 1 to 3,480 m^3s^{-1} (International Boundary Water Commission stream gauge data). Downstream of Dolan Falls the river begins to widen and deepen gradually, with an abundance of long, deep pools alternated with rapids. The terminal 30 km stretch of the Devils River is regularly flooded by the Amistad Reservoir. In addition, several low water dams restrict water flow in the river.

The headwaters of Las Moras Creek are formed by artesian Las Moras Springs located in Fort Clark, Kinney County, Texas. The springs fill a swimming pool built in the beginning of the 20th century (Haenn, 2002). Las Moras Creek runs 60 km downstream to the confluence with the Rio Grande in Maverick County. The flow rates between 1896 and 1978 ranged from a high of 1.7 m^3 per second (m^3s^{-1}) in 1899 to lows of 0.16 and 0.10 m^3s^{-1}in 1964 and 1971 (Brune, 1975). In those two years, the springs dried up completely for a time.

Data collection

To assess the distribution, density and long-term population dynamics of *P. popeii* in the Rio Grande drainage within Texas, both field studies and historical data were used. With additional data collected by our collaborator T. Miller (Laredo Community College) since 2001, we surveyed a total of 250 sample locations (“subsites”) pooled into 42 larger sites (“pooled sites”) within the Rio Grande system (Figure 1) (Karatayev *et al.*, in review, Appendix 1).

Fourteen of these sites were sampled once, while 28 sites were sampled from 2 to 25 times. Over 580 km in remote areas of the Rio Grande, the Devils and the Pecos rivers were sampled using an airboat and a canoe (Photo 1-3). Due to the prevalence of private land in Texas (only 2% of the lands remain in public ownership, TPWD, 1974), some survey sites were selected within state parks, near public boat ramps, or based on accessibility from roads. Landowner Permission was acquired from each property owner, when surveys were conducted from private land, before entering the property. The work was carried out with an appropriate Scientific Research Permit issued by the Texas Parks and Wildlife Department, National Park Service Scientific Permit for Big Bend National Park, and Amistad National Recreational Area Research Permit.

At each site, both live and dead mussels were collected by hand, by snorkeling (at most of the sites), wading in low water, or diving. Reconnaissance sampling (timed search) was used at all sites (Strayer *et al.*, 1997; Vaughn *et al.*, 1997). If *P. popeii* were present, quantitative methods (randomly placed 0.25 m^2 quadrats or area searches) were used for assessments of density. Collected live mussels and shells were counted and measured with calipers to the nearest mm, and then live mussels were carefully bedded back into the sediment from which they were taken. Shell condition of dead mussels was recorded for each shell. Species database with abundance and habitat data was submitted to TPWD Natural Diversity Database, making all data readily available for conservation, monitoring and decision making.

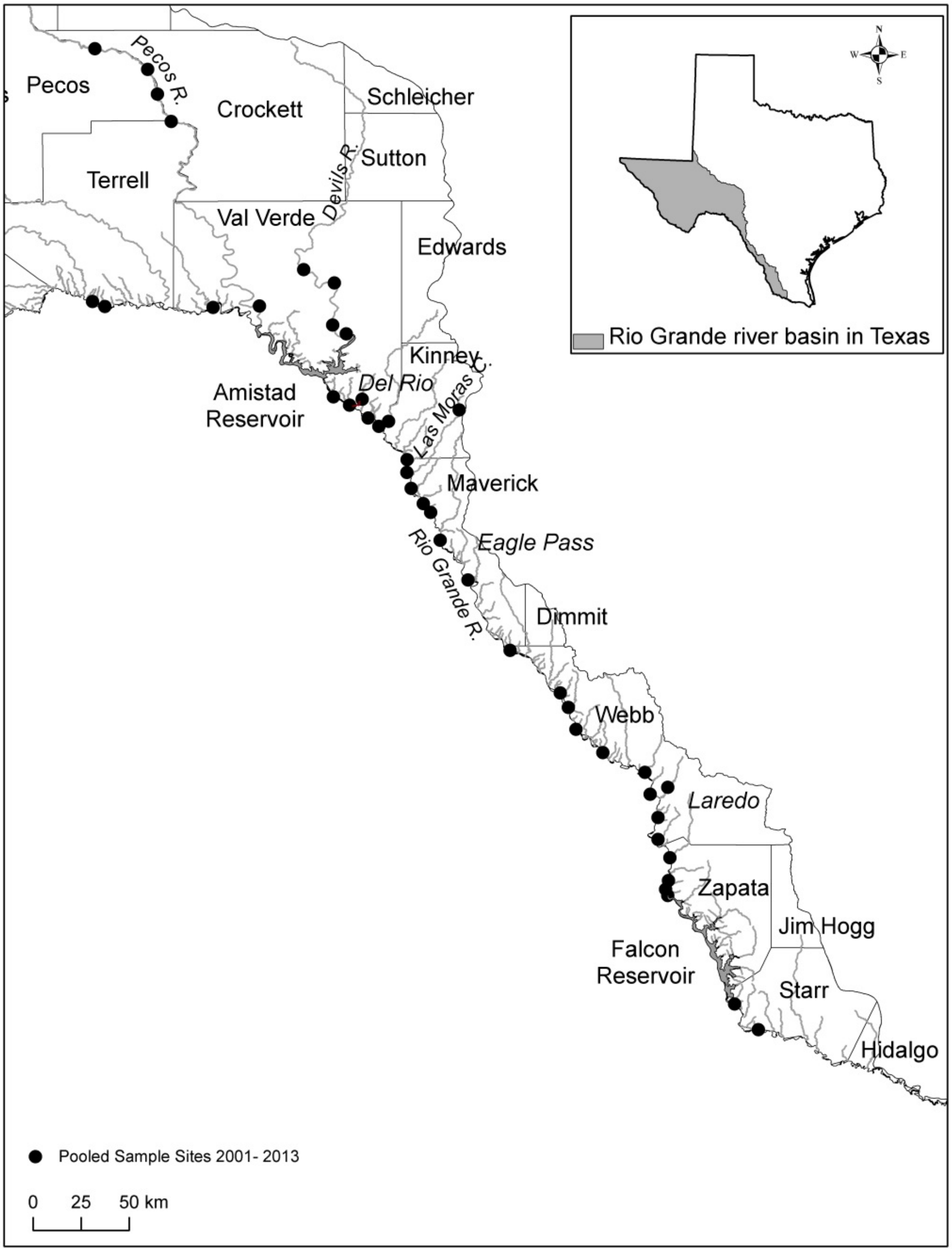

Figure 1. Map of the Rio Grande River basin in Texas with 42 pooled sampling sites surveyed during 2001 - 2014. Texas counties, major cities, reservoirs and rivers are indicated.

Photo 1. Airboat surveys of freshwater mussels in the Rio Grande River. Upper right: S. Barclay, L. Burlakova. Lower right: D. Barclay, S. Barclay, A. Karatayev, and T. Miller.

Photo 2. Texas hornshell survey in the Devils River, Texas, in April 2012. Upper picture, left to right: H. Nichols and T. Nobles (Texas State University, San Marcos), P. Douglas and K. Stubbs (Expedition Outfitters), T. Miller (Environmental Science Center, Laredo Community College), T. Vaughan (Texas International A&M University in Laredo), and L. Burlakova (photo: A. Karatayev). Lower: Texas hornshell found in the Devils River.

Photo 3. Pecos River habitats and shells found, February 2013.

Mark-recapture study

A mark-and-recapture-census was conducted at the La Bota Ranch site in Northern Laredo located ca. 1.4 river miles north of the "World Trade Bridge" in 2011-2014 using methods described by Lang (2001) and Villella *et al.* (2004) (Photo 4). The access to the site was gained from the US Border Patrol boat ramp with owner permission. Following recommendations by Villella *et al.* (2004), we sampled three consecutive days to estimate capture probabilities using closed population models. Each year in late February – early March (depending on water flow that had to be app. 20 ms^{-3} for successful mussel recovery) the same section of the mussel bed with the surface area of 516 m^{2} was surveyed. Sampling for mussels was performed by hand collection by snorkel (up to 2 m depth), and Scuba (up to 3 m only occasionally) applying tactile searches (probing fingers over and into sediment under rocks). All mussels present (new captures, and recaptures) were measured (shell length, width, height (± 0.1 mm)), and wet-weighed. First-time captured individuals were marked with unique numbers assigned by embedding oval (4 x 10 mm) Floy laminated flex tags in Super Glue Gel along the valve hinge posterior to the umbo. Specimens were identified using published taxonomic keys and descriptions (Howells *et al.*, 1996; Johnson, 1999).

A total of 1234 mussels were marked, including 296 in 2011, 413 in 2012, 264 in 2013, and 260 in 2014. We deposited voucher specimens into the Great Lakes Center Invertebrate Collection at Buffalo State College, Buffalo, NY. Each specimen was labeled with a unique number and cataloged in database with the following information: specimen number, name of person who collected and identified the specimen, date of collection, and detailed site information.

Photo 4. Texas hornshell population study at the mark-and-recapture site in La Bota, Laredo, Texas. The mark-recapture site (upper right): note the specific habitat (bedrock, boulders and ledges, red arrow) where the mussels were found. Participants: T. Miller and his students, T. Vaughan and his students, A. Karatayev and L. Burlakova. Lower right: tagged mussels found underneath one rock.

Growth rate

To estimate *P. popeii* growth rate we measured the annual increments for 240 mussels ranging from 32 to 79 mm for mussels found during mark-recapture. We also estimated growth increment for the first 3 years of life from growth rings for 27 mussel shells kept in our collection. Only mussels with the intact periostracum were used for these measurements.

Population dynamics

We used a Barker Robust Design mark-recapture model and a Huggins Closed Captures sample design (Cooch and White, 2014). This allowed us to estimate annual survival rates, annual downstream movement of mussels, as well as the population size in the sampled area for each of the 4 primary sampling occasions (2011-2014). Each primary sampling occasion consisted of 3 secondary sampling occasions on consecutive days, during which we assumed that the population did not change in size (i.e., no recruitment, mortality, or dispersal occurred).

Several key assumptions underlie this model design. First, we assume that the initial capture and tagging of an individual does not impact the probability of recapturing the same individual on a subsequent sampling occasion. To model this we set the initial encounter probability (p) and subsequent encounter probabilities for an individual (c) equal within a primary sampling occasion ($p_t = c_t$). Survival and dispersal probability are assumed to be the same for all animals in the population, regardless of availability for capture, with the exception of differences in survival with mussel length when marked. We also assume that individuals are equivalent across tagging cohorts. For further information on the assumptions of such models, see MARK book.

We constructed *a priori* a parallel set of 64 models under and fit them to our live and dead encounter data using program MARK. These reflected a range of factors affecting rates of survival, dispersal, and encounter probabilities (Table 2 in Results). We also tested for effects of individual size on survival and encounter probabilities, and the effects of handling on survival. In all models, we assumed that dead and live marked mussels had equal encounter probabilities - that is, the probability of an individual alive in a given year *t* being reported as dead between *t* and *t*+1 is equal to the mean encounter probability of mussels at year *t*+1.

The support for each model was determined using the Akaike Information Criterion (AIC), which ranks models by their fit to data, with an added penalty for models with a higher number of parameters (MARK book); models with AICc values which differed by < 2 were deemed equivalent. Models were ranked using AIC weights, which indicate the relative goodness of fit of each model in a nested group. Using robust design, we could not estimate the overdispersion parameter c_{hat}, and therefore we did not adjust AICc values. Finally, we obtained survival, dispersal, and encounter rates by averaging these estimates across all constructed models, weighted by the AIC weight of each model.

Model Parameter	**Description**
a', a"	**Transition rates to unobservable and observable states**; fixed at 0 and 1, respectively, for the analysis as we assume all marked individuals not washed downstream to be available for capture.
R', R"	**Sighting rates of live, marked individuals between years**; fixed at zero for the analysis.
S	**Annual per capita survival probability, with the following variations:** **S(.) / S(t)** - survival rates constant/different across years **S(.+L) / S(t+L)** - survival rates are constant/different across years, and are also affected by length of individual when marked **S(.+h)** - survival rates are constant across years, but differ in the first year following

Model Parameter	**Description**
	marking. This tests for a handling effect, as some individuals which survived year *t* were not recaptured in year *t* (and were encountered only in a subsequent year), whereas all individuals marked in year *t* were handled. **S(t+h)** - similar to above, except that survival rates of the marked population and newly marked individuals differ between themselves and between years. **S(.+h+L) / S(t+h+L)** - same as the previous two variations, except with length of individual when marked as an additional factor affecting survival
1-F	**The annual proportion of mussels in the sample area that are washed downstream**. Parameter variations include **F(.)** and **F(t)**, which assume that dispersal is constant and different among sampled years, respectively.
p	**Encounter probability of a marked mussel, with the following variations**: **p(.) / p(t)** - encounter rates constant/different across years **p(.+L) / p(t+L)** - encounter rates are constant/different across years, and are also affected by length of individual when marked
r	**Encounter rates of dead individuals between years**; the value of r between year *t* and *t*+1 was fixed to the mean encounter probability of marked individuals (p) in year *t*+1.

River discharge

To analyze discharge data (in cubic meters per second) for studied rivers we used the Rio Grande historical mean daily discharge data from International Boundary and Water Commission webpage (http://www.ibwc.state.gov/Water_Data/histflo1.htm). Rio Grande data used for the analysis were from site 08-4590.00 (Rio Grande at Laredo, TX and Nuevo Laredo, Tamaulipas) spanning from 1915 to 2014. Devils River data from were for site 08-4494.00 (Devils River at Pafford Crossing near Comstock, TX) (1960-2014). Historical data for the Black River were extracted from USGS gauge number 08405500 (Black River above Malaga, NM, http://waterdata.usgs.gov/nm/nwis/dv?cb_00060=on&format=rdb&site_no=08405500&referred_module=sw&period=&begin_date=1900-07-27&end_date=2014-07-27) (1947-2014). The Black River discharge data (in ft^3s^{-1}) was converted to m^3s^{-1} for comparison.

Population size calculations

Geographic coordinates for sampled site locations were pooled in Excel and mapped in ESRI ArcGIS 10.1. The 2010 stream segment data published by the Texas Commission on Environmental Quality was used for the analysis. These data represent classified and unclassified stream segments and reservoirs at a scale of 1:250:000. To calculate the distance for sampled areas, the stream segments were split at sampling starting and ending locations (e.g., El Indio Dam below Eagle Pass to Laredo). The distance in kilometers was then generated in ArcGIS for the split stream segments. The details of data calculation please find in Appendix 1.

Genetic analysis (D. Berg, and K. Inoue)

We collected 254 *P. popeii* from three rivers with snorkeling, SCUBA, or other tactile methods. 193 individuals were collected from eight locations in the Black River, NM; 58 individuals from five locations in the Rio Grande, TX; and three individuals from three locations in the Devils River, TX. Due to the small sample size from the Devils River, we pooled them into one "population" for analyses. We sampled non-destructively using tissue swabs of the foot and then returned mussels to the river bottom. Samples were preserved in 95% ethanol and stored at -20˚C. Total genomic DNA was extracted using ArchivePure DNA Cell/Tissue Kits (5 Prime, Gaithersburg, MD), diluted to 10 ng/µL, and used as a template in polymerase chain reactions (PCR) for mtDNA and microsatellite analyses.

Mitochondrial DNA sequencing

We used PRIMER3 (Untergasser *et al.* 2012) to design primers (forward: 5'-TGTGGGGTGAATCATTCCTT-3' and reverse: 5'-TAAACCTCAGGATGCCCAAA-3'), which amplified about 810 basepairs (bp) of the cytochrome oxidase I gene and part of the cytochrome oxidase II gene (hereafter, this region is abbreviated as COI). Conditions for PCR, sequencing, and post-sequencing analyses are described in Inoue *et al.* (2014b).

We estimated population genetic indices from mtDNA sequences using DNASP v5.10 (Librado and Rozas, 2009). We calculated number of haplotypes (H), mean number of basepair differences (K), and mean nucleotide diversity (π) over the pooled dataset and within each locality and river. We built a 95% confidence parsimony network with TCS v1.21 (Clement *et al.*, 2000). Multiple connections between haplotypes were simplified by assigning the shortest path from the most frequent haplotype (Fetzner and Crandall, 2003).

Microsatellite genotyping

We genotyped 20 tetra-nucleotide microsatellite loci (Inoue *et al.*, 2013). Forward primers for each PCR were labeled with a 5' fluorescent tag (6-FAM, NED, PET, or VIC) for visualization. We performed five sets of multiplex PCR (Plex1: Tetra17-19-41; Plex2: Tetra01-09-14-24-30-36; Plex3: Tetra02-03-22-23; Plex4: Tetra05-31-40; and Plex5: Tetra08-15-33-37) designed by multiplexmanager (Holleley and Geerts, 2009). Thermal cycling began with initial denaturing at 95˚C for 2 min; 35 cycles of 94˚C for 30 s, 60˚C for 1.5 min, and 72˚C for 1 min; and final extension at 72˚C for 30 min. We used previously published procedures for fragment analyses, allele scoring, and assignment of integer numbers to DNA fragment sizes (Inoue *et al.*, 2014b).

We tested for the presence of null alleles and large allele dropout using MICRO-CHECKER (van Oosterhout *et al.,* 2004) and checked for microsatellite loci under directional or balancing selection using lositan (Antao *et al.,* 2008). We conducted exact tests for pairwise linkage disequilibrium and deviation from Hardy-Weinberg expectation (HWE) using GENEPOP v4.0.10 (Rousset, 2008) for each locality. We estimated population genetic indices (mean number of alleles per locus, N_A; observed and expected heterozygosities, H_O and H_E; and number of private alleles, N_P) for each locality using GENALEX v6.3 (Peakall and Smouse, 2006). We used rarefaction to correct mean allelic richness (rarefied number of alleles per locus; A_R) to a standardized sample size of six individuals using FSTAT v2.9.3 (Goudet, 1995).

Population genetic structure

We used STRUCTURE v2.3.4 (Pritchard *et al.*, 2000) to evaluate population genetic structure without *a priori* delineation of populations. We used the admixture model with correlated allele frequencies to account for possible ancestral admixture, and used a burn-in period of 500,000 Markov chain Monte Carlo (MCMC) generations followed by 100,000 iterations for $k = 1$ through 10 with 10 replicates for each k. We determined the most likely number of distinct clusters by evaluating the logarithm of the probability of the data and estimating Δk using STRUCTURE HARVESTER (Earl and vonHoldt, 2012). We used CLUMPP (Jakobsson and Rosenberg, 2007) to average each individual's admixture proportions over the 10 replicates for the best k, and then produced graphical display results using DISTRUCT (Rosenberg, 2004). We employed GENALEX to estimate pairwise F_{ST} and D_{EST} among STRUCTURE-defined populations. For both indices, we tested for statistically significance differences from 0 using 9999 permutations. We used only the microsatellite dataset for these indices of genetic differentiation.

Results

Objective 1. Assess the current distribution of Popenaias popeii in Texas.

We found that *P. popeii* still exists in the Rio Grande and the Devils River. Live specimens and shells of this species were found at 26% and 43% of the 42 sites sampled, respectively (Figure 2). A total of 1,801 live *P. popeii* were recorded in our surveys. The species was most commonly found in crevices under large flat boulders of limestone conglomerates resting on bedrock, where small sediment deposits provide stable substrates for mussels in these flow refuges, with over 10 (and up to 40) individuals found under one rock.

In the Devils River, we found only 11 *P. popeii* during 12 years of our surveys within the 66 km stretch of the river above the confluence with Amistad reservoir. We estimated the current density of *P. popeii* in this waterbody to be 40.3 ± 19.8 mussels per river km, for a total population size of 2,660 ± 1,307 mussels (Table 1).

The density of *P. popeii* in the 190 km of the Rio Grande between Eagle Pass and Laredo was the highest in the whole range (1,514 ± 516 per river km, Table 1), and the estimated population size on this stretch of the river was 287,660 ± 98,040.

Between the Big Bend and Del Rio (177 km, excluding the Amistad Reservoir, in which this species has not been found), we estimated a density of 40 mussels per river km (the same as that observed in the Devils River), for a total of 7,133 ± 3,505 *P. popeii* in this river stretch.

Only old dead shells were found at seven sites below the Laredo Sewage Plant waste water discharge to the mouth of the river, although *P. popeii* were very abundant just 10 m upstream of the discharge site (at the mouth of Zapata Creek, Las Palmas Park). Thus, overall, the estimated current population size of *P. popeii* in the Rio Grande is approximately 294,793 ± 98,103 mussels (Table 1).

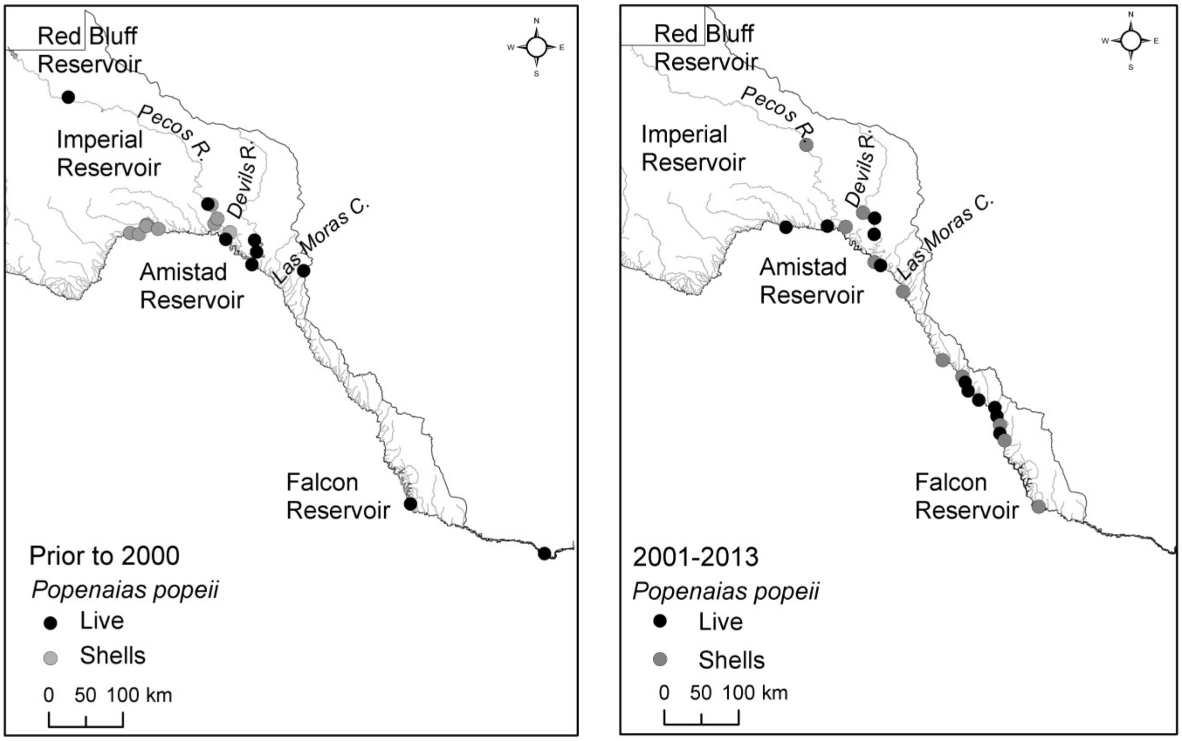

Figure 2. Map of the Rio Grande River basin in Texas with sites where live and/or dead shells of *Popenaias popeii* were found prior to 2000 (based on data from: Singley, 1893; Cockerell, 1902; Strecker, 1931; Taylor, 1967; Metcalf, 1974, 1982; Murray, 1975; Metcalf and Stern, 1976; Neck, 1984; Neck and Metcalf, 1988; Howells, 1994, 2001; Howells *et al.*, 1996, 1997; Johnson, 1999;) and from 2001 to 2013 (authors data).

No live *P. popeii* were found in the Pecos River during our study, although long dead shells were extremely abundant in the lower reaches of the river, where live mussels were reported by Metcalf (1982) prior to the area being flooded by the Amistad Reservoir. In addition, one fragment of a *P. popeii* valve was found in 2011 at one of the four surveyed sites on the Pecos River in Pecos County (near Iraan).

Our study also didn't reveal any live mussels or even dead shells of *P. popeii* in the Las Moras Creek.

Table 1. Current and historical ranges and population size of *Popenaias popeii* in the Rio Grande drainage of Texas.

Waterbody	Current range, km (% from historical)	Historic range, km	Current density, mussels per river km	Historic density, mussels per river km	Current population, mussels (% from historical)	Historic population, mussels
Rio Grande:						
Between the Big Bend and Eagle Pass (excluding Amistad Reservoir)	177	n. c.	40.3 ± 19.8	n. c.	7,133 ± 3,505	n. c.
Between Eagle Pass and Laredo	190	n. c.	1,514 ± 516	n. c.	287,660 ± 98,040	n. c.
The whole Rio Grande (from the San Francisco Creek to Brownsville)	367 (37%)	1000	n. c.	1,514 ± 516	294,793 ± 98,103 (20%)	1,514,000 ± 516,000
Devils River	66 (69%)	96	40.3 ± 19.8	40.3 ± 19.8	2,660 ± 1,307(69%)	3,869 ± 1,901
Pecos River	0 (0%)	679	0	777 ± 516*	0 (0%)	527,583 ± 350,622
Las Moras Creek	0 (0%)	60	0	777 ± 516*	0 (0%)	46,620 ± 30,960
Total	433 (24%)	1835	n. c.	n. c.	297,453 ± 98,111(14%)	2,092,072 ± 624,623

*Density calculated as an average between high density in the Rio Grande and low density in the Devils River

Objective 2. Evaluate long-term changes in distribution range

Historically, *P. popeii* in Texas was reported from the Rio Grande and its tributaries, including the Pecos and Devils rivers and Las Moras Creek (reviewed in Karatayev *et al.*, 2012, Attachment 1). Although two dead *P. popeii* shells were reported from the South Concho and Llano rivers outside the Rio Grande drainage (Strenth *et al.*, 2004), there is no evidence that these records represent extant populations of *P. popeii.*

In the Rio Grande, based on historical records (Howells, 1994; Howells *et al.*, 1996, 1997; Metcalf, 1982) and our shell findings, *P. popeii* in the past occurred throughout the 1,000 km stretch of the river from the mouth of San Francisco Creek in the Big Bend reach (Brewster County) to Brownsville, near the Gulf of Mexico (Figure 1). According to Metcalf and Stern (1976), no living or fossilized unionids were ever reported in the Rio Grande above the mouth of the Rio Conchos. Currently *P. popeii* persists in only two fragments of the Rio Grande: between Big Bend National Park and Del Rio (excluding the Amistad Reservoir), and between Eagle Pass and Laredo - which constitutes only 37% of the species' historical range in the river. Due to the relative similarity of environmental conditions and historical records in the Rio Grande (from the mouth of San Francisco Creek in the Big Bend to the river mouth), we suggest that prior densities of *P. popeii* in the river were similar to those presently found between Laredo and

Eagle Pass. By this approximation, the historical population size of *P. popeii* in the Rio Grande was about 1,514,000 mussels, which indicates that the current abundance of this species in the river is only at 20% of its historical levels.

In the Devils River, *P. popeii* were historically found only in the lower reaches (in Val Verde County, Singley, 1893; Neck, 1984), which are currently flooded by the Amistad Reservoir (Figure 2). As the remaining length of the river is still in pristine conditions, we assume that the historical *P. popeii* density throughout the river was similar to that observed in the present study (40.3 mussels per river km). This is likely an underestimation as in the past mussels were more abundant near the confluence with the Rio Grande. Although the lower reaches of the river are now flooded by the reservoir and no longer support this lotic species, we estimated the historical range of *P. popeii* as the distance between our most upstream record of the species and the mouth of the river (Figure 2, Table 1). According to these conservative assumptions, the Devils River historically supported a total of 3,869 *P. popeii* (Table 1).

In the Pecos River, *P. popeii* was reported by Metcalf (1982) from the lower reaches later flooded by the Amistad Reservoir. In addition, in the past, mussels were recorded in this river in New Mexico (Cockerell, 1902; Metcalf, 1982; Lang, 2001), and are still quite abundant in one of its tributaries (the Black River, Lang, 2001, 2010; Inoue *et al.*, 2014). Therefore, we suggest that the entire span of the Pecos River in Texas was previously populated by *P. popeii*, and its historical density could be an average of the species' current, low density in the Devils River and its high density in the Rio Grande between Laredo and Eagle Pass. According to this assumption, the total number of *P. popeii* that the Pecos River in Texas supported historically was 527,583 (Table 1). This figure is likely an underestimation since the current total population of *P. popeii* in a 14-km stretch of the Black River is 48,006 mussels (Inoue *et al.*, 2014a).

In the Las Moras Creek, Kinney County, *P. popeii* were first recorded in 1892 (Taylor, 1967), and according to Cockerell (1902), at the end of 18th century mussels were abundant in this creek near Fort Clark. However, extensive, repeated surveys along 48 km of the Las Moras Creek in 1971, 1973, and 1975 found no living *P. popeii* (Murray, 1975). Our study also did not reveal any live mussels or even dead shells of *P. popeii* in the Las Moras Creek. To reconstruct the former density of *P. popeii* in the Las Moras Creek, we used the average value between the current densities in the Devils River and the densities in the Rio Grande between Laredo and Eagle Pass. Based on this assumption, the estimated historical abundance of *P. popeii* in the Las Moras Creek was ~46,620 mussels (Table 1). This creek is similar to the Black River where *P. popeii* densities were estimated as 3,429 molluscs per river km (total 48,006 molluscs per 14 km, Inoue *et al*., 2014a), and thus our estimation of population size of the 60-km Las Moras Creek is likely very conservative. In total, 76% of *P. popeii* habitat was lost during the last century, and only 14% of the former population remains in the Rio Grande drainage in Texas (Table 1).

Objective 3. Locate and describe existing populations.

Mark-recapture study at La Bota site, Laredo, Texas

All models which received any AIC weight assumed differences in survival, dispersion, and encounter rates between different time periods (Table 2); consequently, derived estimates of population size also differed greatly (Table 3, Figure 3). Models which further assumed survival and encounter rates to decrease and increase with mussel length, respectively, also received 80% of support among the data (based on AIC weights; Figure 4). Models assuming an effect of handling on survival attained 20% of support among the data; model averaged estimates show survival rates for newly marked individuals to be 1-2% lower than those of previously marked individuals, although these differences were not significant. However, it should be noted that this is only an indirect test for handling effect. Nevertheless, our best, significantly different model did not incorporate a handling effect on survival (Table 2).

Table 2. Results for the five most-parsimonious models for the Barker Robust Design mark-recapture model and Huggins Closed Captures sample design for *P. popeii* La Bota population in 2011-2014.

Model	Delta AICc	AICc Weights	Number of Parameters	Model Deviance
S(t+L) F(t) p(t+L)	0	0.604	20	6308
S(t+h+L) F(t) p(t+L)	2.25	0.196	22	6306
S(t) F(t) p(t+L)	3.40	0.110	19	6313
S(t+h) F(t) p(t+L)	5.79	0.033	21	6311
S(t+L) F(t) p(t)	7.46	0.015	19	6317

Table 3. Model averaged parameter estimates (encounter rates condensed to an average across the 12 sampling occasions).

Parameter	Estimate	SE	LCI	UCI
Survival 2011-12	0.87	0.05	0.72	0.95
Survival 2012-13	0.93	0.03	0.83	0.97
Survival 2013-14	0.97	0.02	0.92	0.99
Dispersal 2011-12	0.16	0.90	0.45	0.04
Dispersal 2012-13	0.44	0.94	0.56	0.33
Dispersal 2013-14	0.51	0.94	0.63	0.39
Abundance 2011	986	145	702	1269
Abundance 2012	1463	134	1201	1725
Abundance 2013	1178	122	940	1416
Abundance 2014	884	82	723	1046
Mean encounter and dead recovery rate, 2011-14	0.13	0.02	0.10	0.17

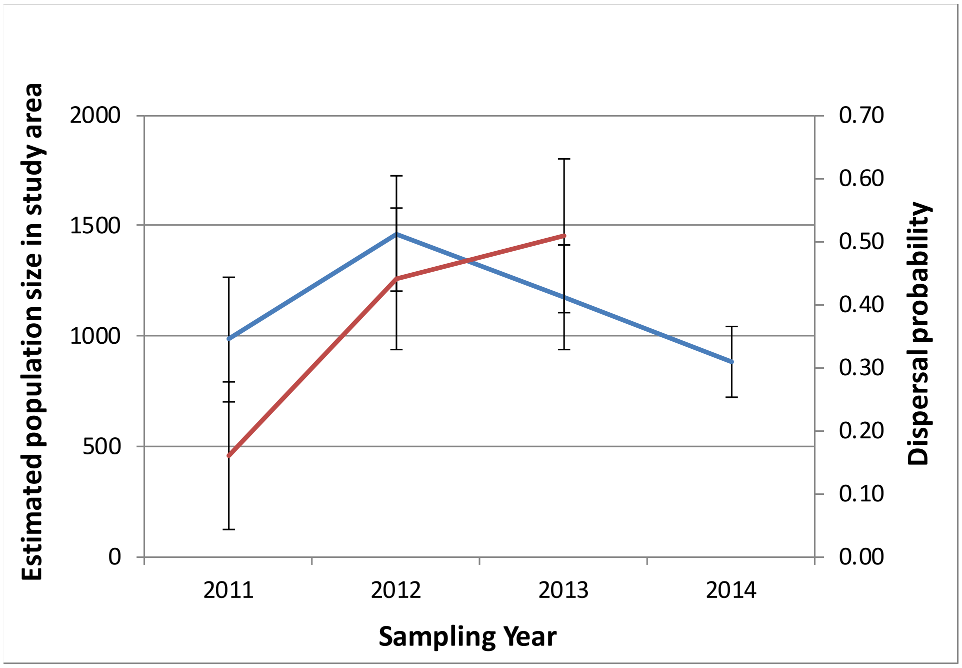

Figure 3. *Popenaias popeii* population size (blue line, left vertical axis) and dispersal rate (red line, right axis) at La Bota mark-recapture site in 2011-2014. Mean ± 95% confidence interval.

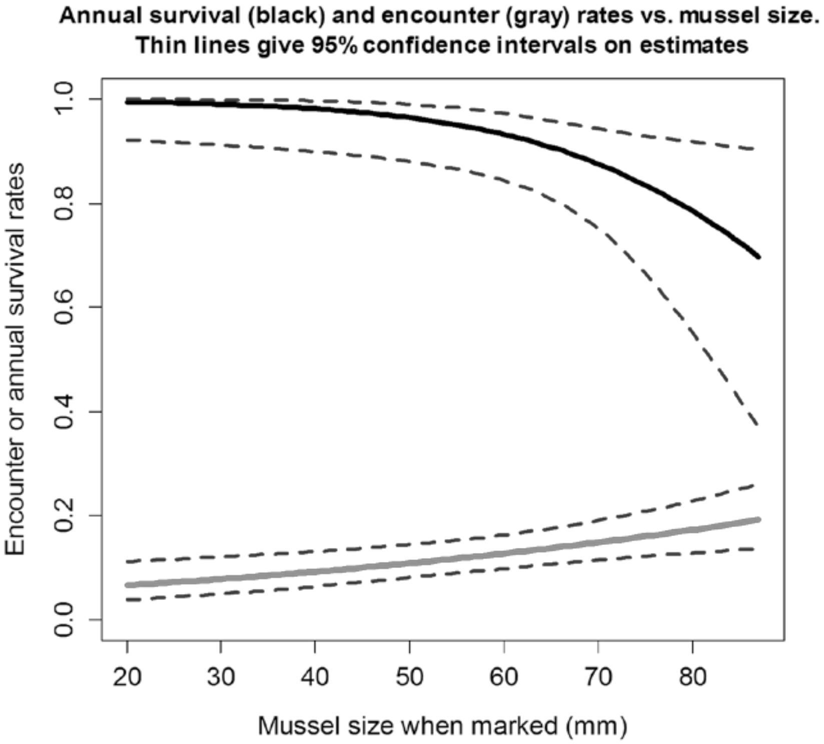

Figure 4. Annual survival (black line) and encounter rates (grey line) of different sizes of *P. popeii* at La Bota mark-recapture site (Laredo, Texas).

Evaluation of growth and survivorship

The average length of live mussels collected at La Bota mark-recapture site (62.06±0.27 mm, range 20.8 - 87.0 mm) was not different (P = 0.23, two-sided t-test) from 10 other sites studied on the Rio Grande (average 61.14 ± 0.75 mm, range 26-92 mm), but was significantly smaller ($P<<0.001$, two-sided t-test) than the lengths of dead mussels (average 67.11±1.01 mm, range 38.0 - 87.0 mm) (Figure 5). The annual growth rate has been decreasing from about 20 mm during the first year to less than 1 mm per year in the largest mussels (Figure 6).

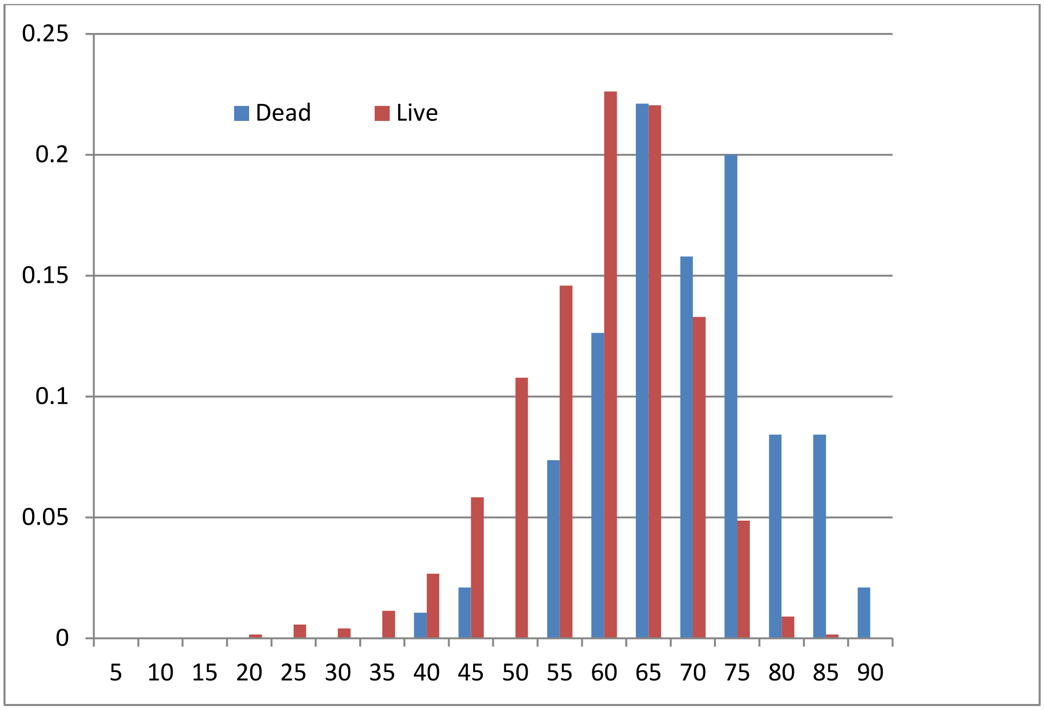

Figure 5. Length-frequency distribution of live and dead *P. popeii* in the La Bota site of the Rio Grande. Live mussels average length 62.02±0.27 mm (n=1234). Dead mussels average length 67.11±1.01 mm (n=95). Axes: vertical – frequency, horizontal – mussel length.

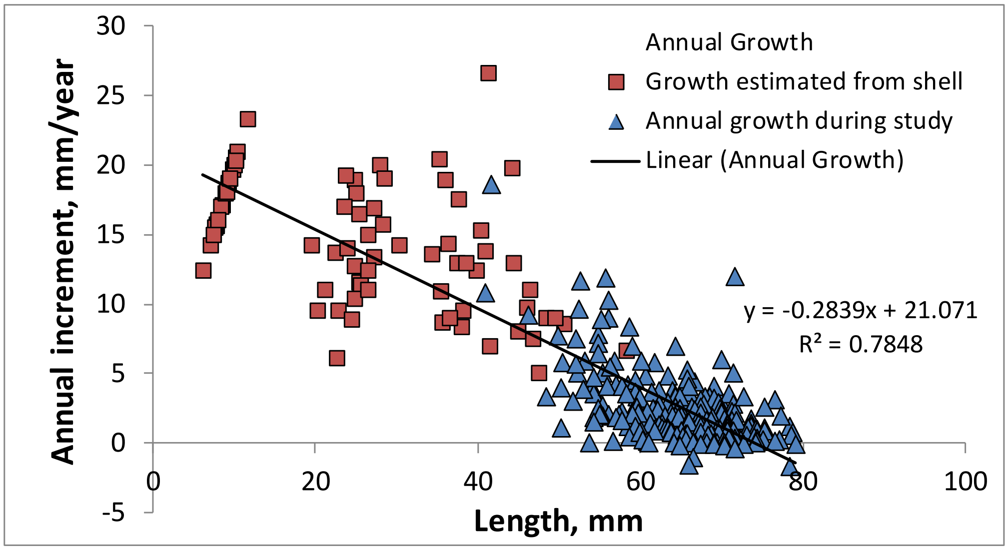

Figure 6. Annual length increments plotted versus different shell length of live *P. popeii* collected in the Rio Grande in 2011-2014. Linear regression and equation is given. Vertical axis represents growth increments (mm), horizontal - mussel length (mm). Growth rate was estimated by measuring live mussels annually at mark-recapture site (blue triangles) and by measuring rings on the shells of dead mussels (red squares).

Based on the growth increments we calculated that the maximum longevity of mussels larger than 85 mm in the Rio Grande may be up to 14 years (Figure 7), however over 80% of mussels died smaller than 75 mm at the age less than 8 years.

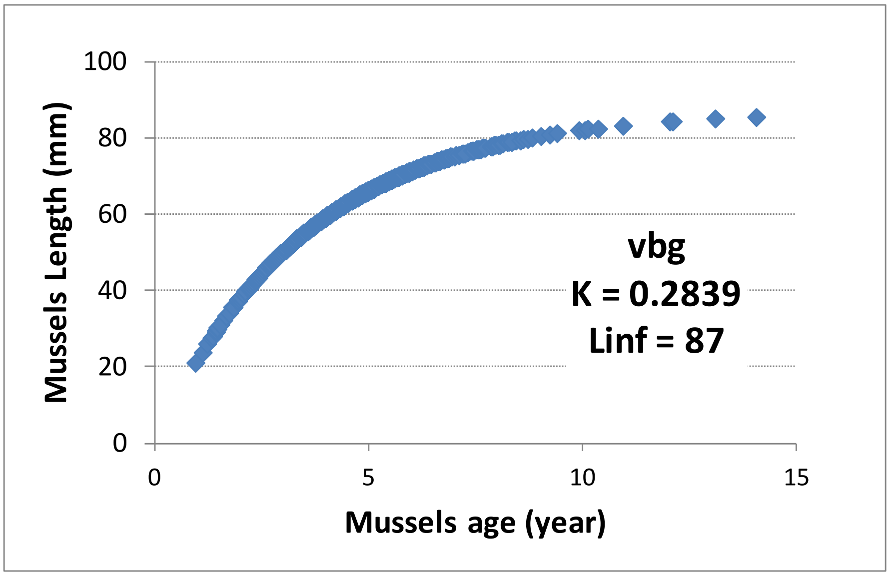

Figure 7. Age-length relationship for *P. popeii* calculated from length increments and by counting external annual rings on the shell. Vertical axis – mussels' length (mm), horizontal – mussels age (year).

Preliminary analysis of *P. popeii* individual growth rate using our measurements of recaptured mussels has shown that the growth is age-dependent, and the increment of shell length (the largest dimension of shell) per day depends on mussel's initial length ($y = 0.0339 - 0.0005x$; $R = -0.6827$, $P < 0.0001$, linear regression, where y = length increment rate (mm/day), and x – shell length, mm) (Table 4, Picture 8). 40-50 mm mussels grew in average 5 mm a year, and up to 15 mm in 2 years (Table 4). The length increment declined with age: it was ~3 mm a year in 50-60 mm mussels, 1.5 mm/year in 60-70 mm, and only 0.6 mm/year in the largest age class (70-80 mm) (Table 4).

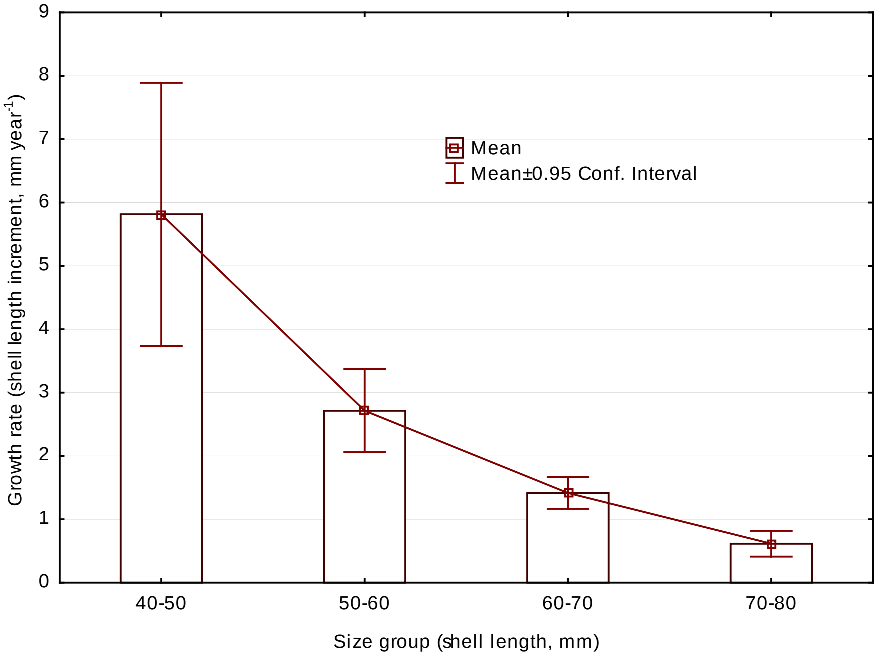

Figure 8. Growth rate of *P. popeii* (average shell length increments of individual mussels per year, mm year^{-1}) depending on the initial size of the shell.

Table 4. Growth rate of *Popenaias popeii* in the Rio Grande River at La Bota mark-recapture site, Laredo, Texas, 2011-2013. Growth duration (days), initial shell length (mm), mean of individual length increments per time period (mm), relative growth (length increment divided by the initial length), and growth rate (length increment divided by the number of days, mm day^{-1}) are given. Mean + standard deviations, sample size (in parentheses) and minimal and maximal values are given for each parameter.

Size group	40-50 mm		50-60 mm		60-70 mm		70-80 mm	
Growth duration, days	348	723	365	724	367	724	355	724
Initial length, mm	48.15+1.02 (6) 46.7-49.5	45.63+4.05 (3) 41.5-49.6	55.57+2.77 (26) 50.1-59.6	55.01+2.64 (8) 51.5-58.2	65.35+2.82 (62) 60.2-69.9	63.71+2.91 (18) 60.2-69.3	72.93+2.56 (27) 70.1-79.1	72.58+1.86 (4) 70.2-74.1
Length increment, mm	4.57+2.26 (6) 1.1-7.5	14.68+4.24 (3) 10.1-18.5	2.81+1.9 (26) 0.1-9	4.72+3.77 (8) 1-12.9	1.47+1.25 (62) -1-7	2.7+1.94 (18) 0-8.9	0.61+0.53 (27) -0.4-1.6	0.92+1.03 (4) 0-2.4
Relative length increment	0.10+0.05 (6) 0.02-0.16	0.33+0.12 (3) 0.2-0.45	0.05+0.04 (26) 0-0.18	0.09+0.07 (8) 0.02-0.25	0.02+0.02 (62) -0.01-0.12	0.04+0.03 (18) 0-0.14	0.01+0.01 (27) -0.01-0.02	0.01+0.01 (4) 0-0.03
Growth rate, mm/day	0.0137+0.0075 (6) 0.003-0.023	0.0203+0.0059 (3) 0.014-0.026	0.0077+0.0052 (26) 0-0.022	0.0065+0.0052 (8) 0.001-0.018	0.0039+0.0032 (62) -0.003-0.017	0.0037+0.0027 (18) 0-0.012	0.0017+0.0016 (27) -0.001-0.005	0.0013+0.0014 (4) 0-0.003

Discharge rates

Long-term discharge rates in Rio Grande at Laredo averaged from daily measurements from May 1900 to December 2011 was 111.8±215.7 m^3 s^{-1} (mean ± standard deviation, n = 36,923), with median of 66 and range from 0 to 16,300 m^3 s^{-1} (International Boundary Water Commission stream gauge data). In June and July of 1953 the Rio Grande in Laredo was dry (water discharge was from 0 to 0.77 m^3s^{-1}, International Boundary Water Commission stream gauge data). Of these 48 days in June – July of 1953, zero flow rate was recorded for 40 days. In contrast, water discharge in the other two rivers and streams that support *P. popeii* populations has never been reduced to zero in the last 50 years.

The mean discharge rates in the Rio Grande significantly decreased after 1969 when the Amistad dam was built ($P << 0.001$, two-sided t-test) from 130.7±265.0 m^3 s^{-1} (median 73.1, range 0 – 16300) to 86.6±115.9 m^3 s^{-1} (median 54.1, range 6.99 – 3,260).

Mean discharge rate in the Devils River (data from January 1960 to December 2011) was 10.2±51.2 m^3 s^{-1} (n = 18,993, median of 6.8 and range from 1.5 to 3,478 m^3 s^{-1} (International Boundary Water Commission stream gauge data). Much smaller mean discharge rate was recorded in the Black River (data from January 1947 and July 2014): 0.34±2.7 m^3 s^{-1} (n = 24,680, median of 0.22 and range from 0.0003 (which was recorded only once on 30 September 1998) to 339.8 m^3 s^{-1} (USGS stream gauge data).

Objective 4. Determine species' habitat requirements.

Species' habitat requirements were determined during surveys when we studied densities of *P. popeii* in different habitats. We found that the suitable habitat for *P. popeii* in the Rio Grande is similar to the preferred habitat for this species in the Black River: low-flow refuges where Texas hornshell remain secure during large volume spates characterized by aggregations of mussels under large boulders of limestone conglomerates, where clay seams provide stable substrata for mussels in low-velocity microhabitats (Lang, 2010). This habitat is different from the soft substrate type preferred by other species commonly found in the Rio Grande such as *C. tampicoensis, T. cognata, M. nervosa,* and *Q. apiculata* (Karatayev *et al*., 2012). Identification of preferred habitat aided in locating mussel beds during our surveys, making possible density estimations and the assessment of current population size in the basin.

Juvenile *P. popeii* were found during in our surveys and mark-recapture study both in the same habitats as adults (see above) and also in nearshore shallow zones in small-grained substrates like sands and silty sands.

P. popeii is a lotic species as it is not known from impoundments (Metcalf, 1982, Neck and Metcalf, 1988, Howells *et al.*, 1996). Burlakova et al. (2011b) found that all common Texas species were able to inhabit both lotic and lentic waters, and most of them were up to six fold more abundant in reservoirs than in rivers and streams. In contrast, none of the very rare species, including *P. popeii,* were found in standing waters. Therefore, the ongoing replacement of lotic waterbodies with lentic ones largely favors very common and common species, and dramatically reduces habitat for endemic unionids (Burlakova *et al.*, 2011b). The presence of low-head dams appears to limit its habitat and distribution in the Black River, possibly by restricting movement of host fish or smothering of habitat by excessive silt deposition (Lang, 2001). These low-head dams apparently preclude opportunities for recolonization by *P. popeii* in upstream riverine reaches of the Black River, while the downstream recolonization is potentially limited by altered physicochemical (salinity gradient) and hydrologic regimes (Lang, 2001). Some of the living species may now be extinct in the Pecos River system because of impoundment of its lowermost part in Amistad Reservoir (Metcalf and Stern, 1976). Creation of Falcon Reservoir most likely decimated the lotic habitat of the bivalves in the lower Rio Grande (Neck and Metcalf, 1988). In southeastern New Mexico, the construction of impoundments (Lake MacMillan, Brantley and Avalon reservoirs) was one of the many factors responsible for extirpation of *P. popeii* from the Pecos River mainstream (Taylor, 1967). The construction of reservoirs also facilitated the introduction and range expansion of common species (*Q. apiculata, P. purpuratus,* and *P. grandis*) nonnative to the Rio Grande river drainage (Metcalf and Smart, 1972; Metcalf, 1982; Johnson, 1999). Any future projects to construct a new dam, or to modify existing low-head dams and associated water diversion structures, both on the Black River or in the Rio Grande River in Laredo could potentially impact *P. popeii*.

Water quality is an important component of *P. popeii* habitat and changes in water quality may have contributed to population loss throughout the historic range. The species is sensitive to elevated salinities, los dissolved oxygen, pollution (reviewed in Carman, 2007). Please find more information on threats in the sections below.

Freshwater mussels require perennially wetted habitats; emersion (stranding) causes dehydration and death, although length of time to mortality varies by species (reviewed in Carman, 2007). Drought has been shown to cause extirpation of several species, both by emersion and loss of fish hosts (Metcalf, 1982).

Fish hosts

Popenaias popeii typically brood mature, infective larvae in Black River (New Mexico) from May through June (Smith *et al.*, 2003). On May 23, June 19 and July 26, 2012, our collaborator Tom Miller and his students sampled fishes in La Bota site using seines and nets.

The most abundant species of fish collected were shiners, including blacktail and red shiners, inland silverside, and minnow (Table 5).

Although laboratory studies described *P. popeii* as a host generalist (i.e., 31 fish species tested representing 11 families, 24 physiological host species identified; Lang, 2001; 2004), Levine et al. (2012) found that in the wild *P. popeii* uses a much smaller subset of all potential hosts that occur in the Black River. Only half of the 20 fish species observed in the river were infested with glochidia (*Cyprinella lutrensis, Pimephales promelas, Carpiodes carpio, Cycleptus elongates, Moxostoma congestum, Ictalurus lupus, Gambusia affinis, Lepomis macrochirus, Lepomis megalotis,* and *Micropterus punctulatus*). Of all naturally infested fishes *Carpiodes carpio, M. congestum,* and *C. lutrensis* represented 80% of all individual fishes infected and carried over 99% of glochidia: 84% of all glochidia recorded were attached to *C. carpio*, 12.9% to *M. congestum* and 2.5% to *C. lutrensis* (Levine *et al.*, 2012). Small-bodied red shiners, *C.*

lutrensis, constituted half of all fishes in this survey with cysts, while large-bodied catostomids (river carpsucker, *Carpiodes carpio*, and gray redhorse, *Moxostoma congestum*) and long-ear sunfish, *Lepomis megalotis*, each accounted for 10 percent of total observed infestations. Of large-bodied fishes, *Carpiodes carpio* and *C. elongatus* exhibited the highest prevalence of infestation (60%). Although *Moxostoma congestum* was infested at lower prevalence (10%), it represented the most frequently captured large-bodied fish (292 captures). *C. lutrensis*, small-bodied fish, was the only species infested consistently and exhibited the highest prevalence (30%) (Levine *et al.*, 2012).

Table 5. Fish collected during seining events in summer 2012 at La Bota site, Laredo.

Fish species	Common name	Individuals collected	Fish host (Levine *et al.*, 2012)*	
			Lab	Field
Cyprinella sp.	shiner	125		
Menidia beryllina	inland silverside	104	U	
Cyprinella venusta	blacktail shiner	79		
Pimephales vigilax	bullhead minnow	77		
Cyprinella lutrensis	red shiner	68	Y	Y
Poecilia Formosa	Amazon molly	53		
Dorosoma petenense	threadfin shad	43		
Dorosoma cepedianum	American gizzard shad	35		U
Oreochromis aureus	blue tilapia**	17		
Hypostomus plecostomus	suckermouth catfish**	11		
Lepomis macrochirus	bluegill	7	Y	Y
Lepomis megalotis	longear sunfish	5	Y	Y
Ictalurus punctatus	channel catfish**	3	Y	U
Cichlasoma cyanoguttatum	Rio Grande cichlid	1		
Moxostoma congestum	gray redhorse	1	Y	Y
Total		**629**		

*Y = yes, produced transformed juveniles, N = no juveniles produced, U = uncertain, glochidia encysted at the time of host death (laboratory) or cysts unclear (field).
** exotic

Only 4 fish species of all collected in the Rio Grande in summer 2012 were found suitable field hosts in the Black River (Levine *et al.*, 2012, Table 5). Of these, only red shiners were abundant (Table 5). None of the fish collected in the La Bota area had been found infested with glochidia in the field. The lack of the glochidia may also indicate that either mussels in the Rio Grande reproduced in different season, or that ecological fish hosts are present in very low densities.

Genetic analysis (D. Berg, and K. Inoue).

We obtained 246 COI sequences and recovered 34 haplotypes (Table 6). The highest diversity was observed from Rio Grande sites, while no nucleotide diversity in COI sequences was found in the Black and Devils rivers, where only single haplotypes were recovered in all sites of each river (Table 6 and Figure 9). The Black River haplotype was also the most common haplotype in the Rio Grande. The haplotype unique to the Devils River differed from the Black River haplotype by a single base pair.

Table 6. Descriptive statistics for COI sequences and 18 microsatellite loci for *Popenaias popeii* populations in the Black River, NM; and the Devils River and Rio Grande, TX.

			COI			Microsatellites				
River	Site ID	N	H	K	π	N_A	A_R	N_P	H_O	H_E
Black	CC	3	1	0	0	2.0	--	0	0.380	0.314
	Fall	24	1	0	0	3.3	2.8	0	0.469	0.483
	Magby	19	1	0	0	3.6	2.9	1	0.469	0.477
	DV	55	1	0	0	4.5	3.1	2	0.503	0.507
	RF5	20	1	0	0	3.9	3.1	0	0.527	0.518
	RF1	41	1	0	0	4.6	3.1	4	0.507	0.532
	BS	10	1	0	0	3.4	3.0	0	0.450	0.480
	RF3	21	1	0	0	3.9	3.1	0	0.513	0.518
	All Black R.	193	1	0	0	6.0	3.0	8	0.495	0.528
Devils	DR	3	1	0	0	3.4	--	2	0.556	0.608
Rio Grande	RG1	29	21	3.56	0.0050	14.2	7.7	40	0.813	0.881
	RG2	5	4	2.00	0.0028	6.2	--	5	0.800	0.793
	RG3	2	2	5.00	0.0070	3.4	--	0	0.800	0.693
	RG4	16	13	3.39	0.0047	11.4	7.6	11	0.805	0.872
	RG5	6	5	4.60	0.0064	6.7	6.7	3	0.731	0.798
	All RG	58	33	3.49	0.0049	16.7	7.3	167	0.806	0.902
Global		254	34	1.41	0.0020	17.3	4.5	68	0.566	0.694

A_R, rarefied allelic richness; COI, cytochrome oxidase subunit I; H, number of haplotypes; H_E, mean expected heterozygosity; H_O, mean observed heterozygosity; K, mean number of base pair differences between all pairs of individuals; n, number of mussels sampled; N_A, mean number of observed alleles; N_P, number of private alleles; π, nucleotide diversity

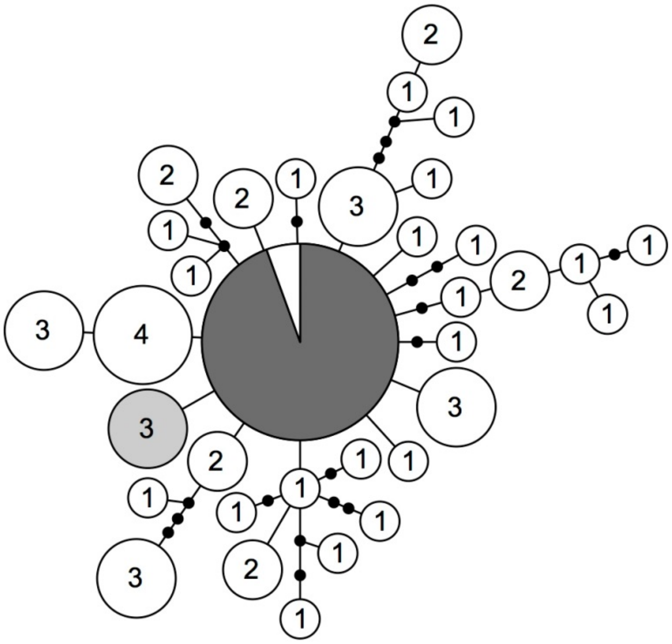

Figure 9. Parsimony network of COI sequences for *Popenaias popeii*. Each circle represents a unique haplotype; lines between haplotypes represent one base pair; black dots are inferred missing haplotypes. Haplotype frequency is relative to the size and number in the circle. The most common haplotype is shared between individuals from the Black River (n = 185) and from the Rio Grande (n = 10). Colors of circles represent localities (Black River = dark gray; Devils River = light gray; Rio Grande = white).

Two microsatellites (Tetra41 and Tetra37) were excluded from analyses because Tetra41 amplified multiple fragments and we were unable to score alleles, and Tetra37 showed signs of positive selection. Over 18 loci, we found some evidence of null alleles (4.8% of all locality-by-locus pairs), no evidence of linkage disequilibrium, and some deviation from HWE (2% of all locality-by-locus pairs) after sequential Bonferroni correction. However, neither the null alleles nor the deviations from HWE showed any pattern across localities or loci, and thus, we included these loci in all subsequent analyses.

The number of alleles per locus ranged from five to 51 (for a total of 349 different alleles over 18 loci). The mean allelic richness across the 18 loci ranged from 2.8 at one Black River site to 7.7 alleles at a Rio Grande site (Table 6). The mean observed heterozygosity ranged from 0.380 at a Black River site to 0.813 at a Rio Grande site, and the mean expected heterozygosity ranged from 0.314 at a Black River site to 0.902 at a Rio Grande site. Overall, the lowest genetic diversity was found in the Black River sites.

Popenaias popeii showed evidence of significant range-wide population genetic structure. The STRUCTURE analysis indicated explicit boundaries between the Black River (hereafter BR) and Devils River/Rio Grande (hereafter RG) at $k = 2$, and further splits of the BR sites into two clusters at $k = 3$ (Figure 10). The BR sites were split between three upstream sites (hereafter BR-u) and five downstream sites (hereafter BR-d) at $k = 3$, where the BR-d had admixture of BR-u genotypes (Figure 10). We found no evidence of admixture between BR and RG populations when $k = 2$ or 3. The simulation for $k = 4$ did not show further split of BR-u, BR-d, or RG (Figure 8). We observed the mode of Δk at $k = 2$ ($\Delta k = 1305.5$, mean Ln P(D) = -12576.0), but Δk was still high at $k = 3$ ($\Delta k = 63.4$, Ln P(D) = -12473.7), suggesting further improvement in the fit of the model at $k = 3$. We considered $k = 3$ to be the most biologically relevant clustering for *P. popeii*.

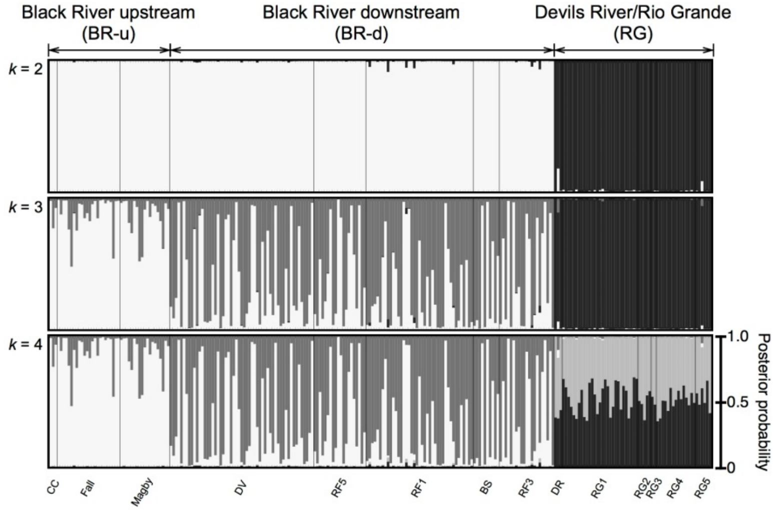

Figure 10. Bar plots obtained from STRUCTURE, assigning individuals into *k* = 2 through 4 clusters. In *k* = 2, clusters consisted of Black River (light gray) and Rio Grande (black) individuals. In *k* = 3, an additional division was observed in the Black River populations (light gray, upstream sites on the Black River; medium gray, downstream sites on the Black River). No further division was shown in either the Black River or Rio Grande populations in *k* = 4. Bottom labels are *a priori* population assignments.

All pairwise comparisons of F_{ST} and D_{EST} were significant. Pairwise F_{ST} values between populations ranged from 0.033 (between BR-u and BR-d) to 0.255 (between BR-d and RG). Similar genetic differentiation was observed for D_{EST} values, ranging from 0.015 (between BR-u and BR-d) to 0.751 (between BR-u and RG).

Conclusions

This study revealed highly structured *P. popeii* populations at both a regional scale among the Rio Grande drainages, and also a more local scale in the Black River. The finer spatial-scale genetic structure within segments of the Black River, might be driven by anthropogenic factors such as a low-water culvert crossing (built in 1932-1936) which can prevent dispersal of fishes due to increased water velocity (Warren and Pardew, 1998). Further studies of host fish movements may help elucidate the means by which fine-scale population structure is maintained in populations of *P. popeii* from the Black River.

Discussion

Since the first description of *Popenaias popeii* by Lea (1857), this species was always considered rare, declining or even endangered (Singley, 1893; Strecker, 1931; Neck, 1984; Stansbery, 1971). Across all published studies of this species in Texas, less than 200 individuals have been recorded, and a quantitative assessment of the species has never been conducted. Our recent discovery of a relatively large population of *P. popeii* in the Rio Grande above Laredo and a small population in the Devils River proved that the species still exists in Texas. Being endemic to the Rio Grande drainage, *P. popeii* was always restricted to a few rivers; over the last century, however, its formerly continuous distribution across this range in Texas has been fragmented and reduced to a few isolated populations.

Using historical data on the distribution of *P. popeii* and records of shells collected during our study, along with the estimation of density of recently discovered populations, we were able to reconstruct the historical distribution range and population size of this species in Texas. We found that *P. popeii* has been extirpated from two of four rivers, and the total length of the rivers populated by the mussel has declined by 76%. The total *P. popeii* population size has declined by 86%. The only area that still supports a high density of *P. popeii* in Texas is the stretch of the Rio Grande between Laredo and Eagle Pass (Figure 11). Similar dramatic decline of *P. popeii* was reported from the New Mexican part of the Rio Grande drainage where the species currently occupies about 12% of its historic range in a 14 km reach of the Black River in Eddy County (Lang, 2001; Carman, 2007). Although the current status of *P. popeii* in Mexico is unknown (USFWS, 2012), it is likely that the Mexican population experienced similar decline. Therefore, we suggest that during the last century across the whole range, *P. popeii* faced range fragmentation, local extirpation, and almost 90% loss of their population.

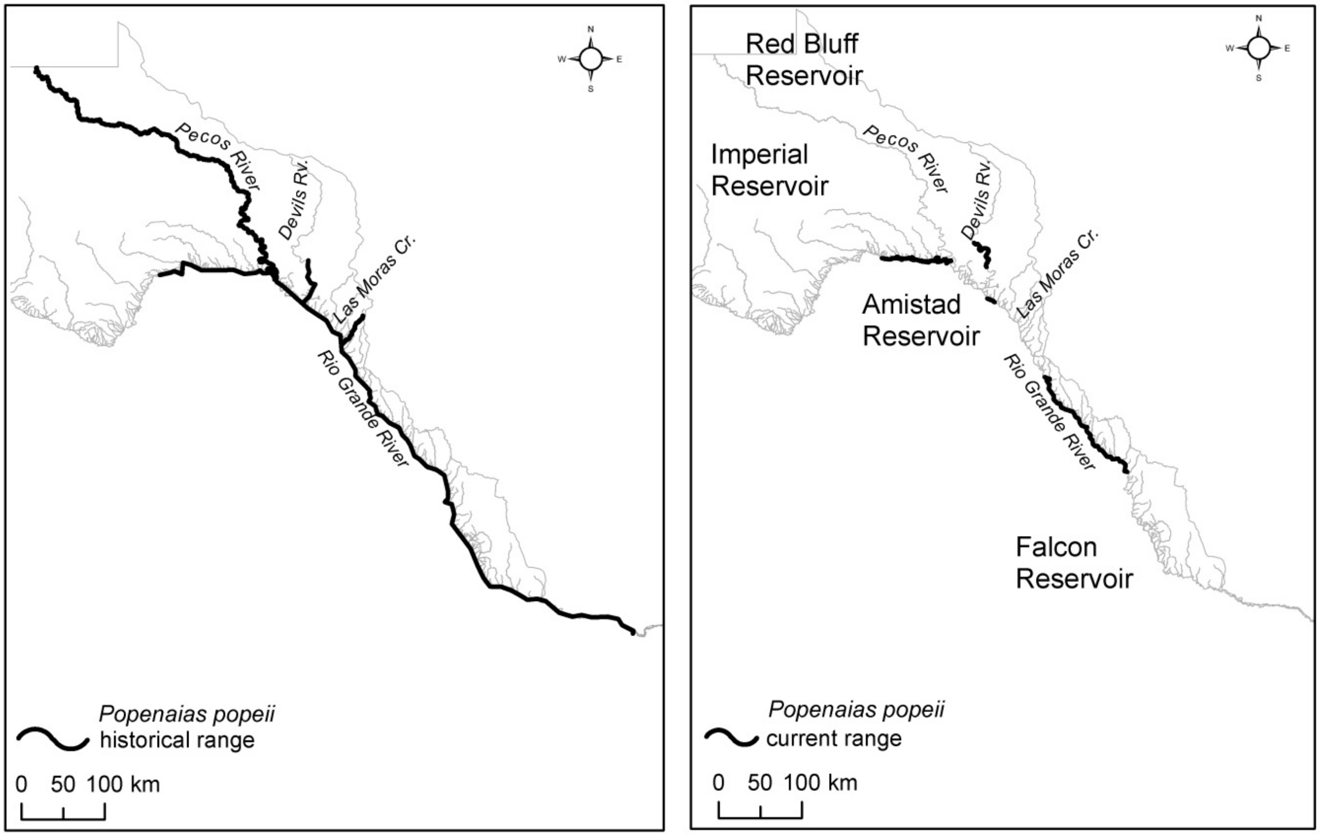

Figure 11. Map of the Rio Grande River basin in Texas with historical and current range of *Popenaias popeii* in Texas.

Growth and longevity

The Rio Grande is one of the largest and the most endangered river system in the North America (Wong *et al.*, 2007). Over the last century the river experienced strong habitat degradation, pollution and water over-extraction which along with the global climate change lead to extremely strong water fluctuation. In contrast to the Rio Grande, the Black River is a much smaller stream and, although experiencing significant human impacts, it may provide more stable environment for mussels. We hypothesize that the reason why *P. popeii* reach much larger sizes in this river (average length 93.6 mm, range 33.9-123.2 mm in the Black River vs. average length 62.1 mm, range 20.8 – 87.0 mm in the Rio Grande) an live longer (20 maximum longevity in the Black River, vs. 14 years in the Rio Grande) may be at least partially explained by the smaller fluctuations in the water discharge rate and more stable environment.

Analysis of historical data for the Rio Grande and the Black River indicated that the mean discharge rate for the Rio Grande is two orders of magnitude higher the average discharge rate of the Black River, even if compared to the post-impoundment period (after the Amistad Reservoir was build and river flows became highly regulated). The peak flow in the Rio Grande was recorded in 1954 (16,300 $m^3\ s^{-1}$) and in 1922 (7,020 $m^3\ s^{-1}$), both before river undulation. The maximum discharge rate in the Black River was recorded only once in 1966 (340 $m^3\ s^{-1}$), and the next record flow was only 90 $m^3\ s^{-1}$. This is an order of magnitude less than the maximum flows in the Rio Grande after the Amistad dam start operations (3,260 $m^3\ s^{-1}$ recorded in 1972).

Population dynamics

According to our preliminary analysis, *P. popeii* population in the Rio Grande experiences large fluctuations in density and high migration rates compared to mussels from the much smaller and more stable Black River in New Mexico (Inoue et al., 2014a). Much larger dataset from the Black River (15 years of monitoring) allowed our collaborators to reveal the importance of habitat heterogeneity and hydrological cycles for population dynamics (Inoue et al., 2014a). They demonstrated that *P. popeii* survival positively correlated with river discharge and was a function of minimum monthly discharge. Considering that flow rates and ranges are much higher in the Rio Grande, we can assume that hydrological cycles will be even more important for the Rio Grande population.

Another large difference between populations in the Black River and in the Rio Grande (La Bota population) was in the migration rate. While in the Black River there was no temporal migration among subpopulations (Inoue et al., 2014a), we evidenced ongoing dispersal in La Bota population (finding marked mussels downstream from our mark-recapture site), and the dispersal rates calculated by our model were very high (Table 3, Figure 5). This dispersal may have facilitated the downstream recolonization of areas of the river affected by severe drought, such as the La Bota habitats after the drought of 1953 when the river in Laredo was dry for almost 2 months (International Boundary Water Commission stream gauge data). Considering that the drought happened during the hottest months (June and July), we hypothesize that population in La Bota may have died out and then was recolonized from upstream, as the upper gage stations (in Eagle Pass and El Indio) have never recorded flow less than 2 m^3s^{-1} (International Boundary Water Commission stream gauge data). In contrast, discharges in the

Black and Devils rivers that support *P. popeii* populations have never been reduced to zero for several days in the last 50 years (since 1947 in the Black River and since 1960 in the Devils River). This high downstream migration in the Rio Grande, however, may have large negative consequences for population as no live *P. popeii* was found below the Laredo Sewage Plant waste water discharge to the mouth of the river despite of abundance of suitable habitat (Figure 11).

High *P. popeii* dispersal rates along with smaller individual size and shorter live span in the Rio Grande compared to *P. popeii* population parameters in the Black River may be at least partially explained by larger fluctuations in the discharge rate and less stable environment in this large river.

Update of Texas Hornshell Recovery Plan and Management Actions for *P. popeii* in Texas

Recovery planning is a process to identify and facilitate the implementation of priority actions to ensure the survival and recovery of species and ecosystems at risk. The first Recovery Plan for Texas Hornshell was developed by S. Carman in 2007, however at the time the only known population was in the Black River in New Mexico. During our study we surveyed the former range of the species in Texas and discovered a large population of *P. popeii* in the Rio Grande, in Laredo, Webb County. Our surveys will add to the background information and situation analysis of this Recovery Plan by providing habitat and population assessment in Texas, identify major threats to the species, and aid in developing management strategy.

Assessment of P. popeii status in Texas

The most efficient means to secure the viability of existing unionid populations is by applying the knowledge of their distribution, biology and ecology towards reducing and preventing threats through regulatory mechanisms and habitat restoration programs. As a first step, information on distribution and population size is required to define conservation priorities. Our 2001-2013 surveys of *P. popeii* in Texas, in combination with recent surveys of the Black River in New Mexico (Inoue *et al.*, 2014a), provided sufficient information on the current population range and size of this species in the USA, and, most importantly, on the historical changes in populations in the last 100 years. **The species currently occupies only 12% of its historic range in New Mexico, and 24% in Texas**. We also calculated that ***P. popeii* in Texas have experienced an 86% decline in population size over the last century**. This confirms the evaluation of *P. popeii* by IUCN as critically endangered meaning that the species is facing an extremely high risk of extinction in the wild.

Decline in population size is the first among the five quantitative criteria which are used to determine whether a taxon is threatened (IUCN, 2014). The measure of species' area of occupancy is important for the estimation of species' decline and is usually obtained by counting the number of occupied cells in a uniform grid (commonly used grid size is 2 km, a cell area of 4 km^2) that covers the entire range of a taxon and then tallying the total area of all occupied cells (IUCN, 2014). The authors recognize that there is concern that grids do not have much ecological meaning for taxa living in "linear" habitats such as in rivers or along coastlines

(IUCN, 2014). For example, in all rivers where it was found, *P. popeii* (in contrast to, for example, amphibians or insects) does not live in small tributaries. Thus the area cell approach, although useful while working with different species for comparable listing, grossly overestimates the area of occupancy for freshwater mussels. The method we used for calculating the change in the historical species range and population size based on species densities per river length instead of area (Appendix 1), made it more appropriate for freshwater molluscs with very restricted "linear" habitat range.

Population fragmentation is another criterion for determining species conservation status (IUCN, 2014). A taxon is considered to be severely fragmented if most (>50%) of its total area of occupancy is in habitat patches that are separated from other habitat patches by a distance larger than the dispersal distance of the taxon (IUCN, 2013). Although laboratory studies described *P. popeii* as a host generalist (24 physiological fish host species identified; Lang, 2001, 2004), Levine et al. (2012) found that in the wild, *P. popeii* uses a much smaller subset of all potential hosts that occur in the Black River, including *Carpiodes carpio, Moxostoma congestum*, and *Cyprinella lutrensis* that represented 80% of all individual fish infected and carried over 99% of glochidia. While large-bodied *C. carpio* exhibited the highest prevalence of infestation (60%), *M. congestum* (10% prevalence) represented the most frequently captured large-bodied fish, and the small-bodied *C. lutrensis* was the only species infested consistently and exhibited the highest prevalence (30%) (Levine *et al.*, 2012). In Texas, only 4 species of 15 collected in the Rio Grande in the summer of 2012 (629 fish caught) were found suitable field hosts as in the Black River (*C. lutrensis, Lepomis macrochirus, L. megalotis,* and *M. congestum*) (authors unpublished data), of which only crevice spawners red shiners (*C. lutrensis*) were abundant. All these four potential *P. popeii* fish hosts do not have long-distance dispersal. Their separation distance for suitable habitat is <15 km (NatureServe, 2014), and thus impoundments can fragment their habitat, limiting dispersal and re-colonization (Mattews and Marsh-Mattews, 2007). Since fish hosts are the main vector for *P. popeii* dispersal, the large gaps (200 km) among the populated segments on the Rio Grande and the Devils River due to the Amistad Reservoir is larger than the dispersal distance of the species. Therefore *P. popeii* population on the Rio Grande drainage can be considered as **severely fragmented**.

Existing populations

The next important step in species protection is to identify the most important drainages and localities having viable populations of the threatened species. Our studies identified that the only highly populated habitat left in the Rio Grande is the 190 km river stretch between Eagle Pass and Laredo, which also supports another rare endemic Rio Grande mussel *Truncilla cognata*. This large *P. popeii* population is viable and reproducing (Karatayev *et al.*, 2012) and, therefore, requires priority protection. However its dramatic decline over the last century warrants immediate species protection. Similar declines in *Margaritifera margaritifera* in Europe led to inclusion of this species in the main European policy that protects wildlife habitats and attracted most of European Community funds devoted to freshwater bivalve conservation (Geist, 2010; Gum *et al.*, 2011; Prié, 2013). Despite the existence of large *M. margaritifera* populations in several European countries (over several million), 28 European LIFE projects were implemented to restore freshwater mussel habitats with funding totalling 64 million Euros. The majority of these funds were directed to the conservation of *M. margaritifera* and *U. crassus* illustrating that restoration of freshwater mussel habitats is possible, but that substantial effort is needed to mitigate any negative impact (Lopes-Lima *et al*. in review).

Although the part of the Rio Grande in and above Laredo has the status of a mussel sanctuary (where mussels harvesting is prohibited, Texas Register 31, 2006), additional protection is urgently necessary as any activity associated with the water flow alternation could potentially impact the remaining habitat of *P. popeii*. The population above Amistad Reservoir is small, but considering the presence of another Rio Grande endemic species (extremely rare *Potamilus metnecktayi*), this habitat is warranted protection as well. Part of the river segment that begins in the Big Bend National Park in Brewster County and continues to the Terrell and Val Verde County border was designated in 1978 as the National Wild and Scenic River (National Parks and Recreation Act of 1978, Public Law 95-625, November 10, 1978). This Wild and Scenic Rivers Act prohibits federal support for construction of dams, water conduits, reservoirs, or other instream activities; but neither prohibits development or gives the federal government control over private property, nor does it affect existing water rights.

Threats

Identifying **direct threats** to mussel habitats is the next step that needs to be considered in conservation planning. Globally, major threats to freshwater biodiversity (including bivalves) include loss, fragmentation and degradation of habitat, overexploitation, pollution, introduction of non-native invasive species, and climate change (Dudgeon *et al.*, 2006; Geist, 2011). The primary threats to the *P. popeii* identified by U. S. Fish and Wildlife Service are habitat alterations such as streambank channelization, impoundments, and diversions for agriculture and flood control; contamination of water by oil and gas activity; alterations in the natural riverine hydrology; and increased sedimentation and flood pulses from prolonged overgrazing and loss of native vegetation (Federal Register 78, 2013).

Water over-extraction

Water over-extraction is responsible for the extinction of *P. popeii* both in the upper and lower reaches of the Rio Grande. Irrigated agriculture is the primary use of the Rio Grande surface flow throughout the basin, and it is further exasperated by evaporation from major reservoirs that exceeds the quantity of water used for municipal purposes in the basin (Dahm *et al.*, 2005). Evapotranspiration, groundwater recharge, and human appropriation of the Rio Grande water has resulted in less than 1% of basin precipitation reaching the mouth (Dahm *et al.*, 2005). Water diversion from the Rio Grande is so high that the riverbed between El Paso and Presidio/Ojinaga often lies dry (Dahm *et al.*, 2005; Wong *et al.*, 2007; Douglas, 2009); and in several years, the river failed to reach the Gulf of Mexico (Dahm *et al.*, 2005). The flow rate of the Las Moras Springs (headwaters of Las Moras Creek) decreased between 1896 and 1978 by an order of magnitude, and in 1964 and 1971 it dried up completely for a time (Brune, 1975). Similarly, the Pecos River, that was once a critical source of water in the Trans-Pecos region in Texas, has dwindled to a trickle in some areas due to water over-extraction (Gregory and Hatler, 2008).

The most important existing threats for the Rio Grande population above Amistad Reservoir as well as for the population in the Devils River are droughts and the decrease in the water table due to ground water over-extraction. Thus, declines over 12 m in the water table have been already recorded in this area (both in Hueco Bolsom and Alluvium Aquifers, El Paso and Reeves

Counties, TWDB, 2014), and the existing groundwater supplies are expected to decline 30% by 2060 (TWDB, 2012).

Impoundments

P. popeii is a lotic species and is not found in reservoirs (Metcalf, 1982; Neck and Metcalf, 1988; Karatayev *et al.*, 2012). Construction of the Amistad and Falcon reservoirs turned otherwise available for the mussel habitats in the Rio Grande into unsuitable environment. The severe *P. popeii* population fragmentation we found in Texas was due to existing impoundments, but there are plans proposed for another low-water diversion dam just downstream of the only large and viable Rio Grande population near Laredo. Impoundments also caused extirpation of *P. popeii* from the lower reaches of the Pecos and Devils rivers where it was previously reported (Singley, 1893; Metcalf, 1974, 1982; Neck, 1984). In addition, low water dams that are common in the Rio Grande and its tributaries may have negative impact on *P. popeii* by restricting movement of host fish or smothering of habitat by excessive silt deposition (Lang, 2001).

Water pollution

Water pollution and salinization are among the important additional threats not identified in the US FWS assessment. Pollution is one of the main threats to most freshwater mussel species (Bogan, 1993; Strayer *et al.*, 2004). Fecal coliforms, nutrients, low dissolved oxygen, pesticides, herbicides, metals, and organic contaminants are significant concerns throughout the Rio Grande basin (Dahm, 2005). Untreated or poorly treated discharges from inadequate wastewater treatment facilities primarily in Mexico are the principal source for fecal coliform bacteria contamination. A secondary source is from nonpoint source pollution on both sides of the river, including poorly constructed or malfunctioning septic and sewage collection systems and improperly managed animal wastes. Contact recreation use of the Rio Grande is not supported downstream of the Amistad Reservoir due to elevated levels of fecal coliform bacteria (TPWD, 2005). We found that waste water discharges from Laredo and Nuevo Laredo wastewater treatment facilities completely wiped out populations of *P. popeii* from downtown Laredo all the way downstream to the Falcon Reservoir. Mussels were abundant in the Rio Grande 10 m above discharge, but absent in over 40 km stretch of the river below the discharge in spite of the presence of abundant suitable habitats. Several live individuals of common unionid species were found only 40 km below Laredo, perhaps owing to the Dolores Creek input of fresh water below the Zapata County line, but no live *P. popeii* was found. Similarly, we found extended good *P. popeii* habitat above Eagle Pass near an irrigation canal overgrown by abundant green benthic filamentus algae (likely indicating pollution from agriculture), but not a single live *P. popeii* was found in the area. Agricultural and oil field pollutants, and blooms of toxic algae have reportedly caused deterioration of the water quality in the Pecos River (TPWD, 1974; Linam and Kleinsasser, 1996). According to Murray (1975), *P. popeii* was extirpated from Las Moras Creek due to the removal of aquatic vegetation, the paving of a portion of the spring, and the chlorination in conjunction with the use of the spring headwater as a swimming pool.

Salinization

While streamflow salinization and loss of freshwater taxa in the Pecos and Rio Grande appear to be regional phenomena associated with upstream agricultural developments, water demand for agriculture will increase in response to global warming, suggesting that the threat of salinization will likewise increase (reviewed in Hoagstrom, 2009). Before the 1950s, irrigation from groundwater in the Texas portion of the Permian Basin was relatively minor, but subsequent groundwater overdraft greatly reduced spring inflows and ultimately reversed the flow direction in some reaches, causing conveyance losses via seepage into the aquifer (Hoagstrom, 2009). Other causes of streamflow salinization include reduced flood frequency and magnitude; increased evapotranspiration; and increased prevalence of natural, high-salinity groundwater over freshwater sprig inflows (Hoagstrom, 2003, 2009). Similar to the lower Pecos River, invertebrate and fish faunas of the salinized Rio Grande downstream from the New Mexico-Texas border have relatively low biodiversity and consist only of salt-tolerant forms (Hubbs *et al.*, 1977; Davis, 1980). High salinity seems to be the major factor limiting *P. popeii* in the Pecos River along with water over-extraction and impoundments. According to Lang (2001), this species show behavioral signs of physiological stress followed by death at a salinity of 7.0 ppt, which is similar to salinity in the Pecos River (ranges from 6.0-7.0 ppt) downstream of it confluence with the Black River (Lang, 2001); and the levels of salinity are even higher (up to 12 ppt) in the Texas part of the Pecos River (Hoagstrom, 2009).

Climate Change

Current climate model simulations suggest that the American southwest could experience a 60-year stretch of heat and drought unseen since the 12th century and that the region is likely to become drier and experience more frequent droughts, with changes accelerating toward the end of the century (Woodhouse *et al.*, 2010). Growing demands for water by agricultural, industrial, and recreational activities may be exacerbated by predicted climatic change trends towards an increased inter-annual variability in precipitation and consequent effects in river flows (Millán, *et al.*, 2005; Milly *et al.*, 2005). Extreme climatic events like droughts and floods are predicted to become more frequent and intense in the future (Diez *et al.*, 2012). Freshwater fauna is particularly vulnerable to the effects of climate change because of the limited dispersal abilities of many species (Woodward *et al.*, 2010), and the expected changes may impact freshwater ecosystems more strongly than past anthropogenic alterations (Doll and Zhang, 2010). Particularly affected may be freshwater mussel populations at the edges of current distribution, e.g. in the southern borders, due to the possible minor tolerance to increased temperatures and also to the low dispersal capacity to more favourable habitats located at higher latitudes or altitude. Thus, the southernmost hydrologic regions, including the Texas Gulf, are predicted to experience on average 30–40% reductions in average annual discharge; and mussel extirpations, due to reductions in both discharge and loss of fish hosts, may result in considerably greater mussel extirpation rates with average losses of 15 species and up to 44 species (Spooner *et al.*, 2011).

Population viability

The large size of *P. popeii* population in the Rio Grande can be a meaningful indicator of population's viability, or population's probability of persistence. However, because of temporal variation in population size and large migration rate found in the La Bota population, estimating population viability is problematic and population size should be considered in concert with other factors, such as quantity and quality of occupied habitat, known history of population persistence, condition (e.g., evidence of successful reproduction for long-lived species), current threats, and landscape context. Size structure of the La Bota population consisted of multiple age-classes, including small mussels suggesting the successful recruitment of juvenile mussels (Figure 5). Our fish surveys also indicated presence of potential host fish in this reach of the river, which is very important for unionid reproduction. However we should stress that the Rio Grande population is severely fragmented (Figure 11), and the La Bota population we monitored occupies likely the only large suitable habitat left in the Rio Grande (the 190 km river stretch between Eagle Pass and Laredo). The dramatic decline in *P. popeii* population size, range and in available habitat in the Rio Grande basin over the last century, severe fragmentation and multiple current threats warrant immediate species protection.

Management strategy

Human-caused habitat modifications (e.g., riverine impoundment, low-head dams, regulated flows, ground- and surface- water withdrawals, and impoverished water quality) have severely reduced the amount of habitat available for *P. popeii* in the Rio Grande in Texas. All management strategies developed for the recovery of *P. popeii* in New Mexico (Carman, 2007) are appropriate and essential for Texas, including the need to:

- increase government agency and public understanding and support for federal, state, local and private programs that will promote and enhance ecosystem integrity of the Rio Grande River and it's watershed for the benefit of the *P. popeii* and other aquatic and aquatic-dependent resources, and land uses;
- work with the TPWD, TWDB, Laredo and Nuevo Laredo sewage plants and other agencies and experts to insure continued adequate water flow and water quality for *P. popeii* in the Rio Grande River,
- prevent construction of additional impoundments and low-water diversion dams in the Rio Grande and Devils River, especially between Eagle Pass and Laredo.
- identify and secure resources to promote habitat restoration and protection under federal, state, and local programs;
- assess the effects of oil and gas operations and groundwater mining on groundwater quality and quantity in the Rio Grande basin.

Our study estimated the changes in the current and historical range and population size of Rio Grande endemic *P. popeii*, identified the most important remnant populations and existing threats. However, future studies have to be conducted in order to fill the gaps in species ecology and biology that will enhance our ability to apply effective management measures. As indicated in the Texas Hornshell Recovery Plan (Issue 5, Needed Research), critical biological information is still lacking for *P. popeii*, which can lead to speculative management and unsupportable goals. Support is needed for research focusing on basic life history, reproductive biology, feeding and nutrition, habitat use by juvenile and adult mussels, impacts on biology by changes in water quality and quantity, population genetics, and population viability analysis (Carman, 2007). More research is needed in Texas to determine ecological host species, their abundance in *P. popeii* habitat, their population biology and viability, and how movement patterns.

Finally, there is a general lack of concern, awareness, and understanding about the ecological and economic value of aquatic resources in the rivers that currently support populations of *P. popeii* (Carman, 2007). Management strategies outlined in the Texas Hornshell Recovery Plan (Issue 6. Outreach Needs) are very important to implement in Texas, including:

- Identification of government agencies, non-government agencies, and private interests that can assist with conservation efforts and gain their support, provide them with educational materials related to the goal, strategies, and progress of this effort; and encourage to participate in conservation activities;
- Develop and implement an informational program, outreach material and specific media that increase public awareness of this threatened endemic mussel and the benefits of maintaining the ecological integrity of the Rio Grande and Devils River ecosystems and their watershed.

Considering all the identified threats and impending climate change, conservation plans should be aimed at promoting river management, including prevention of water over-extraction, pollution, and maintaining flow regime in response to the species needs. Recommendations for *P. popeii* recovery include conservation of current populations through habitat protection and restoration of historical populations and habitats. Recovery actions under the Wildlife Conservation Act and the Recovery Plan are voluntary and cooperative. Therefore, one of the primary recommendations for recovery is coordinating actions across the watershed, including supporting private landowners and land management agencies in habitat management activities that will benefit *P. popeii* (Carman, 2007). Since freshwater mussels are important components of aquatic ecosystems (Vaughn and Hakenkamp, 2001) and some species fulfil criteria of indicator, keystone, flagship and umbrella species, making them ideal targets in aquatic conservation (Geist, 2010), protection of these areas will also guarantee protection of other endemic Rio Grande species.

Publications and Presentations.

Peer-reviewed publications.

One paper was published and one submitted to a journal based on the results of this study:

Karatayev, A. Y., T. D. Miller, and L. E. Burlakova. 2012. Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 rivers at risk. *Aquatic Conservation: Marine and Freshwater Ecosystems.* 22(2): 206-219.

Karatayev, A. Y., L. E. Burlakova T. D. Miller, and Mary F. Perrelli. In review. Long-term changes in the distribution range and population size of endangered Rio Grande endemic mollusc *Popenaias popeii*. Submitted to *Aquatic Conservation: Marine and Freshwater Ecosystems.*

Presentations.

We presented 5 oral talks and one poster at international and local meetings:

(1) Burlakova, L. E., and A. Y. Karatayev. Biogeography and conservation of freshwater mussels (Bivalvia: Unionidae) in Texas. IV International Scientific Conference "Lake Ecosystems: Biological Processes, Anthropogenic Transformation, Water Quality", September 12-17, 2011, Minsk-Naroch, Belarus (oral presentation).

(2) Karatayev, A., Miller, T, and L. Burlakova. Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 Rivers at Risk. International Meeting on Biology and Conservation of Freshwater Bivalves in Braganca, Portugal (September, 4-7, 2012) (oral presentation).

(3) Karatayev, A., Burlakova, L., and T. Miller. Long-term changes in the distribution range and population size of Texas hornshell *Popenaias popeii*. 8th Biennial Symposium of the Freshwater Mollusk Conservation Society, April 10 – 15, 2013. Guntersville, Alabama.

(4) Burlakova, and L. E., Karatayev, A. Y. Biogeography and conservation of freshwater mussels (Bivalvia: Unionidae): drivers of diversity and threats in Texas". Berry College Biology Seminar, Berry College, Rome, Georgia, February 4, 2014.

(5) Karatayev, A. Y., and Burlakova, L. E. Long-term changes in the distribution range and population size of endemic unionid bivalve *Popenaias popeii.* Berry College Biology Seminar, Berry College, Rome, Georgia, February 4, 2014.

(6) Karatayev, A. Y., Miller, T. D., and L. E. Burlakova. Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 rivers at risk 12th Annual 2011 Faculty and Staff Research and Creativity Fall Forum, Buffalo State College, October 2011 (poster).

Significant Deviations

There are no Approach tasks which were planned for this reporting period but which were not addressed.

Appendices

1. Karatayev, A. Y., T. D. Miller, and L. E. Burlakova. 2012. Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 rivers at risk. *Aquatic Conservation: Marine and Freshwater Ecosystems.* 22(2): 206-219.

2. Karatayev, A. Y., L. E. Burlakova T. D. Miller, and Mary F. Perrelli. In review. Long-term changes in the distribution range and population size of endangered Rio Grande endemic mollusc *Popenaias popeii*. Submitted to *Aquatic Conservation: Marine and Freshwater Ecosystems.*

These appendices detail 2010-2014 activities accomplished under Objectives 1-4.

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References

Aldridge DC, Fayle TM, Jackson N. 2007. Freshwater mussel abundance predicts biodiversity in UK lowland rivers. *Aquatic Conservation: Marine and Freshwater Ecosystems* **17**: 554-564.

Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G. 2008. LOSITAN: a workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics* 9, 323.

Bogan AE. 1993. Freshwater bivalve extinctions (Molusca: Unionidae): a search for causes. *American Zoologist* **33**: 599-609.

Brune G. 1975. Major and Historical Springs of Texas, *Technical Report* 189. Texas Water Development Board, Austin.

Burlakova LE, Karatayev AY. 2008. State-wide assessment of unionid diversity in Texas. Interim Performance Report, T-43, State Wildlife Grants Program, Texas Parks and Wildlife Department. 13 pp.

Burlakova LE, Karatayev AY, Karatayev VA, May ME, Bennett DL, Cook MJ. 2011a. Biogeography and conservation of freshwater mussels (Bivalvia: Unionidae) in Texas: patterns of diversity and threats. *Diversity and Distributions* **17**: 393-407.

Burlakova LE, Karatayev AY, Karatayev VA, May ME, Bennett DL, Cook MJ. 2011b. Endemic species: contribution to community uniqueness, effect of habitat alteration, and conservation priorities. *Biological Conservation* **144**: 155-165.

Campbell L. 1959. Basic survey and inventory of species present in the Pecos River of Texas. Dingell-Johnson Project F-5-R-5, Job 13. Texas Game and Fish Commission, Austin, Texas.

Carman SM. 2007. Texas Hornshell *Popenaias popeii* Recovery Plan. New Mexico Department of Game and Fish, Conservation Services Division, Santa Fe, New Mexico.

Chapman EG, Gordon, ME, Walker JM, Lang BK, Campbell DC, Watters GT, Curole JP, Piontkivska H, Hoeh WR. 2008. Evolutionary relationships of *Popenaias popeii* and the early evolution of lampsiline bivalves (unionidae): Phylogenetic analyses of DNA and amino acid sequences from F and M mitochondrial genomes. *Malacologia* 50:303-318.

Clement M, Posada D, Crandall KA 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9, 1657-1659.

Cockerell TDA. 1902. *Unio popei*, Lea, in New Mexico. *Nautilus* 16:69–70.

Cooch E, White G. 2014. Program MARK - A Gentle Introduction. 13th Edition. Available at: http://www.phidot.org/software/mark/docs/book/. Accessed July 22, 2014.

Dahm CN, Edwards RJ, Gelwick FP. 2005. Gulf Coast rivers of the Southewestern United States. In *Rivers of North America*, Arthur CB, Colbert EC (eds). Academic Press: Burlington; 180-228.

Davis JR. 1980. Species composition and diversity of benthic macroinvertebrate populations of the Pecos River, Texas. *Southwestern Naturalist* **25**: 241-56.

Davis JR. 1987. Faunal characteristics of a saline stream in the Northern Chihuahuan Desert. *Contributed Papers of the Second Symposium on Resources of the Chihuahuan Desert Region: United States and Mexico*. Chihuahuan Desert Research Institute, Alpine, Texas.

Diez JM, D'Antonio CM, Dukes JS, Grosholz ED, Olden JD, Sorte CJB, Blumenthal DM, Bradley BA, Early R, Ibáñez I, Jones SJ, Lawler JL, Miller LP. 2012. Will extreme climatic events facilitate biological invasions? *Frontiers in Ecology and the Environment* **10**: 249-257.

Doll P, Zhang J. 2010. Impact of climate change on freshwater ecosystems: a global-scale analysis of ecologically relevant river flow alterations. *Hydrology and Earth System Sciences* **14**: 783-799.

Douglas AJ. 2009. Social, Political, and Institutional Setting: Water Management Problems of the Rio Grande. *Journal of Water Resources Planning and Management* 135: 493-501.

Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Leveque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny MLJ, Sullivan CA. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* **81**: 163-182.

Earl DA, vonHoldt BM. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4, 359-361.

Federal Register 78, 2013. Endangered and Threatened Wildlife and Plants; Review of Native Species That are Candidates for Listing as Endangered or Threatened; Annual Notice of Findings on Resubmitted Petitions; Annual Description of Progress on Listing Actions (Proposed Rules). November 22, 2013: 70128. Available at: http://www.gpo.gov/fdsys/pkg/FR-2013-11-22/pdf/2013-27391.pdf. Accessed: 02/25/14.

Federal Register 73, 2008. Endangered and threatened wildlife and plants; review of native species that are candidates for listing as endangered or threatened; annual notice of findings on resubmitted petitions; annual description of progress on listing actions. Department of the Interior, Fish and Wildlife Service, 50 CFR Part 17, 73(238):75176-75244.

Fetzner JW, Jr., Crandall KA. 2003. Linear habitats and the nested clade analysis: an empirical evaluation of geographic versus river distances using an Ozark crayfish (Decapoda: Cambaridae). *Evolution* 57, 2101-2118.

Geist J. 2010. Strategies for the conservation of endangered freshwater pearl mussels (*Margaritifera margaritifera* L.): a synthesis of conservation genetics and ecology. *Hydrobiologia* **644**: 69-88.

Geist J. 2011. Integrative freshwater ecology and biodiversity conservation. *Ecological Indicators* 11: 1507-1516.

Goudet J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity* 86, 485-486.

Gregory L, Hatler W. 2008. A Watershed Protection Plan for the Pecos River in Texas. Texas State Soil and Water Conservation Board, U.S. Environmental Protection Agency. Available at http://pecosbasin.tamu.edu/media/1923/pecosriverwpp.pdf. Accessed January 15, 2014.

Gum B, Lange M, Geist J. 2011. A critical reflection on the success of rearing and culturing juvenile freshwater mussels with a focus on the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). *Aquatic Conservation: Marine and Freshwater Ecosystems* **21**: 743-751.

Haenn WF. 2002. Fort Clark and Brackettville: Land of Heroes. Arcadia Publishing Chicago IL. ISBN 978-0-7385-2063-6.

Hoagstrom CW. 2003. Historical and recent fish fauna of the lower Pecos River. Aquatic fauna of the northern Chihuahuan desert. *Special publications of Museum of Texas Tech University* **46**: 91-110.

Hoagstrom CW. 2009. Causes and impacts of salinization in the lower Pecos River. *Great Plains Research* **19**: 27-44.

Hoeh, W. R., S. A. Roark, B. K. Lang, M. E. Gordon, and S. I. Guttman. 1999. Allozymic investigations of the enigmatic Texas hornshell, *Popenaias popeii* (Lea, 1957) (Bivalvia: Unionoida: Unionidae). Report to the NMDGF under Professional Services Contract no. 98-516.48. 16 pp.

Hoeh, W. R. 2009. Genetic and species diversity in *Popenaias* (Bivalvia: Unionoida: Unionidae: Lampsilini) populations from New Mexico and Texas. Report to the NMDGF under Share with Wildlife Program 16 pp.

Holleley CE, Geerts PG. 2009. Multiplex Manger 1.0: a cross-platform computer program that plans and optimizes multiplex PCR. *Biotechniques* 46, 511-517.

Howells RG, Mather CM, Bergmann JAM. 1997. Conservation status of selected freshwater mussels in Texas. In *Conservation and Managenment of Freshwater Mussels II (Initiatives for future): Proceedings of a UMRCC Symposium*, Cummings KS, Buchanan AC, Mayer CA, Naimo TJ (eds). Upper Mississippi River Conservation Committee: Rock Island, IL; 117-129.

Howells RG, Neck RW, Murray HD. 1996. *Freshwater Mussels of Texas*. Texas Parks and Wildlife Press: Austin, TX.

Howells RG. 1994. Preliminary distributional surveys of freshwater bivalves in Texas: progress report for 1992. Management Data Series 105. Texas Parks and Wildlife Department: Austin, TX.

Howells RG. 2001. Status of freshwater mussels of the Rio Grande, with some comments on other bivalves. Texas Parks and Wildlife Department, Austin, TX.

Hubbs CR, Miller R, Edwards RJ, Thompson KW, Marsh E, Garrett GP, Powell GL, Morris DJ, Zerr RW. 1977. Fishes inhabiting the Rio Grande, Texas and Mexico, between El Paso and the Pecos confluence, In *Symposium on the importance, preservation and management of the riparian habitat*. July 9, 1977, Tucson, Arizona. pp. 91-97.

Inoue K, Lang BK, Berg DJ. 2013. Development and characterization of 20 polymorphic microsatellite markers for the Texas hornshell, *Popenaias popeii* (Bivalvia: Unionidae), through next-generation sequencing. Conservation Genetics Resources 5, 195-198.

Inoue K, Levine TD, Lang BK, Berg DJ. 2014a. Long-term mark-and-recapture study of a freshwater mussel reveals patterns of habitat use and an association between survival and river discharge. *Freshwater Biology* doi:10.1111/fwb.12389.

Inoue K, Monroe EM, Elderkin CL, Berg DJ. 2014b. Phylogeographic and population genetic analyses reveal Pleistocene isolation followed by high gene flow in a wide-ranging, but endangered, freshwater mussel. *Heredity* 112, 282-290.

International Boundary Water Commission stream gauge data. http://www.ibwc.gov/Water_Data/histflo1.htm. Accessed March 14, 2014.

IUCN. 2013. The International Union for Concervation of Nature Red List of Threatened Species 2013.1, http://www.iucnredlist.org/documents/summarystatistics/2013_1_RL_Stats_Table_3a.pdf

IUCN. 2014. The International Union for Concervation of Nature Standards and Petitions Subcommittee. Guidelines for Using the IUCN Red List Categories and Criteria. Version 11. Prepared by the Standards and Petitions Subcommittee. http://www.iucnredlist.org/documents/RedListGuidelines.pdf. Accessed on March 19, 2014.

Jackson RB, Carpenter SR, Dahm CN, McKnight DM, Naiman RJ, Postel SL, Running SW. 2001. Water in a changing world. *Ecological Applications* 11: 1027-1045.

Jakobsson M, Rosenberg NA. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23, 1801-1806.

Johnson PD, Bogan AE, Brown KM, Burkhead NM, Cordeiro JR, Garner JT, Hartfield PD, Lepitzki DAW, Mackie GL, Pip E, Tarpley TA, Tiemann JS, Whelan NV, Strong EE. 2013. Conservation Status of Freshwater Gastropods of Canada and the United States. *Fisheries* 38: 247-282.

Johnson RI. 1999. The Unionidae of the Rio Grande (Rio Bravo del Norte) system of Texas and Mexico. *Occasional Papers on Mollusks* 6: 1-65.

Karatayev AY, Miller TD, Burlakova LE. 2012. Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 rivers at risk. *Aquatic Conservation: Marine and Freshwater Ecosystems.* 22(2): 206-219.

Kummu M, de Moel H, Ward PJ, Varis O. 2011. How close do we live to water? A global analysis of population distance to freshwater bodies. *PLoS ONE* **6**: e20578.

Lang BK. 2001. Status of the Texas Hornshell and Native Freshwater Mussels (Unionoidea) in the Rio Grande and Pecos River of New Mexico and Texas. Completion Report (E-38) to the Division of Federal Aid, U. S. Fish and Wildlife Service, Region 2. New Mexico Department of Game and Fish, Albuquerque, NM.

Lang BK. 2001. Status of the Texas Hornshell and Native Freshwater Mussels (Unionoidea) in the Rio Grande and Pecos River of New Mexico and Texas. Completion Report (E-38) to the Division of Federal Aid, U.S. Fish and Wildlife Service, Region 2. New Mexico Department of Game and Fish, Albuquerque, NM.

Lang BK. 2004. Artificial culture and captive rearing of the Texas hornshell. New Mexico Department of Game and Fish, Completion Report, E-51, submitted to the Division of Federal Aid, U.S. Fish and Wildlife Service, Albuquerque, New Mexico.

Lang, B. K. 2009. Texas hornshell, *Popenaias popeii* (Bivalvia: Unionidae), population monitoring. New Mexico Department of Game and Fish, Performance Report T-30-P1 submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Albuquerque, NM.

Lang BK. 2010. Texas hornshell, *Popenaias popeii* (Bivalvia: Unionidae), population monitoring. New Mexico Department of Game and Fish, Performance Report T-30-P1 submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Albuquerque, NM.

Lea I. 1857. Description of six new species of fresh water and land shells of Texas and Tamaulipas, from the collection of the Smithsonian Institution. *Proceedings of the Academy of Natural Sciences of Philadelphia* **9**: 101-102.

Levine TD, Lang BK, Berg DJ. 2012. Physiological and ecological hosts of *Popenaias popeii* (Bivalvia: Unionidae): laboratory studies identify more hosts than field studies. *Freshwater Biology* **57**: 1854-1864.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451-1452.

Linam, GW, Kleinsasser LJ. 1996. Relation between fishes and water quality in the Pecos River, Texas. Resource Protection Division, *Texas Parks and Wildlife Department. River Studies Report* **9**: Austin, TX.

Lopes-Lima M, Sousa R, Geist J, Aldridge DC, Araujo R, Bergengren J, Bespalaja Y, Bodis E, Burlakova L, Van Damme D, Douda K, Froufe E, Georgiev D, Gumpinger C, Karatayev A, Kebapci U, Killeen I, Lajtner J, Larsen BM, Lauceri R, Legakis A, Lois S, Lundberg S, Moorkens E, Motte G, Nagel K-O, Ondina P, Outeiro A, Paunovic M, Prie V, von Proschwitz T, Riccardi N, Rudzīte M, Rudzītis M, Scheder C, Seddon M, Sereflisan H, Simić V, Sokolova S, Stoeckl K, Taskinen J, Teixeira A, Thielen F, Trichkova T, Varandas S, Vicentini H, Zajac K, Zajac T, Zogaris S. In review. Conservation Status of Freshwater Mussels in Europe: State of the Art and Future Challenges. Submitted to: *The Quarterly Journal of Biology*.

Mattews WJ, Marsh-Mattews E. 2007. Extirpation of Red Shiner in direct tributaries of Lake Texoma (Oklahoma–Texas): A cautionary case history from a fragmented river–reservoir system. *Transactions of the American Fisheries Society* 136: 1041-1062.

Metcalf AL. 1974. Fossil and living freshwater mussels (Unionacea) from the Pecos River, New Mexico and Texas. *Bulletin of the American Malacalogical Union* 33: 47-48.

Metcalf AL. 1982. Fossil unionacean bivalves from three tributaries of the Rio Grande. In *Symposium on Recent Benthological Investigations in Texas and Adjacent States*, Davis JR (ed). Texas Academy of Science: Austin, Texas; 43-59.

Metcalf AL, Smart RA. 1972. Records of introduced mollusks: New Mexico and Western Texas. The *Nautilus* 85: 144-145.

Metcalf AL, Stern EM. 1976. Notes on unionacean mollusks of the Rio Grande system, United States and Mexico. *Bulletin of the American Malacological Union* 42-43.

Millán MM, Estrela MJ, Miró J. 2005. Rainfall components: variability and spatial distribution in a Mediterranean Area (Valencia Region). *Journal of Climate* **18**: 2682-2705.

Millennium Ecosystem Assessment. 2005. Ecosystem and Human Well-being: Wetlands and Water Synthesis. World Resource Institute, Washington, DC.

Milly PCD, Dunne KA, Vecchia AV. 2005. Global pattern of trends in streamflow and water availability in a changing climate. *Nature* 438: 347-350.

Murray HD. 1975. *Melanoides tuberculata* (Müller), Las Moras Creek, Bracketville, Texas. *Bulletin of the American Malacological Union, Inc* 1975: 43.

Naiman RJ, Turner, MG. 2000. A future perspective on North America’s freshwater ecosystems. *Ecological Applications* 10: 958-970.

NatureServe. 2014. NatureServe Explorer: An online encyclopedia of life [web application] NatureServe, Arlington, Virginia. http://www.natureserve.org/explorer [17 June 2014]

Neck RW. 1984. Restricted and declining nonmarine molluscs of Texas. *Technical Series* 34. Texas Parks and Wildlife Department: Austin, Texas.

Neck RW, Metcalf AL. 1988. Freshwater bivalves of the Lower Rio Grande, Texas. *The Texas Journal of Science* 40: 259-268.

NMDGF 2006. New Mexico Department of Game and Fish. 2006. Comprehensive Wildlife Conservation Strategy for New Mexico. 635 pp.

NMDGF 2010. New Mexico Department of Game and Fish. 2010. Threatened and endangered species of New Mexico: biennial review and recommendations.

Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6, 288-295.

Pope J. 1854. Report of Exploration of a Route for the Pacific Railroad, near the Thirty-Second Parallel of North Latitude from the Red River to the Rio Grande. U.S. Government Printing Office, Washington, DC.

Prié V. 2013. Flagships and umbrellas: unbalanced conservation effort towards charismatic species as potential threat to the unknowns. *Tentacle* **21**: 32-34.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genetype data. *Genetics* 155, 945-959.

Rosenberg NA. 2004. DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes* 4, 137-138.

Rousset F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8, 103-106.

Simpson CT. 1914. *A Descriptive Catalogue of the Naiades, or Pearly Fresh-Water Mussels*. B. Walker: Detroit. Michigan.

Singley JA. 1893. Contributions to the Natural History of Texas. Part I. Texas Mollusca. A preliminary list of the land, fresh water, and marine Mollusca of Texas. Department of Agriculture, Insurance, Statistics, and History, Austin, TX.

Smith, D.G., B.K. Lang, and M.E. Gordon. 2003. Gametogenetic cycle, reproductive anatomy, and larval morphology of *Popenaias popeii* (Unionoida) from the Black River, New Mexico. *The Southwestern Naturalist* 48(3):333-340.

Spooner DE, Xenopoulos MA, Schneider C, Woolnough DA. 2011. Coextirpation of host-affiliate relationships in rivers: the role of climate change, water withdrawal, and host-specificity. *Global Change Biology* **17**: 1720-1732.

Stansbery DH. 1971. Rare and endangered mollusks in the eastern United States. . In *Rare and endangered mollusks (Naiads) of the United States*, Jorgenson SE, Sharp RW (eds). U.S. Fish and Wildlife Service: Twin Cities, Minnesota; 5-18.

Strayer DL, Claypool S, Sprague SJ. 1997. Assessing unionid populations with quadrats and timed searches. In *Conservation and Managenment of Freshwater Mussels II (Initiatives for future): Proceedings of an Upper Mississippi River Conservation Committee (UMRCC) Symposium.*, Cummings KS, Buchanan AC, Mayer CA, Naimo TJ (eds). Upper Mississippi River Conservation Committee: 163–169.

Strayer DL, Downing JA, Haag WR, King TL, Layzer JB, Newton TJ, Nichols SJ. 2004. Changing perspectives on pearly mussels, North America’s most imperiled animals. *Bioscience* 54: 429-439.

Strayer DL, Dudgeon D. 2010. Freshwater biodiversity conservation: recent progress and future challenges. *Journal of the North American Benthological Society* 29: 344-358.

Strecker JK. 1931. The distribution of the naiades or pearly freshwater mussels of Texas. *Baylor University Museum Bulletin* 2: 69.

Strenth NE, Howells RG, Correa-Sandoval A. 2004. New records of the Texas hornshell *Popenaias popeii* (Bivalvia: Unionidae) from Texas and northern Mexico. *The Texas Journal of Science* 56: 223-230.

Taylor DW. 1967. Freshwater mollusks collected by the United States and Mexican Boundary surveys. *The Veliger* 10: 152-158.

Texas Register 31. 2006. Natural resources and conservation. Part 2. Texas Parks and Wildlife Department. Chapter 57. Fisheries §57.157, Proposed Rules concerning Mussels and Clams. June 9, 2006:4699-4704. Texas Secretary of State. http://www.sos.state.tx.us/texreg/pdf/backview/0108/0108adop.pdf [15 April 2014]

Texas Register 35. 2010. Threatened and endangered nongame species. Chapter 65. Wildlife Subchapter G. 31 TAC §65.175. Adopted rules. Texas Register 35, January 8, 2010, 249-251. Texas Secretary of State. http://www.sos.state.tx.us/texreg/pdf/backview/0108/0108adop.pdf [15 February 2010]

TPWD. 1974. An Analysis of Texas Waterways: a Report on the Physical Characteristics of Rivers, Streams and Bayous in Texas. The Texas Agricultural Extension Service, Texas A&M University System. http://www.tpwd.state.tx.us/publications/pwdpubs/pwd_rp_t3200_1047/ [16 May 2014].

TPWD. 2005. Texas Parks and Wildlife Department. Texas Wildlife Action Plan 2005-2010. Texas Parks and Wildlife Department, Austin, TX, USA. PWD PL W7000-1187. http://www.tpwd.state.tx.us/publications/pwdpubs/pwd_pl_w7000_1187a/

TWDB. 2012. Texas Water Development Board. Water for Texas 2012. State Water Plan. Chapter 5. Water Supplies. Texas Water Development Board, http://www.twdb.state.tx.us/publications/state_water_plan/2012/05.pdf. Accessed March 19, 2014

TWDB. 2014. Texas Water Development Board. Hydrological Atlases. Hydrological Athlas 2. http://www.twdb.state.tx.us/publications/reports/HydrologicAtlases/doc/Ha_2.pdf. Accessed March 19, 2014.

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. 2012. Primer3--new capabilities and interfaces. *Nucleic Acids Research* 40, e115.

U. S. Fish and Wildlife Service Endangered Species Recovery Program. May 2009. http://www.fws.gov/endangered/factsheets/recovery.pdf

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-Checker: Software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4, 535-538.

Vaughn CC, Hakenkamp CC. 2001. The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology* 46: 1431-1446.

Vaughn CC, Taylor CM, Eberhard KJ. 1997. A comparison of the effectiveness of timed searches vs. quadrat sampling in mussel surveys. In *Proceedings of an Upper Mississippi River Conservation Committee (UMRCC) Symposium, 16-18 October 1995.*, Cummings KS, Buchanan AC, Mayer CA, Naim TJ (eds). Upper Mississippi River Conservation Committee: Rock Island, IL; 157-162.

Vaughn CC. 2010. Biodiversity losses and ecosystem function in freshwaters: emerging conclusions and research directions. *BioScience* 60: 25-35.

Villella RF, Smith DR, Lemarié D.P. 2003. Estimating survival and recruitment in a freshwater mussel population using mark-recapture techniques. *American Midland Naturalist* 151:114-133.

Warren ML, Pardew MG. 1998. Road crossings as barriers to small-stream fish movement. *Transactions of the American Fisheries Society* 127, 637-644.

Williams JD, Warren ML, Jr., Cummings KS, Harris JL, Neves RJ. 1993. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* 18(9): 6-22.

Wong CM, Williams CE, Pittock J, Collier U, Schelle P. 2007. World's top 10 rivers at risk. Executive Summary. WWF International, Gland, Switzerland.

Woodhouse CA, Meko DM, MacDonald GM, Stahle DW, Cook ER. 2010. A 1,200-year perspective of 21st century drought in southwestern North America. *Proceedings of the National Academy of Sciences, USA* 107: 21283-21288.

Woodward G, Perkins DM, Brown LE. 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society B-Biological Sciences* 365: 2093-2106.

Appendix 1. Manuscript by Karatayev, Burlakova, Miller and Perrelli submitted to *Aquatic Conservation: Marine and Freshwater Ecosystems*

Submitted to: Aquatic Conservation: Marine and Freshwater Ecosystems

***Long-term changes in the distribution range and population size of endangered Rio Grande endemic mollusc* Popenaias popeii**

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Running title: ***Long-term changes in population of endemic mollusc* Popenaias popeii**

ABSTRACT

1. Although almost all endangered bivalve molluscs belong to the freshwater order Unionoida, sufficient data are lacking for the majority of these species. As a result, a species may become rare, endangered, and even extinct before the first population assessment is conducted. This is especially true for endemic species, particularly those limited to remote regions with difficult access.
2. We studied the current distribution and population densities of endemic Rio Grande unionid *Popenaias popeii* in Texas, and developed a method to reconstruct species' historical range and population size to evaluate changes in the population's size and distribution range over the last 100 years.
3. Sampling over 250 sites in four rivers that constitute the entire historical range of *P. popeii* in Texas, we found that the species has been extirpated from two rivers, a 76% decrease in the combined total length of the rivers populated by the mussel. Using our estimations of *P. popeii* density we found an 86% overall decline in the population size of *P. popeii* in the state. The remaining population of this species in the Rio Grande is fragmented, with only one 190 km stretch between Laredo and Eagle Pass still supporting a high density of *P. popeii* in Texas.
4. Conservation plans for *P. popeii* should be aimed at promoting river management, including prevention of water over-extraction, damming, pollution, and maintaining flow regime in response to the species needs.

KEY WORDS: river, distribution, rare species, survey, invertebrates, impoundment, pollution.

INTRODUCTION

Freshwater ecosystems provide many important goods and services such as food, clean water, and flood and erosion control, estimated to have a value of trillions of dollars annually (Millennium Ecosystem Assessment, 2005). Indeed, with ninety percent of the world's population living near fresh water, these unique ecosystems are hotspots of human activity (Kummy *et al*., 2011). At the same time, existing as semi-isolated islands in a terrestrially-dominated landscape, freshwater systems have garnered a disproportionally high number of endemic species compared to marine and terrestrial environments (Strayer and Dudgeon, 2010). Due to steeply rising human demands for water over the past century (Naiman and Turner, 2000; Jackson *et al*., 2001), these ecosystems have suffered a large global decline in biodiversity; many freshwater species are now extremely threatened, possibly more so than species in marine and terrestrial systems (Dudgeon *et al.*, 2006; Strayer and Dudgeon, 2010).

Globally, molluscs (both Bivalvia and Gastropoda) represent 44% of all extinct animals and 24% of those critically endangered (IUCN, 2013). In North America, three quarters of all mollusc species are considered imperiled or extinct, exceeding the imperilment levels of fish (39%) and crayfish (48%) (Williams *et al.*, 1993; Johnson *et al.*, 2013). Nevertheless, at a global scale, only 8% of molluscs have been evaluated for extinction risks, compared to 100% of mammals and birds, 91% of amphibians, and 35% of fish (IUCN, 2013). This lack of attention is ironic not only because the vanishing mollusc species constitute an integral part of the biological diversity in threatened freshwater ecosystems, but also for the reason that they provide important ecological functions and services (Aldridge *et al.*, 2007; Vaughn, 2010).

Among mollusc species, the most threatened and the most data deficient are in the order Unionoida (IUCN, 2013); simultaneously, the lack of information on essential parameters for these species, such as distribution range and population size, greatly hampers the assessment of their conservation status. Often, a species may become rare, endangered, and even extinct before the first population assessment is conducted. This is especially true for endemic species which have a limited range restricted to remote regions with difficult access. However, in contrast to most other invertebrates, dead molluscs (particularly unionid bivalves) leave large calcareous shells that may remain in sediments for decades, providing evidence of former populations and helping to reconstruct their historical range.

In Texas, 40% of all freshwater mussel species are of conservation concern – this is much higher than for all other animal groups – and over 90% of them are Texas or regional endemics (TPWD, 2005). The Rio Grande endemic *Popenaias popeii* (Texas hornshell) was first described by Lea (1857) as *Unio popeii* from the Devils River in Texas and the Río Salado in Mexico. The distribution range of this species is restricted to the Rio Grande drainage in Texas (Singley, 1893; Taylor, 1967; Burlakova *et al.*, 2011a, b; Karatayev *et al.*, 2012), New Mexico (Lang, 2001; Carman, 2007), and several Mexican tributaries (Simpson, 1914; Johnson, 1999; Strenth *et al.*, 2004). Singley (1893) described it as a “rare shell”, Strecker (1931) reported that the species “seems to be rather scarce”, Stansbery (1971) defined it as “rare and endangered”, and Neck (1984) included *P. popeii* in his list of restricted and declining species of Texas. From the mid-1970s until the beginning of our study, no live *P. popeii* have been found in the Rio Grande drainage in Texas (Howells, 2001; Karatayev *et al.*, 2012). Because of this dramatic decline, *P. popeii* has been added to the state’s list of threatened species (Texas Register 35, 2010), is considered as critically endangered by IUCN (www.iucnredlist.org/details/17992/0), and is currently a candidate for listing under the federal Endangered Species Act (Federal Register 78, 2013). Due to the rarity of this species, a quantitative assessment of the *P. popeii* population in Texas has never been conducted, restricting our ability to monitor changes in its historical distribution and protect this species.

The goals and objectives of this paper are to: (1) examine the current distribution and population densities of the Rio Grande endemic *P. popeii* in Texas, (2) reconstruct the historical range and population size of *P. popeii,* and (3) estimate the decline in the distribution range and population size of this species over the last 100 years.

METHODS

Study area

We studied the Rio Grande and its tributaries where *P. popeii* historically had been documented, including the Pecos and Devils rivers, and Las Moras Creek. The Rio Grande (total length: 2,830 km, including 1,470 km in Texas) is the one of the longest rivers in North America, which flows across seven physiographic provinces, from mountain forests and high mountain deserts to desert shrub and grassland (Dahm *et al.*, 2005). In the state of Texas, the Rio Grande forms the border between the United States and Mexico and has been intensively used by both countries during the last century for irrigation, industrial and domestic water consumption (Dahm *et al.*, 2005; Wong *et al.*, 2007). Due to water over-extraction, the riverbed between El Paso and Presidio frequently is dry; and since 2001, the river often fails to reach the Gulf of Mexico (Edwards and Contreras-Balderas, 1991; Contreras-Balderas *et al.*, 2002; Dahm *et al.*, 2005; Wong *et al.*, 2007; Douglas, 2009). In addition, the Rio Grande suffers from persistent drought, an increase in border populations, and subsequent increase in the water pollution and waste water discharge (Dahm *et al.*, 2005; Wong *et al.*, 2007; Douglas, 2009). The river flow is regulated by Amistad Dam (completed in 1969) and Falcon Dam (completed in 1953) that impound the Rio Grande along the border for irrigation and flood control along with several additional low water dams or weirs.

The Pecos River (total length 1,490 km, including 679 km in Texas) is the largest Rio Grande tributary in the United States. It flows from Mora County, New Mexico, southeast through Texas where it joins the Rio Grande. The river flows through flat, semi-arid lands along the upper section, and through canyons and desert terrain in the lower riches. Red Bluff Reservoir, a small lake established for hydroelectric power, is located near the Texas - New Mexico border. Below Red Bluff Reservoir, the Pecos contains only a very limited flow of water, and passes through an arid region of West Texas where rainfall is sporadic and minimal. In the middle of 19th century the river was fast moving and deep, with slightly saline taste (Pope, 1854 cited from Hoagstrom, 2003). Several hydrological changes, initiated in the 1880s, contributed to streamflow salinization, including diminished streamflow due to aquifer depletion, reduced floods, groundwater overdraft, and increased prevalence of natural, high-salinity groundwater (Hoagstrom, 2003, 2009). Along with water overdraft and increasing salinity (Campbell, 1959; Davis, 1987; Gregory and Hatler, 2008), many other factors including contaminated runoff, oil field pollutants and blooms of toxic algae caused deterioration of the water quality (TPWD, 1974) as most of the industrial and agricultural activities in this area solely depend upon the Pecos River.

In contrast to the Pecos River, the Devils River is considered one of the cleanest, naturally flowing streams remaining in Texas due to its remote location and hostile environment (TPWD, 1974). It begins in Sutton County, flows southwest for 151 km through Val Verde County and empties into the northeastern shore of the Amistad Reservoir. The Devils River is intermittent in its upper reaches (from FM 189 to the southernmost crossing of State Highway 163 or Baker's Crossing) due to the arid nature of the region, and in some stretches, the river goes completely underground. Many springs add water to the river throughout its course, and most of the creeks that flow into the river are spring-fed. Therefore, the normal volume of water in the river progressively increases downstream with median daily discharge ~14 $m^3 s^{-1}$, and water levels are subject to extreme fluctuations ranging from 1 to 3,480 $m^3 s^{-1}$ (International Boundary Water Commission stream gauge data). Downriver of Dolan Falls, at a 4.6 m tall natural waterfall located app. 80 km from the river mouth, the river begins to widen and deepen gradually, with an abundance of long, deep pools alternated with rapids. The terminal 30 km stretch of the Devils River is regularly flooded by the Amistad Reservoir. In addition, several low water dams restrict water flow in the river.

The headwaters of Las Moras Creek are formed by artesian Las Moras Springs located in Fort Clark, Kinney County, Texas. The springs fill a swimming pool built in the beginning of the 20th century (Haenn, 2002). Las Moras Creek runs 60 km downstream to the confluence with the Rio Grande in Maverick County. The flow rates between 1896 and 1978 ranged from a high of 1.7 m^3 per second (m^3s^{-1}) in 1899 to lows of 0.16 and 0.10 m^3s^{-1}in 1964 and 1971 (Brune, 1975). In those two years, the springs dried up completely for a time.

Data collection

To assess the distribution, density and long-term population dynamics of *P. popeii* in the Rio Grande drainage within Texas, both field studies and historical data were used. From 2001 to 2013, we surveyed 250 sample locations ("subsites") pooled into 42 larger sites ("pooled sites") within the Rio Grande system (Figure 1). Fourteen of these sites were sampled once, while 28 sites were sampled from 2 to 25 times. Over 580 km in remote areas of the Rio Grande, the Devils and Pecos rivers were sampled using an airboat and a canoe. Due to the prevalence of private land in Texas (only 2% of the lands remain in public ownership, TPWD, 1974), some survey sites were selected within state parks, near public boat ramps, or based on accessibility from roads. Landowner Permission was acquired from each property owner, when surveys were conducted from private land, before entering the property. The work was carried out with an appropriate Scientific Research Permit issued by the Texas Parks and Wildlife Department, National Park Service Scientific Permit for Big Bend National Park, and Amistad National Recreational Area Research Permit.

At each site, both live and dead mussels were collected by hand, by snorkeling (at most of the sites), wading in low water, or diving. Reconnaissance sampling (timed search) was used at all sites (Strayer *et al.*, 1997; Vaughn *et al.*, 1997). If *P. popeii* were present, quantitative methods (randomly placed 0.25 m^2 quadrats or area searches) were used for assessments of density. Collected live mussels and shells were counted and measured with calipers to the nearest mm, and then live mussels were carefully bedded back into the sediment from which they were taken. Shell condition of dead mussels was recorded for each shell.

Data analysis

Population size calculations

Geographic coordinates for sampled site locations were pooled in Excel and mapped in ESRI ArcGIS 10.1. The 2010 stream segment data published by the Texas Commission on Environmental Quality was used for the analysis. These data represent classified and unclassified stream segments and reservoirs at a scale of 1:250:000. To calculate the distance for sampled areas, the stream segments were split at sampling starting and ending locations (e.g., El Indio Dam below Eagle Pass to Laredo). The distance in kilometers was then generated in ArcGIS for the split stream segments.

Devils River. The Devils River was sampled in 2011 (at 6 subsites, one *P. popeii* found; Karatayev et al., 2012) and in 2012-2013 from Baker's crossing to the Amistad Reservoir for

2,223 man hours of search effort. Due to low density of mussels, quantitative methods were not applied, and reconnaissance sampling (timed search) was used to reveal the presence of mussels. In 2011-2013, we searched 34 subsites totaling 7.15 km along the river and found five live *P. popeii.* Due to varying width of the river in different locations, we estimated the relative density of mussels per river kilometer as the number of live mussels found at each site per length of the river searched at the site. Then we calculated the ratio of time search effort (in man hours) spent at each site and the average time effort for all sites. The average ratio on the sites where we found mussels (0.51 ± 0.11, mean ± standard error here and elsewhere unless noted) indicated that extra effort was not applied to these sites. We then weighed the relative density by dividing the number of molluscs per river km at each site per effort ratio at the site. To compensate for potential error associated with difficulty in finding mussels, we adjusted the density by the probability of detection (or correction factor) calculated for this species using the average estimation from La Bota mark-recapture study (0.072 ± 0.009). The estimated mean *P. popeii* density in the Devils River was 40.3 ± 19.8 molluscs per river km. Using this data, we calculated the current total population of *P. popeii* in the Devils River considering that the similar habitat is spreading over 66 km of the river from Baker's Crossing to the Rough Canyon - the upper part of the river not undulated by the Amistad Reservoir. The historical population size of *P. popeii* in the Devils River was estimated using a distance of 96 km from the Baker's Crossing and the confluence with the Rio Grande.

Rio Grande. The most populated by *P. popeii* stretch of the Rio Grande was from El Indio Dam below Eagle Pass to Laredo, totaling 190 river km, 100 km of which were searched. A total of 14 *P. popeii* mussel beds (areas with large rocks on top of bedrock) were found in this 100 km stretch, with no mussels between the beds. At six *P. popeii* mussel beds, we recorded the total area of the bed and conducted quadrat (up to 15 0.25 m^2 quadrats) or area searches (3 to 4 area searches per mussel bed, areas from 1 to 12 m^2). The average densities (mussel m^{-2}) and the total area of the bed (m^2) were then used to calculate the total number of mussels in each of these six mussel beds, and the number of mussels per river km at each bed (as the total number of mussels in the bed divided by the length of the bed). The estimated average number of mussels per mussel bed (8,020 ± 1,896) corresponded well with our assessment of the population size of *P. popeii* in La Bota area (Laredo, Webb Co.) (8,700 mussels, Karatayev et al., 2012). In the other eight mussel beds, we used time searches to confirm mussel presence without quantitative assessment of mussel density. For population estimations we treated this part of the Rio Grande as 2 strata: one with mussels (14 mussel beds) that occupied about 3 km of the river, and the remaining strata among the beds where we did not detect any mussels (97 km). However, as the effort at this mussel-poor strata (quadrat- and time searches at 10 sites) was lower than in the Devils River (34 sites), to correct for potential undetection of mussel we assumed that their density in the strata was likely similar to the Devils River density (40.3 ± 19.8 mussels per river km). The mean overall mussel density per river km at this stretch and its standard error was calculated following formulae for stratified random design (Manly, 2009). To estimate the total population size of mussels between Laredo and Eagle Pass, we multiplied this density by 190 km (distance between El Indio Dam below Eagle Pass and Laredo).

In other parts of the Rio Grande, density of *P. popeii* was very low. In 2008 and 2011 two mussels were found in the river below the Big Bend National Park at John’s Marina (south of Dryden, Terrell County, 29.80237˚N, 102.14025˚W). Another single *P. popeii* was found in our survey of 177 km of the river downstream from the Foster Dam (near Langtry, Val Verde County, 29.78011N, 101.75774W) to the upper reaches of the Amistad National Park (29.7714N, 101.40126W). Two more molluscs were found near Del Rio (Val Verde County, 29.32672 N, 100.921934W). Bottom substrates in this stretch of the Rio Grande were similar to those in the Devils River (mainly bedrock with gravel riffles), and both rivers at these locations have comparable median water flows (International Boundary Water Commission stream gauge data). Therefore to calculate the current *P. popeii* density in the stretches of the Rio Grande from the Big Bend National Park to the upper part of the Amistad Reservoir and near Del Rio (total 177 km), we used the densities estimated for the Devils River (40.3 ± 19.8 mussels per river km). No live mussels were found between Del Rio and Eagle Pass.

Downstream of El Paso/Juarez area, flow in the Rio Grande is extremely limited (Dahm et al., 2005), and the river may stay dry for several months of the year before it reaches the confluence with Rio Concho. Considering this, as well as the lack of historical records from this area, we excluded the river stretch from El Paso to Big Bend National Park from the currently occupied habitat. Similarly, we excluded from the current range all areas below Laredo as no live *P. popeii* were found downstream from the Laredo Sewage Plant to the Falcon Reservoir in spite of the abundance of excellent habitat; and only long dead shells were found in areas below the reservoir (T. Miller, unpublished data).

Historical distribution. To reconstruct the historical distribution range of *P. popeii* in Texas we used data from museum collections, web-based searches, and published accounts (Singley, 1893; Cockerell, 1902; Strecker, 1931; Taylor, 1967; Murray, 1975; Metcalf and Stern, 1976; Metcalf, 1974, 1982; Neck, 1984; Neck and Metcalf, 1988; Howells *et al.*, 1996; Johnson, 1999; Howells, 2001, 2000, 1999, 1994; Strenth *et al.*, 2004) along with our records of shells in the Rio Grande and its tributaries. We made the following assumptions: 1) historically *P. popeii* were present in Texas waterbodies where live or dead shells were ever recorded; 2) historically *P.*

popeii were present in the whole river stretch between two of the most distant points where live or dead shells were ever recorded; 3) if the status of a recorded *P. popeii* was not reported in the paper used for historical analysis, we assumed that the specimen was found alive; 4) if the date of collection was not reported in the paper, we assumed that the mussel was recorded one year earlier preceding the publication year (excluding papers where museum collections were analyzed and mussels had collection dates on their labels); 5) the historic *P. popeii* population size in pristine rivers was similar to the current; 6) in disturbed rivers, the historic *P. popeii* population size was similar to the current at stretches that appear to be unaffected (e.g. the Rio Grande above Laredo where *P. popeii* are present in high density in every suitable habitat).

RESULTS

Current distribution

We found that *P. popeii* still exists in the Rio Grande and the Devils River. Live specimens and shells of this species were found at 26% and 43% of the 42 sites sampled, respectively (Figure 2). A total of 1,801 live *P. popeii* were recorded in our surveys. The species was most commonly found in crevices under large flat boulders of limestone conglomerates resting on bedrock, where small sediment deposits provide stable substrates for mussels in these flow refuges, with over 10 (and up to 40) individuals found under one rock.

In the Devils River, we found only 11 *P. popeii* during 12 years of our surveys within the 66 km stretch of the river above the confluence with Amistad reservoir. We estimated the current density of *P. popeii* in this waterbody to be 40.3 ± 19.8 mussels per river km, for a total population size of approximately 2,660 ± 1,307 mussels (Table 1).

The density of *P. popeii* in the 190 km of the Rio Grande between Eagle Pass and Laredo was the highest in the whole range (1,514 ± 516 per river km, Table 1), and the estimated population size on this stretch of the river was 287,660 ± 98,040. Between the Big Bend and Del Rio (177 km, excluding the Amistad Reservoir, in which this species has not been found), we estimated a density of 40.3 mussels per river km (the same as that observed in the Devils River), for a total of 7,133 ± 3,505 *P. popeii* in this river stretch. Only old dead shells were found at seven sites below the Laredo Sewage Plant waste water discharge to the mouth of the river, although *P. popeii* were very abundant just 10 m upstream of the discharge site (at the mouth of Zapata Creek, Las Palmas Park). Thus, overall, the estimated current population size of *P. popeii* in the Rio Grande is approximately 294,793 ± 98,103 mussels (Table 1).

No live *P. popeii* were found in the Pecos River during our study, although long dead shells were extremely abundant in the lower reaches of the river, where live mussels were reported by Metcalf (1982) prior to the area being flooded by the Amistad Reservoir. In addition, one fragment of a *P. popeii* valve was found in 2011 at one of the four surveyed sites on the Pecos River in Pecos County (near Iraan). Our study also didn’t reveal any live mussels or even dead shells of *P. popeii* in the Las Moras Creek.

Historical range and population size

Historically, *P. popeii* in Texas was reported from the Rio Grande and its tributaries, including the Pecos and Devils rivers and Las Moras Creek (reviewed in Karatayev *et al.*, 2012). Although two dead *P. popeii* shells were reported from the South Concho and Llano rivers outside the Rio Grande drainage (Strenth *et al.*, 2004), there is no evidence that these records represent extant populations of *P. popeii.*

In the Rio Grande, based on historical records (Howells, 1994; Howells *et al.*, 1996, 1997; Metcalf, 1982) and our shell findings, *P. popeii* in the past occurred throughout the 1,000 km stretch of the river from the mouth of San Francisco Creek in the Big Bend reach (Brewster

County) to Brownsville, near the Gulf of Mexico (Fig. 1). According to Metcalf and Stern (1976), no living or fossilized unionids were ever reported in the Rio Grande above the mouth of the Rio Conchos. Currently *P. popeii* persists in only two fragments of the Rio Grande: between Big Bend National Park and Del Rio (excluding the Amistad Reservoir), and between Eagle Pass and Laredo - which constitutes only 37% of the species' historical range in the river. Due to the relative similarity of environmental conditions and historical records in the Rio Grande (from the mouth of San Francisco Creek in the Big Bend to the river mouth), we suggest that prior densities of *P. popeii* in the river were similar to those presently found between Laredo and Eagle Pass. By this approximation, the historical population size of *P. popeii* in the Rio Grande was about 1,514,000 mussels, which indicates that the current abundance of this species in the river is only at 20% of its historical levels.

In the Devils River, *P. popeii* were historically found only in the lower reaches (in Val Verde County, Singley, 1893; Neck, 1984), which are currently flooded by the Amistad Reservoir (Fig. 2). As the remaining length of the river is still in pristine conditions, we assume that the historical *P. popeii* density throughout the river was similar to that observed in the present study (40.3 mussels per river km). This is likely an underestimation as in the past mussels were more abundant near the confluence with the Rio Grande. Although the lower reaches of the river are now flooded by the reservoir and no longer support this lotic species, we estimated the historical range of *P. popeii* as the distance between our most upstream record of the species and the mouth of the river (Fig. 1, Table 1). According to these assumptions, the Devils River historically supported a total of 3,869 *P. popeii* (Table 1).

In the Pecos River, *P. popeii* was reported by Metcalf (1982) from the lower reaches later flooded by the Amistad Reservoir. In addition, in the past, mussels were recorded in this river in

New Mexico (Cockerell, 1902; Metcalf, 1982; Lang, 2001), and are still quite abundant in one of its tributaries (the Black River, Lang, 2001, 2010; Inoue *et al.*, 2014). Therefore, we suggest that the entire span of the Pecos River in Texas was previously populated by *P. popeii*, and its historical density could be an average of the species' current, low density in the Devils River and its high density in the Rio Grande between Laredo and Eagle Pass. According to this assumption, the total number of *P. popeii* that the Pecos River in Texas supported historically was 527,583 (Table 1). This figure is likely an underestimation since the current total population of *P. popeii* in a 14-km stretch of the Black River is 48,006 mussels (Inoue *et al.*, 2014).

In the Las Moras Creek, Kinney County, *P. popeii* were first recorded in 1892 (Taylor, 1967), and according to Cockerell (1902), at the end of 18th century mussels were abundant in this creek near Fort Clark. However, extensive, repeated surveys along 48 km of the Las Moras Creek in 1971, 1973, and 1975 found no living *P. popeii* (Murray, 1975). Our study also did not reveal any live mussels or even dead shells of *P. popeii* in the Las Moras Creek. To reconstruct the former density of *P. popeii* in the Las Moras Creek, we used the average value between the current densities in the Devils River and the densities in the Rio Grande between Laredo and Eagle Pass. Based on this assumption, the estimated historical abundance of *P. popeii* in the Las Moras Creek 46,620 mussels (Table 1). This creek is similar to the Black River where *P. popeii* densities were estimated as 3,429 molluscs per river km (total 48,006 molluscs per 14 km, Inoue *et al*., 2014), and thus our estimation of population size of the 60-km Las Moras Creek is likely very conservative.

In total, 76% of *P. popeii* habitat was lost during the last century, and only 14% of the former population remains in the Rio Grande drainage in Texas (Table 1).

DISCUSSION

Since the first description of *Popenaias popeii* by Lea (1857), this species was always considered rare, declining or even endangered (Singley, 1893; Strecker, 1931; Neck, 1984; Stansbery, 1971). Across all published studies of this species in Texas, less than 200 individuals have been recorded, and a quantitative assessment of the species has never been conducted. Our recent discovery of a relatively large population of *P. popeii* in the Rio Grande above Laredo and a small population in the Devils River proved that the species still exists in Texas. Being endemic to the Rio Grande drainage, *P. popeii* was always restricted to a few rivers; over the last century, however, its formerly continuous distribution across this range in Texas has been fragmented and reduced to a few isolated populations.

Using historical data on the distribution of *P. popeii* and records of shells collected during our study, along with the estimation of density of recently discovered populations, we were able to reconstruct the historical distribution range and population size of this species in Texas (Fig. 3). We found that *P. popeii* has been extirpated from two of four rivers, and the total length of the rivers populated by the mussel has declined by 76%. The total *P. popeii* population size has declined by 86%. The only area that still supports a high density of *P. popeii* in Texas is the stretch of the Rio Grande between Laredo and Eagle Pass. Similar dramatic decline of *P. popeii* was reported from the New Mexican part of the Rio Grande drainage where the species currently occupies about 12% of its historic range in a 14 km reach of the Black River in Eddy County (Lang, 2001; Carman, 2007). Although the current status of *P. popeii* in Mexico is unknown (USFWS, 2012), it is likely that the Mexican population experienced similar decline. Therefore, we suggest that during the last century across the whole range, *P. popeii* faced range fragmentation, local extirpation, and almost 90% loss of their population.

Conservation and management

The most efficient means to secure the viability of existing unionid populations is by applying the knowledge of their distribution, biology and ecology towards reducing and preventing threats through regulatory mechanisms and habitat restoration programs.

As a first step, information on distribution and population size is required to define conservation priorities. Our 2001-2013 surveys of *P. popeii* in Texas, in combination with recent surveys of the Black River in New Mexico (Inoue *et al.*, 2014), provided sufficient information on the current population range and size of this species in the USA; and, most importantly, on the historical changes in populations in the last 100 years. The species currently occupies only 12% of its historic range in New Mexico, and 24% in Texas. We also calculated that *P. popeii* in Texas have experienced an 86% decline in population size over the last century. This confirms the evaluation of *P. popeii* by IUCN as critically endangered meaning that the species is facing an extremely high risk of extinction in the wild.

Decline in population size is the first among the five quantitative criteria which are used to determine whether a taxon is threatened (IUCN, 2014). The measure of species' area of occupancy is important for the estimation of species' decline and is usually obtained by counting the number of occupied cells in a uniform grid (commonly used grid size is 2 km, a cell area of 4 km^2) that covers the entire range of a taxon and then tallying the total area of all occupied cells (IUCN, 2014). The authors recognize that there is concern that grids do not have much ecological meaning for taxa living in "linear" habitats such as in rivers or along coastlines (IUCN, 2014). For example, in all rivers where it was found, *P. popeii* (in contrast to, for example, amphibians or insects) does not live in small tributaries. Thus the area cell approach, although useful while working with different species for comparable listing, grossly overestimates the area of occupancy for freshwater mussels. The method we used for calculating the change in the historical species range and population size based on species densities per river length instead of area, made it more appropriate for freshwater molluscs with very restricted "linear" habitat range.

Population fragmentation is another criterion for determining species conservation status (IUCN, 2014). A taxon is considered to be severely fragmented if most (>50%) of its total area of occupancy is in habitat patches that are separated from other habitat patches by a distance larger than the dispersal distance of the taxon (IUCN, 2013). Although laboratory studies described *P. popeii* as a host generalist (24 physiological fish host species identified; Lang, 2001, 2004), Levine et al. (2012) found that in the wild, *P. popeii* uses a much smaller subset of all potential hosts that occur in the Black River, including *Carpiodes carpio*, *Moxostoma congestum*, and *Cyprinella lutrensis* that represented 80% of all individual fish infected and carried over 99% of glochidia. While large-bodied *C. carpio* exhibited the highest prevalence of infestation (60%), *M. congestum* (10% prevalence) represented the most frequently captured large-bodied fish, and the small-bodied *C. lutrensis* was the only species infested consistently and exhibited the highest prevalence (30%) (Levine *et al.*, 2012). In Texas, only 4 species of 15 collected in the Rio Grande in the summer of 2012 (629 fish caught) were found suitable field hosts as in the Black River (*C. lutrensis, Lepomis macrochirus, L. megalotis,* and *M. congestum*) (authors unpublished data), of which only crevice spawners red shiners (*C. lutrensis*) were abundant. All these four potential *P. popeii* fish hosts do not have long-distance dispersal. Their separation distance for suitable habitat is <15 km (NatureServe, 2014), and thus impoundments can fragment their habitat, limiting dispersal and re-colonization (Mattews and Marsh-Mattews, 2007). Since fish hosts are the main vector for *P. popeii* dispersal, the large gaps (200 km) among the populated segments on the Rio Grande and the Devils River due to the Amistad Reservoir is larger than the dispersal distance of the species. Therefore *P. popeii* population on the Rio Grande drainage can be considered as severely fragmented.

Identifying direct threats to mussel habitats is the next step that needs to be considered in conservation planning. Globally, major threats to freshwater biodiversity (including bivalves) include loss, fragmentation and degradation of habitat, overexploitation, pollution, introduction of non-native invasive species, and climate change (Dudgeon *et al.*, 2006; Geist, 2011). The primary threats to the *P. popeii* identified by U. S. Fish and Wildlife Service are habitat alterations such as streambank channelization, impoundments, and diversions for agriculture and flood control; contamination of water by oil and gas activity; alterations in the natural riverine hydrology; and increased sedimentation and flood pulses from prolonged overgrazing and loss of native vegetation (Federal Register 78, 2014).

Water over-extraction is responsible for the extinction of *P. popeii* both in the upper and lower reaches of the river. Irrigated agriculture is the primary use of the Rio Grande surface flow throughout the basin, and it is further exasperated by evaporation from major reservoirs that exceeds the quantity of water used for municipal purposes in the basin (Dahm *et al.*, 2005). Evapotranspiration, groundwater recharge, and human appropriation of Rio Grande water has resulted in less than 1% of basin precipitation reaching the mouth (Dahm *et al.*, 2005). Water diversion from the Rio Grande is so high that the riverbed between El Paso and Presidio/Ojinaga often lies dry (Dahm *et al.*, 2005; Wong *et al.*, 2007; Douglas, 2009); and in several years, the river failed to reach the Gulf of Mexico (Dahm *et al.*, 2005). The flow rate of the Las Moras Springs (headwaters of Las Moras Creek) decreased between 1896 and 1978 by an order of magnitude, and in 1964 and 1971 it dried up completely for a time (Brune, 1975). Similarly, the Pecos River, that was once a critical source of water in the Trans-Pecos region in Texas, has dwindled to a trickle in some areas due to water over-extraction (Gregory and Hatler, 2008).

Impoundments. *P. popeii* is a lotic species and is not found in reservoirs (Metcalf, 1982; Neck and Metcalf, 1988; Karatayev *et al.*, 2012). Construction of the Amistad and Falcon reservoirs turned otherwise available for the mussel habitats in the Rio Grande into unsuitable environment. The severe *P. popeii* population fragmentation we found in Texas was due to existing impoundments, but there are plans proposed for another low-water diversion dam just downstream of the only large and viable Rio Grande population near Laredo. Impoundments also caused extirpation of *P. popeii* from the lower reaches of the Pecos and Devils rivers where it was previously reported (Singley, 1893; Metcalf, 1974, 1982; Neck, 1984). In addition, low water dams that are common in the Rio Grande and its tributaries may have negative impact on *P. popeii* by restricting movement of host fish or smothering of habitat by excessive silt deposition (Lang, 2001).

Water pollution and salinization are among the important additional threats not identified in the US FWS assessment. Pollution is one of the main threats to most freshwater mussel species (Bogan, 1993; Strayer *et al.*, 2004). Fecal coliforms, nutrients, low dissolved oxygen, pesticides, herbicides, metals, and organic contaminants are significant concerns throughout the Rio Grande basin (Dahm, 2005). Untreated or poorly treated discharges from inadequate wastewater treatment facilities primarily in Mexico are the principal source for fecal coliform bacteria contamination. A secondary source is from nonpoint source pollution on both sides of the river, including poorly constructed or malfunctioning septic and sewage collection systems and improperly managed animal wastes. Contact recreation use of the Rio Grande is not supported downstream of the Amistad Reservoir due to elevated levels of fecal coliform bacteria (TPWD, 2005). We found that waste water discharges from Laredo and Nuevo Laredo wastewater treatment facilities completely wiped out populations of *P. popeii* from downtown Laredo all the way downstream to the Falcon Reservoir. Mussels were abundant in the Rio Grande 10 m above discharge, but absent in over 40 km stretch of the river below the discharge in spite of the presence of abundant suitable habitats. Several live individuals of common unionid species were found only 40 km below Laredo, perhaps owing to the Dolores Creek input of fresh water below the Zapata County line, but no live *P. popeii* was found. Similarly, we found extended good *P. popeii* habitat above Eagle Pass near an irrigation canal overgrown by abundant green benthic filamentus algae (likely indicating pollution from agriculture), but not a single live *P. popeii* was found in the area. Agricultural and oil field pollutants, and blooms of toxic algae have reportedly caused deterioration of the water quality in the Pecos River (TPWD, 1974; Linam and Kleinsasser, 1996). According to Murray (1975), *P. popeii* was extirpated from Las Moras Creek due to the removal of aquatic vegetation, the paving of a portion of the spring, and the chlorination in conjunction with the use of the spring headwater as a swimming pool.

Salinization. While streamflow salinization and loss of freshwater taxa in the Pecos and Rio Grande appear to be regional phenomena associated with upstream agricultural developments, water demand for agriculture will increase in response to global warming, suggesting that the threat of salinization will likewise increase (reviewed in Hoagstrom, 2009). Before the 1950s, irrigation from groundwater in the Texas portion of the Permian Basin was relatively minor, but subsequent groundwater overdraft greatly reduced spring inflows and ultimately reversed the flow direction in some reaches, causing conveyance losses via seepage into the aquifer (Hoagstrom, 2009). Other causes of streamflow salinization include reduced flood frequency and magnitude; increased evapotranspiration; and increased prevalence of natural, high-salinity groundwater over freshwater sprig inflows (Hoagstrom, 2003, 2009). Similar to the lower Pecos River, invertebrate and fish faunas of the salinized Rio Grande downstream from the New Mexico-Texas border have relatively low biodiversity and consist only of salt-tolerant forms (Hubbs *et al.*, 1977; Davis, 1980 from Hoagstrom, 2009). High salinity seems to be the major factor limiting *P. popeii* in the Pecos River along with water over-extraction and impoundments (Table 2). According to Lang (2001), this species show behavioral signs of physiological stress followed by death at a salinity of 7.0 ppt, which is similar to salinity in the Pecos River (ranges from 6.0-7.0 ppt) downstream of it confluence with the Black River (Lang, 2001); and the levels of salinity are even higher (up to 12 ppt) in the Texas part of the Pecos River (Hoagstrom, 2009).

The next important step in species protection is to identify the most important drainages and localities having viable populations of the threatened species. Our studies identified that the only highly populated habitat left in the Rio Grande is the 190 km river stretch between Eagle Pass and Laredo, which also supports another rare endemic Rio Grande mussel *Truncilla cognata*. This large *P. popeii* population is viable and reproducing (Karatayev *et al.*, 2012) and, therefore, requires priority protection. Although the part of the Rio Grande in and above Laredo has the status of a mussel sanctuary (where mussels harvesting is prohibited, Texas Register 31, 2006), additional protection is urgently necessary as any activity associated with the water flow alternation could potentially impact the remaining habitat of *P. popeii*. The population above Amistad Reservoir is small, but considering the presence of another Rio Grande endemic species (extremely rare *Potamilus metnecktayi*), this habitat is warranted protection as well. Part of the river segment that begins in the Big Bend National Park in Brewster County and continues to the Terrell and Val Verde County border was designated in 1978 as the National Wild and Scenic

River (National Parks and Recreation Act of 1978, Public Law 95-625, November 10, 1978). This Wild and Scenic Rivers Act prohibits federal support for construction of dams, water conduits, reservoirs, or other instream activities; but neither prohibits development or gives the federal government control over private property, nor does it affect existing water rights. The most important existing threats for this area as well as for the population in the Devils River are droughts and the decrease in the water table due to ground water over-extraction. Thus, declines over 12 m in the water table have been already recorded in this area (both in Hueco Bolsom and Alluvium Aquifers, El Paso and Reeves Counties, TWDB, 2014), and the existing groundwater supplies are expected to decline 30% by 2060 (TWDB, 2012).

Current climate model simulations suggest that the American southwest could experience a 60-year stretch of heat and drought unseen since the 12th century and that the region is likely to become drier and experience more frequent droughts, with changes accelerating toward the end of the century (Woodhouse *et al.*, 2010). Growing demands for water by agricultural, industrial, and recreational activities may be exacerbated by predicted climatic change trends towards an increased inter-annual variability in precipitation and consequent effects in river flows (Millán, *et al.*, 2005; Milly *et al.*, 2005). Extreme climatic events like droughts and floods are predicted to become more frequent and intense in the future (Diez *et al.*, 2012). Freshwater fauna is particularly vulnerable to the effects of climate change because of the limited dispersal abilities of many species (Woodward *et al.*, 2010), and the expected changes may impact freshwater ecosystems more strongly than past anthropogenic alterations (Doll and Zhang, 2010). Particularly affected may be freshwater mussel populations at the edges of current distribution, e.g. in the southern borders, due to the possible minor tolerance to increased temperatures and also to the low dispersal capacity to more favourable habitats located at higher latitudes or altitude. Thus, the southernmost hydrologic regions, including the Texas Gulf, are predicted to experience on average 30–40% reductions in average annual discharge; and mussel extirpations, due to reductions in both discharge and loss of fish hosts, may result in considerably greater mussel extirpation rates with average losses of 15 species and up to 44 species (Spooner *et al.*, 2011).

Therefore, conservation plans should be aimed at promoting river management, including prevention of water over-extraction, pollution, and maintaining flow regime in response to the species needs. Since freshwater mussels are important components of aquatic ecosystems (Vaughn and Hakenkamp, 2001) and some species fulfil criteria of indicator, keystone, flagship and umbrella species, making them ideal targets in aquatic conservation (Geist, 2010), protection of these areas will also guarantee protection of other endemic Rio Grande species.

Although we found a large *P. popeii* population still existing in the Rio Grande drainage, its dramatic decline over the last century warrants immediate species protection. Similar declines in *Margaritifera margaritifera* in Europe led to inclusion of this species in the main European policy that protects wildlife habitats and attracted most of European Community funds devoted to freshwater bivalve conservation (Geist, 2010; Gum *et al*., 2011; Prié, 2013). Despite the existence of large *M. margaritifera* populations in several European countries (over several million), 28 European LIFE projects were implemented to restore freshwater mussel habitats with funding totalling 64 million Euros. The majority of these funds were directed to the conservation of *M. margaritifera* and *U. crassus* illustrating that restoration of freshwater mussel habitats is possible, but that substantial effort is needed to mitigate any negative impact (Lopes-Lima *et al*. in review).

Our study estimated the changes in the current and historical range and population size of Rio Grande endemic *P. popeii*, identified the most important remnant populations and existing threats. However, future studies have to be conducted in order to fill the gaps in species ecology and biology; including understanding the limiting factors in the life cycle of the species, tolerance to pollution, host requirement, and availability. This will enhance our ability to apply effective management measures.

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REFERENCES

Federal Register 78, 2013. Endangered and Threatened Wildlife and Plants; Review of Native Species That are Candidates for Listing as Endangered or Threatened; Annual Notice of Findings on Resubmitted Petitions; Annual Description of Progress on Listing Actions

(Proposed Rules). November 22, 2013: 70128. Available at: http://www.gpo.gov/fdsys/pkg/FR-2013-11-22/pdf/2013-27391.pdf. Accessed: 02/25/14.

Aldridge DC, Fayle TM, Jackson N. 2007. Freshwater mussel abundance predicts biodiversity in UK lowland rivers. *Aquatic Conservation: Marine and Freshwater Ecosystems* **17**: 554-564.

Bogan AE. 1993. Freshwater bivalve extinctions (Molusca: Unionidae): a search for causes. *American Zoologist* **33**: 599-609.

Brune G. 1975. Major and Historical Springs of Texas, *Technical Report* **189**. Texas Water Development Board, Austin.

Burlakova LE, Karatayev AY, Karatayev VA, May ME, Bennett DL, Cook MJ. 2011a. Biogeography and conservation of freshwater mussels (Bivalvia: Unionidae) in Texas: patterns of diversity and threats. *Diversity and Distributions* **17**: 393-407.

Burlakova LE, Karatayev AY, Karatayev VA, May ME, Bennett DL, Cook MJ. 2011b. Endemic species: contribution to community uniqueness, effect of habitat alteration, and conservation priorities. *Biological Conservation* **144**: 155-165.

Campbell L. 1959. Basic survey and inventory of species present in the Pecos River of Texas. Dingell-Johnson Project F-5-R-5, Job 13. Texas Game and Fish Commission, Austin, Texas.

Carman SM. 2007. Texas hornshell *Popenaias popeii* recovery plan. New Mexico Department of Game and Fish, Santa Fe, NM.

Cockerell TDA. 1902. *Unio popei*, Lea, in New Mexico. *Nautilus* **16**: 69-70.

Contreras-Balderas S, Edwards RJ, Lozano-Vilano MD, Garcia-Ramirez ME. 2002. Fish biodiversity changes in the Lower Rio Grande/Rio Bravo, 1953-1996 - A review. *Reviews in Fish Biology and Fisheries* **12**: 219-240.

Dahm CN, Edwards RJ, Gelwick FP. 2005. Gulf Coast rivers of the Southewestern United States. In *Rivers of North America*, Arthur CB, Colbert EC (eds). Academic Press: Burlington; 180-228.

Davis JR. 1980. Species composition and diversity of benthic macroinvertebrate populations of the Pecos River, Texas. *Southwestern Naturalist* **25**: 241-56.

Davis JR. 1987. Faunal characteristics of a saline stream in the Northern Chihuahuan Desert. *Contributed Papers of the Second Symposium on Resources of the Chihuahuan Desert Region: United States and Mexico*. Chihuahuan Desert Research Institute, Alpine, Texas.

Diez JM, D'Antonio CM, Dukes JS, Grosholz ED, Olden JD, Sorte CJB, Blumenthal DM, Bradley BA, Early R, Ibáñez I, Jones SJ, Lawler JL, Miller LP. 2012. Will extreme climatic events facilitate biological invasions? *Frontiers in Ecology and the Environment* **10**: 249-257.

Doll P, Zhang J. 2010. Impact of climate change on freshwater ecosystems: a global-scale analysis of ecologically relevant river flow alterations. *Hydrology and Earth System Sciences* **14**: 783-799.

Douglas AJ. 2009. Social, Political, and Institutional Setting: Water Management Problems of the Rio Grande. *Journal of Water Resources Planning and Management* **135**: 493-501.

Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Leveque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny MLJ, Sullivan CA. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* **81**: 163-182.

Edwards RJ, Contreras-Balderas S. 1991. Historical changes in the ichthyofauna of the Lower Rio Grande (Río Bravo del Norte), Texas and Mexico. *The Southwestern Naturalist* **36**: 201-212.

Geist J. 2010. Strategies for the conservation of endangered freshwater pearl mussels (*Margaritifera margaritifera* L.): a synthesis of conservation genetics and ecology. *Hydrobiologia* **644**: 69-88.

Geist J. 2011. Integrative freshwater ecology and biodiversity conservation. *Ecological Indicators* **11**: 1507-1516.

Gregory L, Hatler W. 2008. A Watershed Protection Plan for the Pecos River in Texas. Texas State Soil and Water Conservation Board, U.S. Environmental Protection Agency. Available at http://pecosbasin.tamu.edu/media/1923/pecosriverwpp.pdf. Accessed January 15, 2014.

Gum B, Lange M, Geist J. 2011. A critical reflection on the success of rearing and culturing juvenile freshwater mussels with a focus on the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). *Aquatic Conservation: Marine and Freshwater Ecosystems* **21**: 743-751.

Haenn WF. 2002. Fort Clark and Brackettville: Land of Heroes. Arcadia Publishing Chicago IL. ISBN 978-0-7385-2063-6.

Hoagstrom CW. 2003. Historical and recent fish fauna of the lower Pecos River. Aquatic fauna of the northern Chihuahuan desert. *Special publications of Museum of Texas Tech University* **46**: 91-110.

Hoagstrom CW. 2009. Causes and impacts of salinization in the lower Pecos River. *Great Plains Research* **19**: 27-44.

Howells RG. 1994. Preliminary distributional surveys of freshwater bivalves in Texas: progress report for 1992. *Management Data Series* **105**. Texas Parks and Wildlife Department: Austin, TX.

Howells RG. 1999. Distributional surveys of freshwater bivalves in Texas: progress report for 1998. *Management Data Series* **161**. Texas Parks and Wildlife Department: Austin, TX.

Howells RG. 2000. Distributional surveys of freshwater bivalves in Texas: progress report for 1999. *Management Data Series* **170**. Texas Parks and Wildlife Department: Austin, TX.

Howells RG. 2001. Status of freshwater mussels of the Rio Grande, with some comments on other bivalves. Texas Parks and Wildlife Department, Austin, TX.

Howells RG, Neck RW, Murray HD. 1996. *Freshwater Mussels of Texas*. Texas Parks and Wildlife Press: Austin, TX.

Howells RG, Mather CM, Bergmann JAM. 1997. Conservation status of selected freshwater mussels in Texas. In *Conservation and Managenment of Freshwater Mussels II (Initiatives for future): Proceedings of a UMRCC Symposium*, Cummings KS, Buchanan AC, Mayer CA, Naimo TJ (eds). Upper Mississippi River Conservation Committee: Rock Island, IL; 117-129.

Hubbs CR, Miller R, Edwards RJ, Thompson KW, Marsh E, Garrett GP, Powell GL, Morris DJ, Zerr RW. 1977. Fishes inhabiting the Rio Grande, Texas and Mexico, between El Paso and the Pecos confluence, In *Symposium on the importance, preservation and management of the riparian habitat*. July 9, 1977, Tucson, Arizona. pp. 91-97.

Inoue K, Levine TD, Lang BK, Berg DJ. 2014. Long-term mark-and-recapture study of a freshwater mussel reveals patterns of habitat use and an association between survival and river discharge. *Freshwater Biology* doi:10.1111/fwb.12389.

International Boundary Water Commission stream gauge data. http://www.ibwc.gov/Water_Data/histflo1.htm. Accessed March 14, 2014.

IUCN. 2013. The International Union for Concervation of Nature Red List of Threatened Species 2013.1, http://www.iucnredlist.org/documents/summarystatistics/2013_1_RL_Stats_Table_3a.pdf

IUCN. 2014. The International Union for Concervation of Nature Standards and Petitions Subcommittee. Guidelines for Using the IUCN Red List Categories and Criteria. Version 11. Prepared by the Standards and Petitions Subcommittee. http://www.iucnredlist.org/documents/RedListGuidelines.pdf. Accessed on March 19, 2014.

Jackson RB, Carpenter SR, Dahm CN, McKnight DM, Naiman RJ, Postel SL, Running SW. 2001. Water in a changing world. *Ecological Applications* **11**: 1027-1045.

Johnson RI. 1999. The Unionidae of the Rio Grande (Rio Bravo del Norte) system of Texas and Mexico. *Occasional Papers on Mollusks* **6**: 1-65.

Johnson PD, Bogan AE, Brown KM, Burkhead NM, Cordeiro JR, Garner JT, Hartfield PD, Lepitzki DAW, Mackie GL, Pip E, Tarpley TA, Tiemann JS, Whelan NV, Strong EE. 2013. Conservation Status of Freshwater Gastropods of Canada and the United States. *Fisheries* **38**: 247-282.

Karatayev AY, Miller TD, Burlakova LE. 2012. Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 rivers at risk. *Aquatic Conservation: Marine and Freshwater Ecosystems* **22**: 206-219.

Kummu M, de Moel H, Ward PJ, Varis O. 2011. How close do we live to water? A global analysis of population distance to freshwater bodies. *PLoS ONE* **6**: e20578.

Lang BK. 2001. Status of the Texas Hornshell and Native Freshwater Mussels (Unionoidea) in the Rio Grande and Pecos River of New Mexico and Texas. Completion Report (E-38) to the

Division of Federal Aid, U.S. Fish and Wildlife Service, Region 2. New Mexico Department of Game and Fish, Albuquerque, NM.

Lang BK. 2004. Artificial culture and captive rearing of the Texas hornshell. New Mexico Department of Game and Fish, Completion Report, E-51, submitted to the Division of Federal Aid, U.S. Fish and Wildlife Service, Albuquerque, New Mexico.

Lang BK. 2010. Texas hornshell, *Popenaias popeii* (Bivalvia: Unionidae), population monitoring. New Mexico Department of Game and Fish, Albuquerque, NM.

Lea I. 1857. Description of six new species of fresh water and land shells of Texas and Tamaulipas, from the collection of the Smithsonian Institution. *Proceedings of the Academy of Natural Sciences of Philadelphia* **9**: 101-102.

Levine TD, Lang BK, Berg DJ. 2012. Physiological and ecological hosts of *Popenaias popeii* (Bivalvia: Unionidae): laboratory studies identify more hosts than field studies. *Freshwater Biology* **57**: 1854-1864.

Linam, GW, Kleinsasser LJ. 1996. Relation between fishes and water quality in the Pecos River, Texas. Resource Protection Division, *Texas Parks and Wildlife Department. River Studies Report* **9**: Austin, TX.

Lopes-Lima M, Sousa R, Geist J, Aldridge DC, Araujo R, Bergengren J, Bespalaja Y, Bodis E, Burlakova L, Van Damme D, Douda K, Froufe E, Georgiev D, Gumpinger C, Karatayev A, Kebapci U, Killeen I, Lajtner J, Larsen BM, Lauceri R, Legakis A, Lois S, Lundberg S, Moorkens E, Motte G, Nagel K-O, Ondina P, Outeiro A, Paunovic M, Prie V, von Proschwitz T, Riccardi N, Rudzīte M, Rudzītis M, Scheder C, Seddon M, Sereflisan H, Simić V, Sokolova S, Stoeckl K, Taskinen J, Teixeira A, Thielen F, Trichkova T, Varandas S, Vicentini H, Zajac K, Zajac T, Zogaris S. In review. Conservation Status of Freshwater

Mussels in Europe: State of the Art and Future Challenges. Submitted to: *The Quarterly Journal of Biology*.

Manly BFJ. 2009. Statistics for Environmental Science and Management, Second Edition. Chapman & Hall/CRC, Taylor & Francis Group, LLC, Boca Raton, FL, USA. 295 pp.

Mattews WJ, Marsh-Mattews E. 2007. Extirpation of Red Shiner in direct tributaries of Lake Texoma (Oklahoma–Texas): A cautionary case history from a fragmented river–reservoir system. *Transactions of the American Fisheries Society* **136**: 1041-1062.

Metcalf AL. 1974. Fossil and living freshwater mussels (Unionacea) from the Pecos River, New Mexico and Texas. *Bulletin of the American Malacalogical Union* **33**: 47-48.

Metcalf AL. 1982. Fossil unionacean bivalves from three tributaries of the Rio Grande. In *Symposium on Recent Benthological Investigations in Texas and Adjacent States*, Davis JR (ed). Texas Academy of Science: Austin, Texas; 43-59.

Metcalf AL, Stern EM. 1976. Notes on unionacean mollusks of the Rio Grande system, United States and Mexico. *Bulletin of the American Malacological Union* 42-43.

Millán MM, Estrela MJ, Miró J. 2005. Rainfall components: variability and spatial distribution in a Mediterranean Area (Valencia Region). *Journal of Climate* **18**: 2682-2705.

Millennium Ecosystem Assessment. 2005. Ecosystem and Human Well-being: Wetlands and Water Synthesis. World Resource Institute, Washington, DC.

Milly PCD, Dunne KA, Vecchia AV. 2005. Global pattern of trends in streamflow and water availability in a changing climate. *Nature* **438**: 347-350.

Murray HD. 1975. *Melanoides tuberculata* (Müller), Las Moras Creek, Bracketville, Texas. *Bulletin of the American Malacological Union, Inc* **1975**: 43.

NatureServe. 2014. NatureServe Explorer: An online encyclopedia of life [web application] NatureServe, Arlington, Virginia. http://www.natureserve.org/explorer [17 June 2014]

Naiman RJ, Turner, MG. 2000. A future perspective on North America's freshwater ecosystems. *Ecological Applications* **10**: 958-970.

Neck RW. 1984. Restricted and declining nonmarine molluscs of Texas. *Technical Series* **34**. Texas Parks and Wildlife Department: Austin, Texas.

Neck RW, Metcalf AL. 1988. Freshwater bivalves of the Lower Rio Grande, Texas. *The Texas Journal of Science* **40**: 259-268.

Pope J. 1854. Report of Exploration of a Route for the Pacific Railroad, near the Thirty-Second Parallel of North Latitude from the Red River to the Rio Grande. U.S. Government Printing Office, Washington, DC.

Prié V. 2013. Flagships and umbrellas: unbalanced conservation effort towards charismatic species as potential threat to the unknowns. *Tentacle* **21**: 32-34.

Simpson CT. 1914. *A Descriptive Catalogue of the Naiades, or Pearly Fresh-Water Mussels*. B. Walker: Detroit. Michigan.

Singley JA. 1893. Contributions to the Natural History of Texas. Part I. Texas Mollusca. A preliminary list of the land, fresh water, and marine Mollusca of Texas. Department of Agriculture, Insurance, Statistics, and History, Austin, TX.

Spooner DE, Xenopoulos MA, Schneider C, Woolnough DA. 2011. Coextirpation of host-affiliate relationships in rivers: the role of climate change, water withdrawal, and host-specificity. *Global Change Biology* **17**: 1720-1732.

Stansbery DH. 1971. Rare and endangered mollusks in the eastern United States. . In *Rare and endangered mollusks (Naiads) of the United States*, Jorgenson SE, Sharp RW (eds). U.S. Fish and Wildlife Service: Twin Cities, Minnesota; 5-18.

Strayer DL, Claypool S, Sprague SJ. 1997. Assessing unionid populations with quadrats and timed searches. In *Conservation and Managenment of Freshwater Mussels II (Initiatives for future): Proceedings of an Upper Mississippi River Conservation Committee (UMRCC) Symposium.*, Cummings KS, Buchanan AC, Mayer CA, Naimo TJ (eds). Upper Mississippi River Conservation Committee: 163–169.

Strayer DL, Downing JA, Haag WR, King TL, Layzer JB, Newton TJ, Nichols SJ. 2004. Changing perspectives on pearly mussels, North America’s most imperiled animals. *Bioscience* **54**: 429-439.

Strayer DL, Dudgeon D. 2010. Freshwater biodiversity conservation: recent progress and future challenges. *Journal of the North American Benthological Society* **29**: 344-358.

Strecker JK. 1931. The distribution of the naiades or pearly freshwater mussels of Texas. *Baylor University Museum Bulletin* **2**: 69.

Strenth NE, Howells RG, Correa-Sandoval A. 2004. New records of the Texas hornshell *Popenaias popeii* (Bivalvia: Unionidae) from Texas and northern Mexico. *The Texas Journal of Science* **56**: 223-230.

Taylor DW. 1967. Freshwater mollusks collected by the United States and Mexican Boundary surveys. *The Veliger* **10**: 152-158.

TPWD. 1974. An Analysis of Texas Waterways: a Report on the Physical Characteristics of Rivers, Streams and Bayous in Texas. The Texas Agricultural Extension Service, Texas

A&M University System.
http://www.tpwd.state.tx.us/publications/pwdpubs/pwd_rp_t3200_1047/ [16 May 2014].

TPWD. 2005. Texas Parks and Wildlife Department. Texas Wildlife Action Plan (Contreras) 2005-2010. Texas Parks and Wildlife Department, Austin, TX, USA. PWD PL W7000-1187. http://www.tpwd.state.tx.us/publications/pwdpubs/pwd_pl_w7000_1187a/

TWDB. 2012. Texas Water Development Board. Water for Texas 2012. State Water Plan. Chapter 5. Water Supplies. Texas Water Development Board, http://www.twdb.state.tx.us/publications/state_water_plan/2012/05.pdf. Accessed March 19, 2014

TWDB. 2014. Texas Water Development Board. Hydrological Atlases. Hydrological Athlas 2. http://www.twdb.state.tx.us/publications/reports/HydrologicAtlases/doc/Ha_2.pdf. Accessed March 19, 2014.

Texas Register 31. 2006. Natural resources and conservation. Part 2. Texas Parks and Wildlife Department. Chapter 57. Fisheries §57.157, Proposed Rules concerning Mussels and Clams. June 9, 2006:4699-4704. Texas Secretary of State. http://www.sos.state.tx.us/texreg/pdf/backview/0108/0108adop.pdf [15 April 2014]

Texas Register 35. 2010. Threatened and endangered nongame species. Chapter 65. Wildlife Subchapter G. 31 TAC §65.175. Adopted rules. Texas Register 35, January 8, 2010, 249-251. Texas Secretary of State. http://www.sos.state.tx.us/texreg/pdf/backview/0108/0108adop.pdf [15 February 2010]

Vaughn CC. 2010. Biodiversity losses and ecosystem function in freshwaters: emerging conclusions and research directions. *BioScience* **60**: 25-35.

Vaughn CC, Hakenkamp CC. 2001. The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology* **46**: 1431-1446.

Vaughn CC, Taylor CM, Eberhard KJ. 1997. A comparison of the effectiveness of timed searches vs. quadrat sampling in mussel surveys. In *Proceedings of an Upper Mississippi River Conservation Committee (UMRCC) Symposium, 16-18 October 1995.*, Cummings KS, Buchanan AC, Mayer CA, Naim TJ (eds). Upper Mississippi River Conservation Committee: Rock Island, IL; 157-162.

Williams JD, Warren ML, Jr., Cummings KS, Harris JL, Neves RJ. 1993. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* **18(9)**: 6-22.

Wong CM, Williams CE, Pittock J, Collier U, Schelle P. 2007. World’s top 10 rivers at risk. Executive Summary. WWF International, Gland, Switzerland.

Woodhouse CA, Meko DM, MacDonald GM, Stahle DW, Cook ER. 2010. A 1,200-year perspective of 21st century drought in southwestern North America. *Proceedings of the National Academy of Sciences, USA* **107**: 21283-21288.

Woodward G, Perkins DM, Brown LE. 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society B-Biological Sciences* **365**: 2093-2106.

Table 1**.** Current and historical ranges and population size of *Popenaias popeii* in the Rio Grande drainage of Texas.

Waterbody	Current range, km (% from historical)	Historic range, km	Current density, mussels per river km	Historic density, mussels per river km	Current population, mussels (% from historical)	Historic population, mussels
Rio Grande:						
Between the Big Bend and Eagle Pass (excluding Amistad Reservoir)	177	n. c.	40.3 ± 19.8	n. c.	7,133 ± 3,505	n. c.
Between Eagle Pass and Laredo	190	n. c.	1,514 ± 516	n. c.	287,660 ± 98,040	n. c.
The whole Rio Grande (from the San Francisco Creek to Brownsville)	367 (37%)	1000	n. c.	1,514 ± 516	294,793 ± 98,103 (20%)	1,514,000 ± 516,000
Devils River	66 (69%)	96	40.3 ± 19.8	40.3 ± 19.8	2,660 ± 1,307(69%)	3,869 ± 1,901
Pecos River	0 (0%)	679	0	777 ± 516*	0 (0%)	527,583 ± 350,622
Las Moras Creek	0 (0%)	60	0	777 ±	0 (0%)	46,620 ±

				516*		30,960
Total	433 (24%)	1835	n. c.	n. c.	297,453 ± 98,111(14%)	2,092,072 ± 624,623

*Density calculated as an average between high density in the Rio Grande and low density in the Devils River

FIGURE LEGENDS

Figure 1. Map of the Rio Grande River basin in Texas with 42 pooled sampling sites surveyed during 2001 - 2013. Texas counties, major cities, reservoirs and rivers are indicated.

Figure 2**.** Map of the Rio Grande River basin in Texas with sites where live and/or dead shells of *Popenaias popeii* were found prior to 2000 (based on data from: Singley, 1893; Cockerell, 1902; Strecker, 1931; Taylor, 1967; Metcalf, 1974, 1982; Murray, 1975; Metcalf and Stern, 1976; Neck, 1984; Neck and Metcalf, 1988; Howells, 1994, 2001; Howells *et al.*, 1996, 1997; Johnson, 1999;) and from 2001 to 2013 (authors data).

Figure 3. Map of the Rio Grande River basin in Texas with historical and current range of *Popenaias popeii* in Texas.

Figure 1

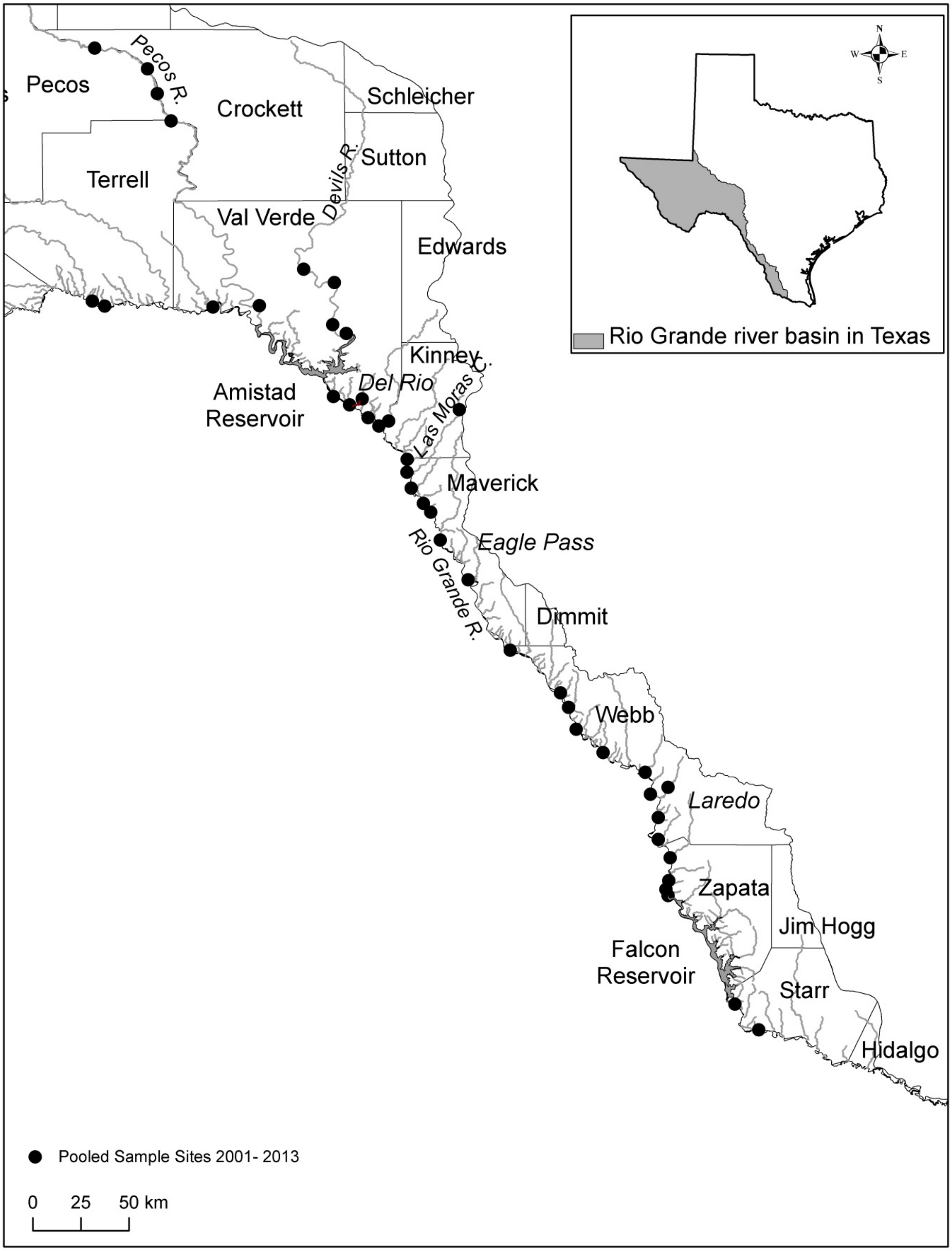

Pecos
Pecos R.
Crockett
Schleicher
Terrell
Sutton
Devils R.
Val Verde
Edwards
Kinney
Amistad Reservoir
Del Rio
Las Moras C.
Maverick
Eagle Pass
Rio Grande R.
Dimmit
Webb
Laredo
Zapata
Jim Hogg
Falcon Reservoir
Starr
Hidalgo
N
W
E
S
Rio Grande river basin in Texas
● Pooled Sample Sites 2001- 2013
0
25
50 km

Figure 2

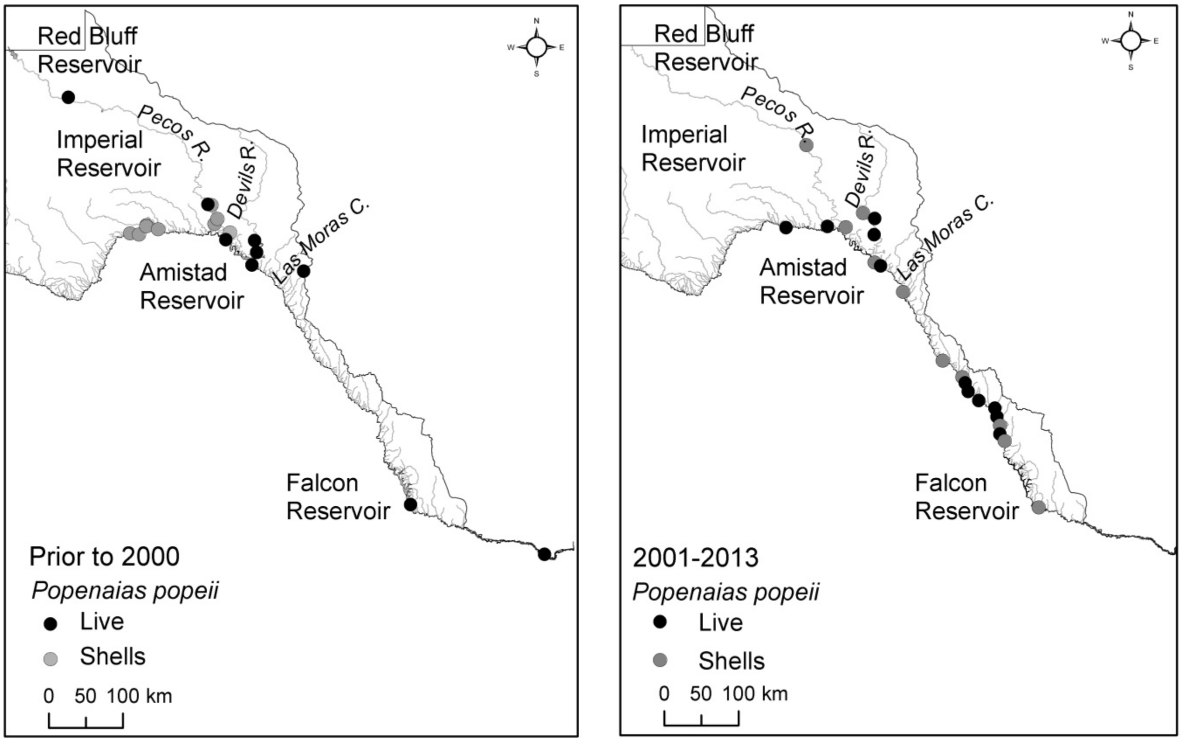
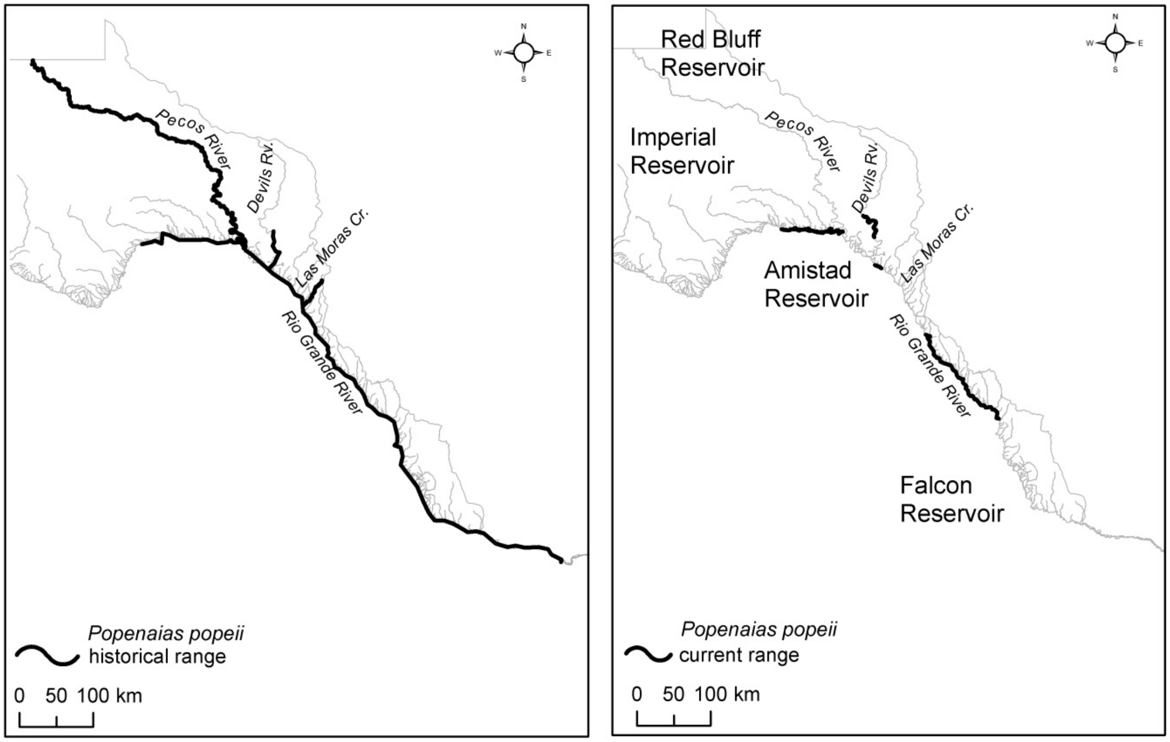

Red Bluff Reservoir
Imperial Reservoir
Pecos R.
Devils R.
Las Moras C.
Amistad Reservoir
Falcon Reservoir
Prior to 2000
Popenaias popeii
● Live
● Shells
0 50 100 km
N
S
E
W
Red Bluff Reservoir
Imperial Reservoir
Pecos R.
Devils R.
Las Moras C.
Amistad Reservoir
Falcon Reservoir
2001-2013
Popenaias popeii
● Live
● Shells
0 50 100 km
N
S
E
W

Figure 3


N
W
E
S
Pecos River
Devils Rv.
Las Moras Cr.
Rio Grande River
Popenaias popeii
historical range
0 50 100 km
Red Bluff Reservoir
Imperial Reservoir
Amistad Reservoir
Falcon Reservoir
Popenaias popeii
current range
0 50 100 km

Appendix 2. Manuscript by Karatayev, Miller, and Burlakova "Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 rivers at risk" published in *Aquatic Conservation: Marine and Freshwater Ecosystems*, 2012.

AQUATIC CONSERVATION: MARINE AND FRESHWATER ECOSYSTEMS
Aquatic Conserv: Mar. Freshw. Ecosyst. **22**: 206–219 (2012)

Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 rivers at risk

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ABSTRACT

1. According to the World Wildlife Fund, the Rio Grande is the most endangered river system in the North American continent and one of the World's top 10 rivers at risk, but is globally important for freshwater biodiversity. Unionid bivalves of the Rio Grande river basin used to be represented by a unique assemblage, including four endemic species (*Truncilla cognata, Potamilis metnecktayi*, *Popenaias popeii*, and *Quadrula couchiana*); however, surveys from 1998–2001 failed to recover any live endemic unionid species suggesting a sharp decrease in their populations and potential of extinction.

2. Intensive surveys (162 sites sampled) conducted by the authors from 2001–2011 on the Rio Grande and its tributaries in Texas recovered live *T. cognata, P. metnecktayi*, and the largest population of *P. popeii* ever reported. Overall the unionid assemblage of the Rio Grande basin has changed considerably during the last century.

3. Decline in species diversity, range fragmentation, local extirpations, and introduction of widespread common species were documented. Two species (*Q. couchiana* and *Quincuncina mitchelli*) are locally extinct. *Potamilus metnecktayi* and *T. cognata* have been extirpated from the Pecos River and their ranges in the Rio Grande have been reduced. *Popenaias popeii* has been extirpated from the Pecos River and Las Moras Creek along with the reduction and fragmentation of its range in the Devils River and Rio Grande.

4. Among the environmental factors responsible for the degradation of unionid assemblages in the Rio Grande river basin, the most important are impoundments, habitat degradation, salinization, pollution, and over-extraction of water.

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KEY WORDS: river; biodiversity; distribution; rare species; invertebrates; impoundments agriculture; salinity; Unionidae; threats

INTRODUCTION

A continuing dramatic increase in pollution, habitat destruction and introduction of invasive species is resulting in simplification and homogenization of ecosystems and a loss of biodiversity worldwide (Mckinney and Lockwood, 1999). Biodiversity loss is especially large in fresh waters, where many species are far more imperilled than their marine or terrestrial counterparts (Jackson *et al*., 2001; Strayer and Dudgeon, 2010). This loss of diversity results from widespread habitat degradation, pollution, flow regulation, and water extraction, and these activities are predicted to increase in the

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future (Naiman and Turner, 2000; Jackson *et al.*, 2001; Strayer and Dudgeon, 2010). The opportunity to conserve much of the remaining biodiversity in fresh waters may vanish if trends in human demands for fresh water remain unaltered and species losses continue at present rates (Dudgeon *et al.*, 2006).

The Rio Grande is a globally important river for freshwater biodiversity, supporting numerous endemic fishes, birds, and molluscs (Groombridge and Jenkins, 1998; Revenga *et al.*, 1998, 2000; Johnson, 1999); however, because of the level of impacts affecting the Rio Grande at present (Dahm *et al.*, 2005), many of these species are now extinct and others are facing a sharp decrease in their population density or fragmentation in their range. Focusing analysis on river basins with high ecological importance and those with large human populations, the World Wildlife Fund recognized the Rio Grande River as the most endangered river in the North American continent, and one of the world's top 10 rivers at risk (Wong *et al.*, 2007).

The Rio Grande/Rio Bravo River (length: 2830 km, river basin area: 870 000 km^2) is one of the longest in North America, flowing from its headwaters in Colorado through New Mexico and then forming the shared border between Texas and Mexico before it empties into the Gulf of Mexico near Brownsville, Texas (Dahm *et al.*, 2005). It traverses seven physiographic provinces with a variety of habitats, but most of the basin is arid or semiarid with either desert shrubland or desert grassland (Dahm *et al.*, 2005). The Rio Conchos, the Pecos River and the Devils River historically contributed the main flow of the Rio Grande in the stretch between their confluences and Amistad Reservoir, although these flows have been reduced substantially and are stored at Amistad International Reservoir. Amistad Dam (completed in 1969) and Falcon Dam (completed in 1953) impound the Rio Grande along the border for irrigation and flood control. Evaporation from major reservoirs has been estimated to exceed the quantity of water used for municipal purposes in the basin, which constitute up to 5% of the agricultural consumption. From Laredo to the mouth of the Rio Grande, the river constitutes the primary source of drinking water for communities in both Mexico and the USA (Dahm *et al.*, 2005). Over 10 million people live in the Rio Grande basin, and urban areas are growing fast, particularly in border towns between the USA and Mexico. By 2060 the area from Eagle Pass to Brownsville is projected to almost triple in population (Texas Water Development Board, 2007). Irrigated agriculture is the primary use of the Rio Grande surface flow throughout the basin and accounts for more than 80% of all water taken from the river (Dahm *et al.*, 2005). The river bed between El Paso and Presidio frequently is dry, owing to water over-extraction for irrigation and domestic consumption, and since 2001 the river often fails to reach the Gulf of Mexico (Edwards and Contreras-Balderas, 1991; Contreras-Balderas *et al.*, 2002; Dahm *et al.*, 2005; Wong *et al.*, 2007; Douglas, 2009). Many other factors have contributed to the recent status of the Rio Grande, including persistent drought, increase in border populations, and subsequent declines in water quantity and quality (Dahm *et al.*, 2005; Wong *et al.*, 2007; Douglas, 2009).

Freshwater bivalves in the order Unionoida are considered to be one of the most endangered groups of animals in North America (Bogan, 1993; Lydeard *et al.*, 2004) with over 76% of the North American Unionidae and Margaritiferidae presumed extinct, threatened, endangered, or deemed of special concern (Williams *et al.*, 1993). Unionid bivalves of the Rio Grande river basin represent a unique assemblage and are distinct from the rest of Texas (Neck, 1982; Neck and Metcalf, 1988; Burlakova *et al.*, 2011a, b). The first data on unionid bivalves of the Rio Grande and its tributaries were published at the turn of the 19th century (Singley, 1893; Simpson, 1900, 1914). In the second half of the 20th century, numerous studies conducted on the Rio Grande system were summarized by Johnson (1999), who provided a detailed description of historical records and current distribution of all 15 species of unionids reported from this system. Extensive surveys done by Texas Parks and Wildlife Department in 1998–2001 failed to recover any live endemic unionid species from the Rio Grande, and Howells (2001) suggested that a sharp decrease in their populations may have put them on the edge of extinction. However, subsequent intensive surveys done by the authors in 2001–2011 recovered live *Truncilla cognata, Potamilis metnecktayi*, and the largest population of *Popenaias popeii* ever reported, proving that at least three endemic unionid species are still present in the river. The goals of this paper are: (1) to analyse the changes in the unionid assemblage of the Rio Grande river basin over 100 years; (2) to study the current distribution of the endemic species and estimate, whenever possible, their population densities; (3) to discuss major factors affecting unionid diversity and distribution in the Rio Grande.

METHODS

Data collection

To assess the current distribution of unionids, mussels were surveyed at 162 sample sites (subsites) pooled into 28 larger sites within the Rio Grande system during 2001–2011 (Figure 1). Fifteen of these sites were sampled once, while 13 sites were sampled from 2–25 times. Survey sites were often selected within state parks, near public boat ramps, or based on accessibility from roads that either crossed or approached a water body owing to the prevalence of private land in Texas, where only 2% of the lands remain in public ownership (Texas Parks and Wildlife Department, 1974). In addition, numerous sites were reached by canoe or kayak. When site surveys were conducted from private land, a Landowner Permission for wildlife research was acquired from each property owner before entering the property. The work was carried out with an appropriate Scientific Research Permit issued by the Texas Parks and Wildlife Department.

Sampling was completed by hand collection of both live and dead mussels, by wading in low water and by snorkelling or diving. Reconnaissance sampling (timed searches) and random searches were used at most sites to reveal the presence of mussels and species diversity (Strayer *et al.*, 1997; Vaughn *et al.*, 1997) and to compare with historical data. If significant mussel assemblages were present, quantitative methods (randomly placed 0.25 m^2 quadrats, mark-and-recapture surveys, or area searches) were used for assessments of density (Dunn, 2000; Strayer and Smith, 2003). Collected live mussels and shells were taxonomically identified, counted, and measured with calipers to the nearest millimetre. After measurements live mussels were carefully bedded into the sediment from which they were taken. Shell condition of dead mussels was recorded for each specimen.

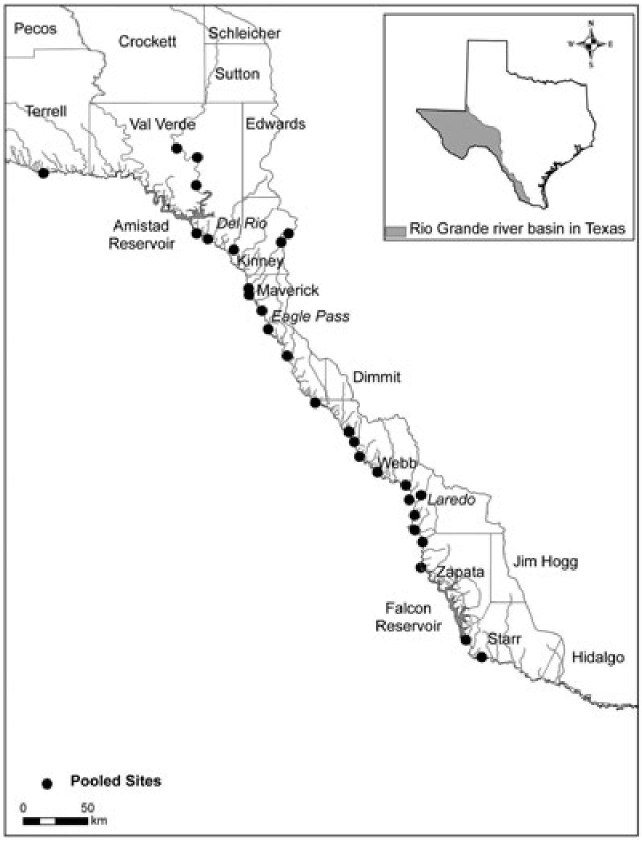

Figure 1. Map of the Rio Grande river basin in Texas with 28 pooled sampling sites surveyed during 2001–2011. Texas counties, major cities (in italics) and reservoirs are indicated.

A mark-and-recapture census was conducted at the La Bota Ranch site in Northern Laredo (Webb County) in March 2011 using methods described by Lang (2001) and Villella *et al.* (2004). Following recommendations by Villella *et al.* (2004), three consecutive days were sampled to estimate capture probabilities using closed population models. All mussels present (new captures, and recaptures) were measured (shell length, width, height ($\pm$ 0.1 mm)), and wet-weighed. First-time captured individuals were marked with unique numbers assigned by embedding oval (4×10 mm) Floy laminated flex tags in Super Glue Gel along the valve hinge posterior to the umbo, to one valve.

Specimens were identified using published taxonomic keys and descriptions (Howells *et al.*, 1996; Johnson, 1998). Voucher specimens were deposited into the Great Lakes Center Invertebrate Collection at Buffalo State College, Buffalo, NY. Each specimen was labelled with a unique number and catalogued in a database with the following information: specimen number, species name, name of person who collected and identified the specimen, date of collection, and detailed site information.

Data analysis

To estimate population density at the mark–recapture site the Schnabel method, an extension of the Petersen method to analyse a series of samples, was used (Krebs, 1999). To evaluate the total size of the *P. popeii* population the average density in the mark–recapture site and the estimation of available habitat area at the La Bota site near Laredo were used.

Differences in community structure were assessed with nonparametric multivariate statistical techniques on data matrices of all live species and their relative densities (as catch-per-unit of effort data, i.e. the number of live mussels for each species found per time search effort at each sampling site (mussels per person per hour). A square root transformation was used to normalize relative densities for the analysis. Similarity of the community composition was summarized by calculating Bray–Curtis distances – a measure of similarity with values ranging from 0 (identical samples) to 1, which is not influenced by rare species as other indices (Bray and Curtis, 1957; Clarke, 1993). To visualize the differences among assemblages, a non-metric multidimensional scaling (NMDS) was used, which calculates a set of metric coordinates for samples, most closely approximating their non-metric distances. Differences among communities were assessed by analysis of similarities (ANOSIM), a resampling technique that uses permutation/randomization methods on Bray–Curtis similarity matrices to identify differences among groups of samples (Clarke, 1993). These analyses were performed using PRIMER 6 software (Plymouth Routines in Multivariate Ecological Research, Version 6.1.6, Primer E-Ltd. 2006). All tests effects were considered significant if $P < 0.05$.

To analyse the historical data a database containing information of unionid species name, water body name, location, recorded date, and the collector's name was created using all available data including published records, museum collections, and web-based searches. Unionid assemblages in the Rio Grande system were analysed using the following time periods: (1) initial reports (before 1931), including collections made by the United States and Mexico Boundary Surveys mostly conducted in 1892 (Taylor, 1967), and data from Singley (1893), Ellis *et al*. (1930), and Strecker (1931); (2) 1968–1990 based mostly on data from Metcalf and Neck (Metcalf, 1974, 1982; Murray, 1975; Neck, 1984; 1987; Neck and Howells, 1984; Neck and Metcalf, 1988); (3) 1992–1999 based on Howells' data (Howells 1994, 1996a, b, 1997a, 1998, 1999, 2000); and (4) 2001–2011 based on the authors' data. Several assumptions were made in the analysis. If the status of a recorded unionid was not reported in the paper used for historical analysis, it was assumed that the specimen was found alive; if the date of collection was not reported in the paper, it was assumed that the mussel was recorded one year earlier preceding the publication year (excluding papers where museum collections were analysed).

RESULTS

Unionid diversity

This study showed that the Rio Grande still supports most of the unionid species previously reported from this river, including the regional endemics *Potamilus metnecktayi, Popenaias popeii*, and *Truncilla cognata* (Table 1). During the current study the most common unionid species were *Cyrtonaias tampicoensis* and *Quadrula*

Table 1. Historical and current records of live unionids (L) and their dead shells (D) from the Rio Grande drainage (excluding the Rio Grande River itself, RGD) and the Rio Grande River (including Falcon and Amistad reservoirs, RG) in Texas. n. r. - not recorded. Total number of species found dead is in parentheses

	Before 1931		1968–1990		1992–1999		2001–2011	
Species	RGD	RG	RGD	RG	RGD	RG	RGD	RG
Cyrtonaias tampicoensis	L	n. r.	L	L	L	L	L	L
Lampsilis teres	L	L	L	L	D	D	n. r.	L
Megalonaias nervosa	L	n. r.	n. r.	D	n. r.	D	n. r.	L
Potamilus metnecktayi[a]	n. r.	n. r.	L	L	n. r.	D	n. r.	L
Popenaias popeii[a]	L	n. r.	L	L	D	D	L	L
Potamilus purpuratus[b]	n. r.	n. r.	n. r.	n. r.	n. r.	L	n. r.	n. r.
Pyganodon grandis[b]	L	n. r.	L	n. r.	n. r.	n. r.	L	n. r.
Quadrula apiculata[b]	n. r.	n. r.	L	L	L	L	L	L
Quadrula couchiana[a]	L	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
Quincuncina mitchelli[a]*	D	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.	n. r.
Toxolasma parvus	L	n. r.	n. r.	n. r.	L	L	n. r.	n. r.
Toxolasma texasensis	L	n. r.	L	n. r.	n. r.	n. r.	L	n. r.
Truncilla cognata[a]	n. r.	n. r.	L	L	n. r.	n. r.	n. r.	L
Uniomerus sp.	n. r.	n. r.	L	n. r.	n. r.	n. r.	n. r.	n. r.
Utterbackia imbecillis	L	n. r.	L	L	L	L	L	L
Total	9 (1)	1	10	7 (1)	4 (2)	5 (4)	6	8

[a] Regional endemics
[b] Introduced species
*Only fossil and greatly weathered specimens are known from Texas part of Rio Grande drainage.

apiculata, found alive at 28.6% of sites sampled (Table 2). The percentage of sites where live molluscs were found compared with the total number of sites where live and dead specimens were found was the greatest for *Q. apiculata* (73%), *Megalonaias nervosa* and *P. popeii* (58% each), and the lowest for *T. cognata* (17%) and *P. metnecktayi* (13%). The rarest species was *P. metnecktayi*, which was found alive at only one location. Other rarely recorded species were *Utterbackia imbecillis* and *Toxolasma texasensis*, which were found mostly in tributaries (Table 2). The highest diversity of unionids was found in a 24 km stretch of the Rio Grande above Laredo (Figure 2(C), 3(B), 4(D)). No live mussels were found below Amistad Reservoir and few below Laredo. Two distinct unionid assemblages depending on the substrate type were found in the Rio Grande above Laredo (Figure 5, R = 0.942, *P* = 0.001, one-way ANOSIM). On soft and unconsolidated sediments (silt, sand, small gravel, and combinations of these) unionid assemblages were dominated by *Q. apiculata*, and *C. tampicoensis*; additional species were *M. nervosa* and *T. cognata*. On bedrock and boulders the dominant species was *P. popeii*. This species was most commonly found in crevices under flat boulders resting on the bedrock. Often up to 10 individuals were found under one rock. Additional unionids found in this habitat included *Lampsilis teres, Q. apiculata*, and *T. cognata*.

Endemic species account

Potamilus metnecktayi

Nineteen live *P. metnecktayi* were found in the Rio Grande at the John's Marina site, south of Dryden, Terrell County in 2003–2008 (Figure 2(C)). Mussels were generally found along the shores, in soft sediments (in a mixture of silt and clay) at 0.5–1.2 m depth (at low flows ~30 m^3 s^{-1}). Their size varied from 63 to 124 mm, averaging 87.1 mm (±17.6 standard deviation). Dead shells of *P. metnecktayi* were found at seven more sites. *P. metnecktayi* had the lowest percentage of sites where live mussels were found, from the total number of sites where shells of the species were recorded (13%). At 15 sites below Lake Amistad, only 50 long-dead or sub-fossil valves

Table 2. Occurrence of unionid species in the Rio Grande river drainage, and separately in the river main stem and its tributaries based on 2001–2011 surveys. In total, 28 pooled sites were studied in the Texas part of the drainage, including 21 sites in the Rio Grande River (excluding reservoirs) and seven sites on tributaries. Species occurrence was calculated as a number of sites where the species was found, and percentage occurrence was calculated as the percentage of sites where the species was found. Single valves were counted as half of a shell

	Rio Grande drainage			Rio Grande River			Tributaries only		
Species	Total found	Occurrence (number of pooled sites)	Percentage occurrence	Total	Occurrence (number of pooled sites)	Percentage occurrence	Total	Occurrence (number of pooled sites)	Percentage occurrence
Live mussels									
Cyrtonaias tampicoensis	89	8	28.6	29	7	33.3	60	1	14.3
Lampsilis teres	17	2	7.1	17	2	9.5	0	0	0
Megalonaias nervosa	34	7	25.0	34	7	33.3	0	0	0
Popenaias popeii	656	7	25.0	649	5	23.8	7	2	28.6
Potamilus metnecktayi	19	1	3.6	19	1	4.8	0	0	0
Quadrula apiculata	204	8	28.6	129	7	33.3	75	1	14.3
Toxolasma texasensis	11	1	3.6	0	0	0	11	1	14.3
Truncilla cognata	19	2	7.1	19	2	9.5	0	0	0
Utterbackia imbecillis	7	1	3.6	0	0	0	7	1	14.3
Total live mussels	**1056**	**14**	**50.0**	**896**	**11**	**52.4**	**160**	**3**	**42.9**
Shells									
Cyrtonaias tampicoensis	789	20	71.4	788	19	90.5	1	1	14.3
Lampsilis teres	84.5	9	32.1	84.5	9	42.9	0	0	0
Megalonaias nervosa	180.5	12	42.9	180.5	12	57.1	0	0	0
Popenaias popeii	473.5	12	42.9	465	11	52.4	8.5	1	14.3
Potamilus metnecktayi	159.5	8	28.6	159.5	8	38.1	0	0	0
Quadrula apiculata	533.5	11	39.3	533	10	47.6	0.5	1	14.3
Toxolasma texasensis	1	1	3.6	0	0	0	1	1	14.3
Truncilla cognata	291	12	42.9	291	12	57.1	0	0	0
Utterbackia imbecillis	57	10	35.7	17	**7**	33.3	40	3	42.9
Total shells	**2569.5**	**21**	**75.0**	**2518.5**	**19**	**90.5**	**51**	**3**	**42.9**

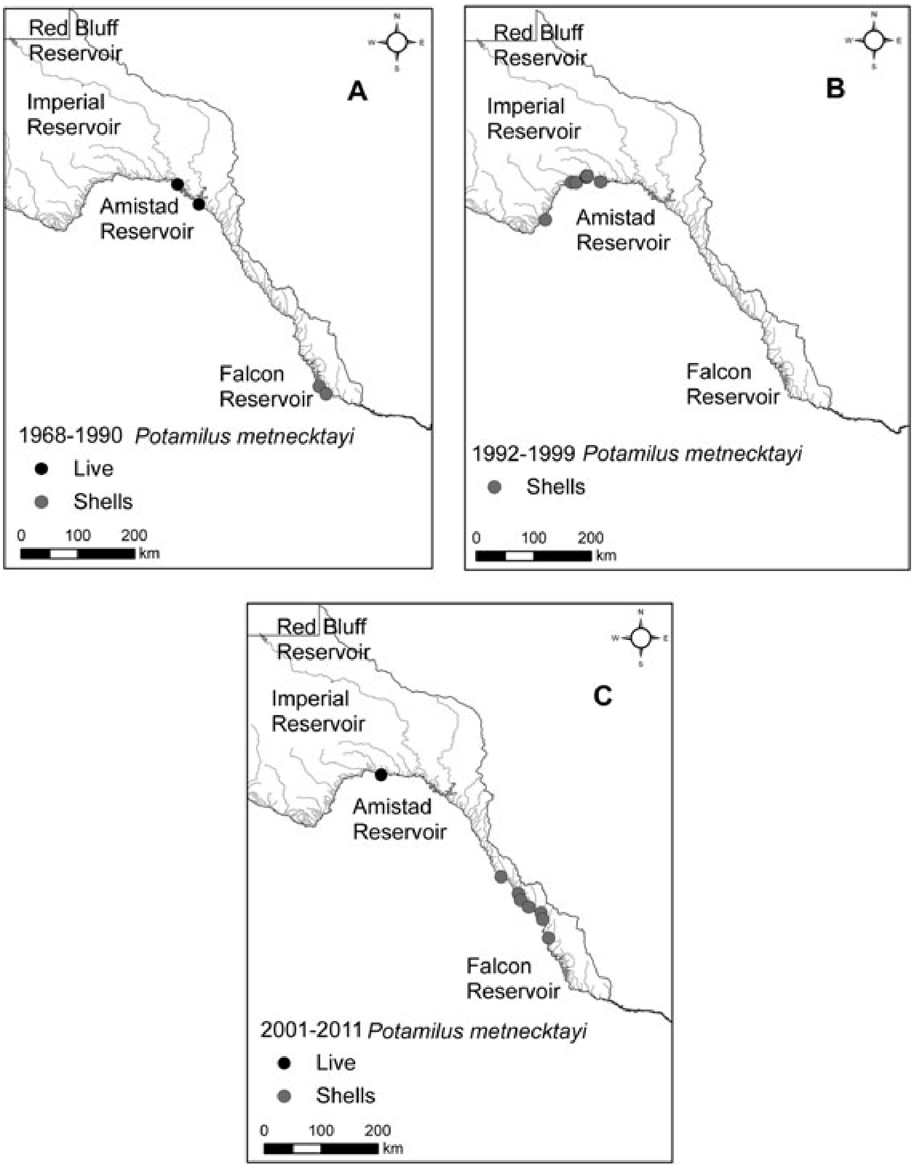

Figure 2. Map of the Rio Grande river basin in Texas with sites where live and/or dead shells of *Potamilus metnecktayi* were found in 1968–1990 (Metcalf, 1974, 1982; Murray, 1975; Neck and Howells, 1984; Neck, 1987; Neck and Metcalf, 1988) (A); in 1992–1999 (Howells 1994, 1996a,b, 1997a, 1998, 1999, 2000) (B); and from 2001 to 2011 (authors' data) (C).

were found, possibly indicating a once widespread population.

Truncilla cognata

In total, 19 live *T. cognata* were found from 2001 to 2011 in the Rio Grande River in Laredo, Webb County. Most molluscs were found down to 15–20 cm deep in a mixture of gravel and sand, and between large boulders. Because of its small size, it was difficult to distinguish *T. cognata* from gravel, adding to the difficulty of detecting this cryptic species. Many excavations were made below the Water Treatment Plant in Laredo, but no live mussels were found there. In 2011 12 *T. cognata* were found at five subsites examined in and above Laredo. Most of them were found in unconsolidated sediments (sand with some silt), captured in shallow protected areas adjacent to gravel riffles. Their size varied from 20.5 mm to 33 mm (average 28.4 ± 4.1 mm). Dead shells of *T. cognata* were found at 12 sites (Figure 3(B)). Very recently dead specimens (i.e. shells with flesh, to 51 mm) were found at four subsites below Laredo into Zapata County. Based on these data, it is likely that additional specimens may be found in Pinto Valle Creek (Webb County) and Dolores Creek (Zapata County). All of the 19 live *T. cognata* from the current study have been found at the confluences of Santa Isabel, Sombrerito, and Zacate Creeks above

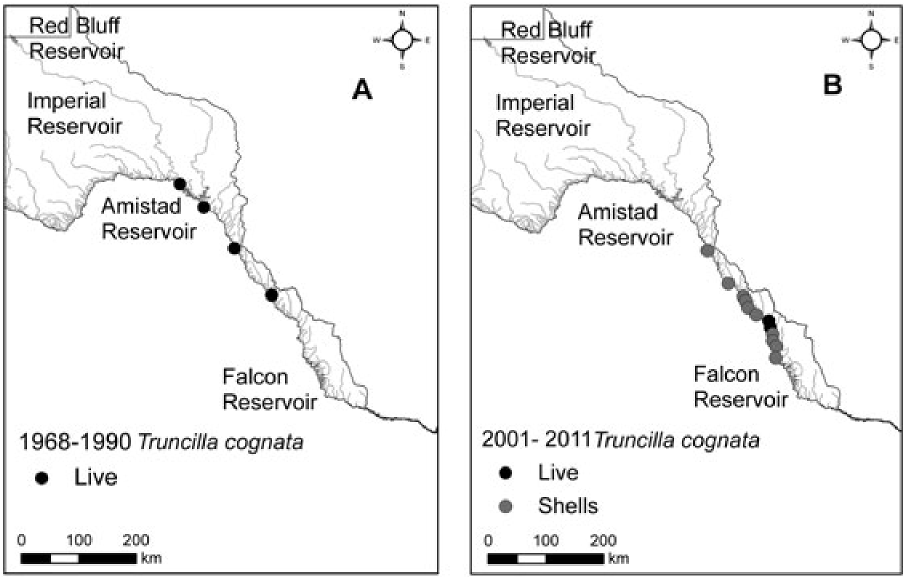

Figure 3. Map of the Rio Grande river basin in Texas with sites where live and/or dead shells of *Truncilla cognata* were found in 1968–1990 (data from Metcalf, 1974, 1982; Murray, 1975, Neck and Howells, 1984; Neck, 1987; Neck and Metcalf, 1988) (A), and from 2001 to 2011 (authors data) (B).

Laredo. Their presumed habitat preference of small gravel/sand/silt mixed substrates is also well known as each of these areas has or had a sand and gravel excavation site nearby.

Popenaias popeii

During 10 years of the current survey, one live *P. popeii* was found in the Rio Grande River in Terrell County (John's Marina), seven live in the Devils River, and 648 live in the Rio Grande near Laredo. Live mussels were found at seven sites, and dead shells were found at a further five sites (Figure 4(D)). Most live mussels were found at the La Bota mark-and-recapture subsite (in Laredo) which had an abundance of low-flow refuges occurring under large boulders, where sand and clay seams provide substrates for mussels. At this mark–recapture site (area sampled *c.* 1000 m^2) 406 live *P. popeii* were found. The recovery rate was 11.7% (18 of 154 mussels marked) on the second day, and was 6.5% (17 of 260 mussels marked) on the third day (9.1% in average). Therefore, the total population may be near 1500 at the site, with a density of ~1.5 m^{-2}. This population consisted of multiple age-classes, with shell lengths ranging from 33.2 to 87 mm (63 ± 1, mean ± 95% confidence interval). Over a third of the mussels measured were less than 60 mm, and 12 individuals were less than 45 mm in length. Considering that the total area of similar substrate upstream of this site was ~ 3200 m^2, and assuming similar densities, up to 4700 individuals of this species may be in this area. At three other subsites located *c.* 1.6 km downstream from this mark-and-recapture locality, 182 live *P. popeii* were found in 3 person-hours of timed searches. These subsites were located along a 280 m river stretch, and may contain up to 4000 more mussels. Therefore, the total population of *P. popeii* in the La Bota area may contain up to 8700 mussels.

DISCUSSION

Long-term changes in unionid diversity

The unionid assemblage of the Rio Grande drainage has changed significantly over the last century (Table 1, 3). Although the Rio Grande itself still supports the majority of unionid species ever reported alive in this river, its unionid assemblage has faced decline in species diversity, range fragmentation, local extirpations, and introduction of widespread common species. Two species (*Quadrula couchiana* and *Quincuncina mitchelli*) are already extinct from the Texas part of the Rio Grande basin. The most drastic changes were recorded during the last 40 years (Table 3).

Several streams and rivers of the Rio Grande drainage have lost all or a significant number of unionid species, including Las Moras Creek in Fort Clark (Brackettville, Kinney County), the Devils River and the Pecos River (Table 4). Along with the local extirpation of rare and endemic species from the Rio Grande drainage, the unionid

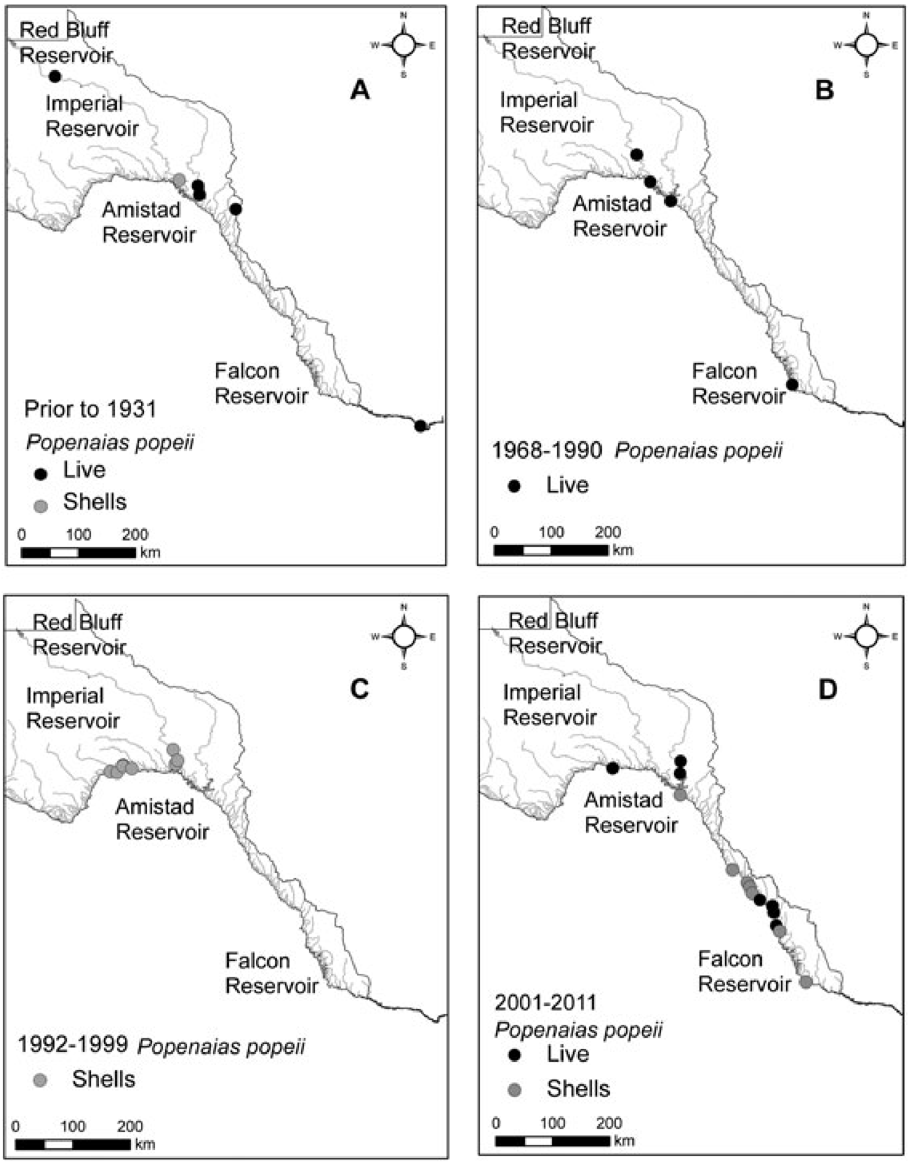

Figure 4. Map of the Rio Grande river basin in Texas with sites where live and/or dead shells of *Popenaias popeii* were found before 1931 (based on data from Singley, 1893; Ellis *et al*., 1930; Strecker, 1931; Taylor, 1967) (A); in 1968–1990 (Metcalf, 1974, 1982; Murray, 1975, Neck and Howells, 1984; Neck, 1987; Neck and Metcalf, 1988) (B); in 1992–1999 (Howells 1994, 1996a, b 1997a, 1998, 1999, 2000) (C); and from 2001 to 2011 (authors' data) (D).

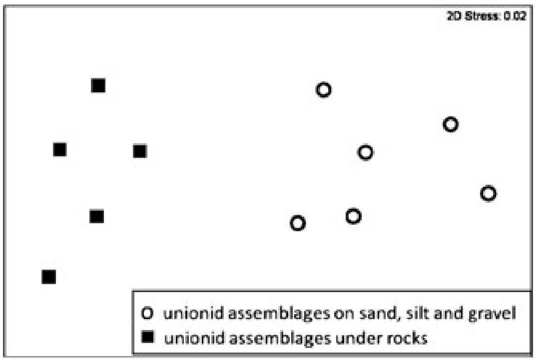

Figure 5. NMDS ordination plot of the unionid assemblages in the Rio Grande near Laredo found on sand, silt and gravel and under rocks. Relative density data (mussels per person per hour) for live molluscs collected at all sampled sites (excluding sites where fewer than two species were collected) were square-root transformed and converted to a similarity matrix using the Bray–Curtis similarity index. There was a significant difference in assemblage structure among the two substrates (Global R = 0.942, $P = 0.001$, one-way ANOSIM).

assemblage was reshaped by the introduction of common species (*Q. apiculata, P. purpuratus*, and *P. grandis*) non-native to this drainage (Metcalf and Smart, 1972; Metcalf, 1982; Johnson, 1999). In the 20th century *Q. apiculata* became very common in the Rio Grande and its tributaries. Previous research noted a lack of fossil *Q. apiculata* (Metcalf, 1982), and no fossil specimens were found during this study. Current data indicate slow, upriver range extension of *Q. apiculata* with greater abundance in Casa Blanca and Falcon reservoirs. *Potamilus purpuratus* has been recorded in the Amistad Reservoir in 1994, 1995 and 1998 (Howells, 1997b, 1999). Historical records of *P. purpuratus* from the Rio Grande river basin (Singley, 1893) have been shown to be

Table 3. Long-term changes in unionid diversity in the Texas part of the Rio Grande drainage

Time period	Changes
Before 1900	Extinction of *Quadrula couchiana* from the Rio Grande drainage Introduction of *Pyganodon grandis*
1900–1970	Extinction of *Q. mitchelli* from the Rio Grande drainage Introduction of *Q. apiculata*
1970–2010	Local extirpations of *Popenaias popeii, Potamilus metnecktayi, Truncilla cognata* Range fragmentation of *P. popeii, P. metnecktayi, T. cognata* Introduction of *Potamilus purpuratus* Range expansion of *Q. apiculata*

misidentified specimens of *C. tampicoensis* (Neck and Metcalf, 1988; Johnson, 1999). Another introduced species, *P. grandis*, was reported from the Granjeno Lake in 1892 (Singley, 1893) and canals in Hidalgo County (Ellis *et al*., 1930), from the El Toro Cement Agency Lake in El Paso in 1969 (Johnson, 1999), and in the Topaz Power Plant cooling pond, Laredo in 2006 (T. Miller unpublished data).

Endemic species accounts

Potamilus metnecktayi

This regional endemic was reported to be extremely rare in the Rio Grande in Texas (Neck and Metcalf, 1988), uncommon even at the fossil localities sampled in New Mexico and Mexico (Metcalf, 1982), and it has been recently added to the state's list of threatened species (Texas Register 35, 2010). Live specimens in the USA were collected in Texas only by Metcalf on the Rio Grande 9.7 km west of Del Rio in 1972, and by Taylor in the Pecos River 1.28 km above its mouth at the former crossing of US Hwy 90 in 1968 (Metcalf, 1982) (Table 4, Figure 2). No live or dead *P. metnecktayi* were found in the Del Rio area during sampling in 2008. Only dead shells of *P. metnecktayi* were found in Texas since the mid-1970s (Howells, 1994, 1999, 2000; Howells *et al.*, 1997; Figure 2(B)). Our discovery of 19 live and numerous shells of *P. metnecktayi* in the Rio Grande by Johnson Marina, Terrell County, proves that this species still exists in the middle Rio Grande, although its distribution range was significantly reduced during the 20th century. Additional studies are urgently needed to estimate the current distribution and population size of *P. metnecktayi* in the Rio Grande considering the subsequent catastrophic floods in 2008 and 2010, and to develop appropriate measures for the species' conservation.

Truncilla cognata

Truncilla cognata is another regional endemic that was described from the Devils River, Texas, and Rio Salado, Nuevo Leon, Mexico (Lea, 1857; Johnson, 1999). This species has a NatureServe

Table 4. Historical and current records of live *Potamilus metnecktayi, Truncilla cognata*, and *Popenaias popeii* in the Texas part of the Rio Grande drainage

Water body	Historical collections	Current status
Potamilus metnecktayi		
Rio Grande, 9.7 km West of Del Rio	1972 (Metcalf, 1982)	No live mussels were found
Rio Grande, Johnson Marina, Terrell County	No historical records from this location	19 live specimens were collected by the authors 2003–2008
Pecos River, 1.28 km above its mouth at the former US Hwy 90 crossing	1968 (Metcalf, 1982)	Flooded by Amistad Reservoir. No live mussels were found
Truncilla cognata		
Rio Grande, 9.7 km West of Del Rio	1972 (Metcalf, 1982)	No live mussels found
Rio Grande, Laredo	No historical records from this location	19 mussels total were found by the authors at two sites 2001–2011
Pecos River, 1.28 km above its mouth at the former US Hwy 90 crossing	1968 (Metcalf, 1982)	Flooded by Amistad Reservoir (population probably extirpated)
Popenaias popeii		
Las Moras Creek, Kinney County	1892 (Taylor, 1967)	No live mussels were found. Population extirpated (Murray, 1975)
Devils River, Val Verde County	1892 (Singley, 1893)	7 live mussels were found by authors 2008–2011
Pecos River, Val Verde County	1903, 1968, 1972, 1973 (Metcalf, 1982)	Flooded by Amistad Reservoir. No live mussels were found
Rio Grande, 9.7 km West of Del Rio	1972 (Metcalf, 1982)	No live mussels were found
Rio Grande, 2.3 km downstream of Falcon Dam, Starr County	1975 (Neck and Metcalf, 1988)	No live mussels were found
Rio Grande, Laredo	No historical records from this location	645 live mussels were found by the authors in 2002–2011
Rio Grande, Johnson Marina, Terrell County	No historical records from this location	1 live specimen was collected by the authors in 2008

global status of 'critically imperilled' (NatureServe, 2009), is considered endangered by the American Fisheries Society, and has recently been added to the state's list of threatened species (Texas Register 35, 2010). *Truncilla cognata* is currently under consideration for federal listing by the US Fish and Wildlife Service (USFWS) (Federal Register 74, 2009). In the USA, *T. cognata* was reported only from a few sites in Texas (Table 4, Figure 3) with no living or dead specimens collected since 1972 (Howells *et al.*, 1997; Howells, 2001). Again it is likely that the Pecos River population of *T. cognata* is already extirpated and the 19 live specimens that were found in the Rio Grande near Laredo in 2001–2011 represent the only known population of this species left in the US.

Popenaias popeii

Popenaias popeii is known from the Rio Grande drainage in Texas (Singley, 1893; Taylor, 1967; Neck, 1987), Black River in New Mexico (Lang, 2001; Carman, 2007), and several Mexican tributaries of the Rio Grande (Simpson, 1914; Johnson, 1999; Strenth *et al.*, 2004). Strecker (1931) reported that *P. popeii* 'seems to be rather scarce', Stansbery (1971) listed this species as 'rare and endangered', and Neck (1984) included it in his list of restricted and declining species of Texas. NatureServe ranks *P. popeii* as critically imperilled across its range (NatureServe, 2009). This species has recently been added to the state's list of threatened species (Texas Register 35, 2010), and is currently considered a candidate for listing (priority 8) under the federal Endangered Species Act.

In Texas, live *P. popeii* were reported from Las Moras Creek (Taylor, 1967), the Devils (Singley, 1893) and Pecos Rivers (Metcalf, 1982), and from two distinct areas in the Rio Grande (Metcalf, 1982; Neck and Metcalf, 1988) (Table 4, Figure 4). Only two dead shells of *P. popeii* were reported in Texas outside the Rio Grande drainage in the South Concho and Llano Rivers (Strenth *et al.*, 2004). There is no evidence that these records represent extant populations of *P. popeii*.

No live *P. popeii* had been found in the Rio Grande since the mid-1970s (Howells, 2001). Our discovery of seven live *P. popeii* in the Devils River in 2008–2011, and 45 live *P. popeii* in 2002–2008 in the Rio Grande River confirmed that the species was still present in Texas. However, more significant was the discovery of a large population (604 live specimens recorded) of *P. popeii* in 2011 in Laredo. The conservative estimate of more than 8000 individuals made this Laredo population by far the largest ever reported from Texas, New Mexico or Mexico. This population consisted of multiple age-classes suggesting the recruitment of juvenile mussels and thus a healthy reproducing population. This also implies that a healthy host fish population occurs in this reach of the river, which is very important for unionid reproduction, and future population survival.

These particular refuges in upper Laredo may be vulnerable to excess water fluctuations including periods of low water and flood. During a low-flow period ($22.6\,m^3\,s^{-1}$) in December, 2002, snowy egrets (*Egreta thula*) were observed feeding on *P. popeii*. Another site on Zacate Creek (Las Palmas Park, a TPWD mussel sanctuary) where more than 50 live mussels of six species (including numerous *P. popeii*) were found over the years, has been smothered by cobble deposited by the July 2010 flood. No live mussels were recorded at this site since this last flood. Specimens of *P. popeii* do not appear to survive well in the Rio Grande downstream of Zacate Creek (Las Palmas Park, Laredo). Only one live mussel and two shells have been found in numerous shore surveys along the 80 km downstream reach of the river to Falcon Lake.

Another important finding was suitable habitat for *P. popeii* in the Rio Grande. This is similar to the preferred habitat for this species in the Black River: low-flow refuges characterized by aggregations of mussels under large boulders of limestone conglomerates, where clay seams provide stable substrates for mussels in low-velocity microhabitats (Lang, 2010). This habitat is different from the soft substrate type preferred by other species such as *C. tampicoensis*, *T. cognata*, *M. nervosa*, and *Q. apiculata* (Figure 5).

Environmental factors affecting unionids

The Rio Grande is at present one of the most impaired rivers in the world, with both water quantity and water quality issues being the major concerns (Dahm *et al.*, 2005). We suggest that among various types of human activities on the Rio Grande drainage, most destructive for unionid assemblages are impoundments, habitat degradation, salinization, pollution, and over-extraction of water (Table 5).

Impoundments

Some species may now be extinct in the Pecos system because of impoundment of its lowermost part by Amistad Reservoir (Metcalf and Stern, 1976). Creation of Falcon Reservoir most likely decimated the lotic habitat of the bivalves in the lower Rio Grande (Neck and Metcalf, 1988). In south-eastern New Mexico, the construction of impoundments (Lake MacMillan, Brantley and Avalon reservoirs) was one of the many factors responsible for extirpation of *P. popeii* from the Pecos River mainstem (Taylor, 1967). The construction of reservoirs also facilitated the introduction and range expansion of common species (*Q. apiculata, P. purpuratus*, and *P. grandis*) non-native to the Rio Grande river drainage (Metcalf and Smart, 1972; Metcalf, 1982; Johnson, 1999). Low-head dams on the Black River apparently preclude opportunities for recolonization by *P. popeii* in upstream riverine reaches and with downstream recolonization potentially limited by altered physicochemical (salinity gradient) and flow regimes (Lang, 2001). Any future projects to construct a new dam, or to modify existing low-head dams and associated water diversion structures, both on the Black River or in the Rio Grande River in Laredo, could potentially have impacts on *P. popeii*.

Salinity

Salinity concentrations in the Rio Grande are the result of both human activities and natural conditions: the naturally salty waters of the Pecos River are a major source of the salts that flow into Amistad Reservoir and continue downstream. Salinity may be the major factor limiting *P. popeii* distribution in the Pecos River and in the Rio Grande below its confluence with the Pecos River. In laboratory studies *P. popeii* shows behavioural signs of physiological stress, followed by death, at a salinity of 7.0 psu (Lang, 2001). Salinity in the area of the Black River occupied by *P. popeii* is approximately 0.9 psu. It increases significantly downstream to 2.8 psu and, in the Pecos River, ranges from 6.0–7.0 psu downstream of the confluence with the Black River (Lang, 2001). This increased salinity may have prevented populations becoming established in the main stem of the Pecos River even before its impoundment.

Table 5. Environmental pressures affecting unionid assemblages in the Texas part of the Rio Grande drainage

Environmental pressure	Effect
Impoundments	• Extirpation of *P. metnecktayi, T. cognata* and *P. popeii* from the lower Pecos River flooded by Amistad Reservoir • Decreased range of *P. metnecktayi* and *T. cognata* in the Rio Grande • Introduction of *P. grandis*, and *P. purpuratus*
Habitat degradation and pollution	• Extirpation of all unionids, including *Q. mitchelli* and *P. popeii* from Las Moras Creek • Decreased or fragmented range of all unionids, including *P. popeii, P. metnecktayi*, and *T. cognata* in the Rio Grande
Salinization	• Extirpation of all unionids, including *P. popeii* from the Pecos River
Over-extraction of water	• Decreased or fragmented range of all unionids, including *P. popeii, P. metnecktayi*, and *T. cognata* in the Rio Grande

Over-extraction, habitat destruction, and pollution

Water diversion from the middle Rio Grande is so high that the river bed between El Paso and Presidio/Ojinaga often lies dry (Dahm *et al*., 2005; Wong *et al*., 2007; Douglas, 2009). Evapotranspiration, groundwater recharge, and human appropriation of Rio Grande water has resulted in less than 1% of basin precipitation reaching the mouth, and failures to reach the Gulf of Mexico were recorded in much of 2002 and 2003 (Dahm *et al*., 2005). Growth in water demand from agricultural economic activity near the Mexican border and regional maquiladoras (manufacturing or export assembly plants in northern Mexico that produce parts and products for the USA) resulted in more than a 5-fold loss of lower Rio Grande stream flow between 1905–1934 and 1951–1980 (reviewed in Douglas, 2009). The population in the basin was about 13 million inhabitants in 1990, and increased along the Texas border by 27% between 1980 and 1990, and by 26% on the Mexican side. As a result of low water levels, the concentration of pollutants is very high; salinization has already displaced 32 native freshwater fish species, while marine fish species are invading as far as 400 km upstream (Contreras and Lozano, 1994). In addition to salinization, water quality problems include elevated nutrients, bacteria, metals, pesticides, herbicides, and organic solvents (Dahm *et al*., 2005). Another major change in the Rio Grande in recent years has been the disconnection of the river from the floodplain (Molles *et al*., 1998);

the fragmentation of river channels by dams, diversions and depletions has eliminated the natural flood pulse, reducing productivity and altering the structure of riparian ecosystems.

Protection

All three endemic species (*P. metnecktayi, T. cognata*, and *P. popeii*) have been added by the Texas Parks and Wildlife Department (TPWD) to the list of state-threatened species in 2010 (Texas Register 35, 2010). However, the state protection only prohibits the 'take' of a state-threatened species. Since 2009 these species are under consideration for federal listing by the USFWS (Federal Register 74, 2009), but listing of these species has not yet been warranted (Federal Register 76, 2011). *Popenaias popeii* was petitioned to the Candidate list as a Federally Endangered Species with Critical Habitat in 2004, and is currently considered a Candidate Species under the Federal Endangered Species Act (Federal Register 71, 2006). Although USFWS encourages conservation of these species, candidate species receive no statutory protection under the Endangered Species Act.

In 1978, a 315 km stretch of the United States side of the Rio Grande along the Mexican border was designated as a National Wild and Scenic River (National Parks and Recreation Act of 1978, Public Law 95–625, November 10, 1978). The river segment begins in, and is administered from, the Big Bend National Park in Brewster County and continues to the Terrell and Val Verde County border, thereby encompassing the area where the extremely rare *P. metnecktayi* and a few specimens of *P. popeii* were found. The Wild and Scenic Rivers Act prohibits federal support for construction of dams, water conduits, reservoirs, or other instream activities that would harm the river's free-flowing condition, water quality, or outstanding resource values. However, the designation neither prohibits development nor gives the federal government control over private property, and does not affect existing water rights. Although the part of the Rio Grande in and above Laredo where we found the only large known population of *P. popeii* has the status of a mussel sanctuary (where mussel harvest is prohibited) (Texas Register 31, 2006), additional protection is urgently necessary as any activity associated with water flow alteration could potentially damage the remaining habitat of *P. popeii*.

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REFERENCES

Bogan AE. 1993. Freshwater bivalve extinctions (Mollusca: Unionidae): a search for causes. *American Zoologist* **33**: 599–609.

Bray JR, Curtis JT. 1957. An ordination of the upland forest communities of Southern Wisconsin. *Ecological Monographs* **27**: 326–349.

Burlakova LE, Karatayev AY, Karatayev VA, May ME, Bennett DL, Cook MJ. 2011a. Biogeography and conservation of freshwater mussels (Bivalvia: Unionidae) in Texas: patterns of diversity and threats. *Diversity and Distributions* **17**: 393–407.

Burlakova LE, Karatayev AY, Karatayev VA, May ME, Bennett DL, Cook MJ. 2011b. Endemic species: contribution to community uniqueness, effect of habitat alteration, and conservation priorities. *Biological Conservation* **144**: 155–165.

Carman SM. 2007. Texas hornshell *Popenaias popeii* recovery plan. New Mexico Department of Game and Fish, Santa Fe, NM. http://www.wildlife.state.nm.us/conservation/threatened_endangered_species/documents/TXHornshellRecoveryPlanFinal.pdf [16 March 2009].

Clarke KR. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**: 117–143.

Contreras-Balderas S, Edwards RJ, Lozano-Vilano MD, Garcia-Ramirez ME. 2002. Fish biodiversity changes in the Lower Rio Grande/Rio Bravo, 1953–1996 – a review. *Reviews in Fish Biology and Fisheries* **12**: 219–240.

Contreras S, Lozano ML. 1994. Water, endangered fishes, and development perspectives in arid lands of Mexico. *Conservation Biology* **8**: 379–387.

Dahm CN, Edwards RJ, Gelwick FP. 2005. Gulf Coast rivers of the Southewestern United States. In *Rivers of North America*, Arthur CB, Colbert EC (eds). Academic Press: Burlington; 180–228.

Douglas AJ. 2009. Social, political, and institutional setting: Water management problems of the Rio Grande. *Journal of Water Resources Planning and Management* **135**: 493–501.

Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Leveque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny MLJ, Sullivan CA. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* **81**: 163–182.

Dunn HL. 2000. Development of strategies for sampling freshwater mussels (Bivalvia: Unionidae). In *Freshwater Mollusk Symposia Proceedings. Part II. Proceedings of the 1st Freshwater Mollusk Conservation Society Symposium*, Tankersley RA, Warmolts DI, Watters GT, Armitage BJ, Johnson PD, Butler RS (eds). Ohio Biological Survey: Columbus, OH; 161–167.

Edwards RJ, Contreras-Balderas S. 1991. Historical changes in the ichthyofauna of the Lower Rio Grande (Río Bravo del Norte), Texas and Mexico. *The Southwestern Naturalist* **36**: 201–212.

Ellis MM, Merrick AD, Ellis MD. 1930. The blood of North American freshwater mussels under normal and adverse conditions. *Bulletin of the U.S. Bureau of Fisheries* **46**: 509–542.

Federal Register 71. 2006. Endangered and Threatened Wildlife and Plants – Proposed Critical Habitat Designations. Proposed Rules. September 12, 2006: 53755–53835. Department of the Interior, Fish and Wildlife Service, 50 CFR Part 17. http://www.fws.gov/policy/library/2006/06-7375.html [13 October 2011].

Federal Register 74. 2009. Endangered and Threatened Wildlife and Plants; 90-Day Finding on Petitions to List Nine Species of Mussels from Texas as Threatened or Endangered with Critical Habitat. Proposed Rules. December 15, 2009: 66261–66271. Department of the Interior, Fish and Wildlife Service, 50 CFR Part 17. http://www.fws.gov/policy/library/2009/E9-29698.html [16 December 2009].

Federal Register 76. 2011. Endangered and Threatened Wildlife and Plants; 12-Month Finding on a Petition to List Texas Fatmucket, Golden Orb, Smooth Pimpleback, Texas Pimpleback, and Texas Fawnsfoot as Threatened or Endangered. Proposed Rules. October 6, 2011: 62166–62212. Department of the Interior, Fish and Wildlife Service, 50 CFR Part 17. http://www.gpo.gov/fdsys/pkg/FR-2011-10-06/pdf/2011-25471.pdf [7 October 2011].

Groombridge B, Jenkins M. 1998. *Freshwater Biodiversity: a Preliminary Global Assessment*. World Conservation Monitoring Centre: Cambridge, U.K.

Howells RG. 1994. Preliminary distributional surveys of freshwater bivalves in Texas: progress report for 1992. *Management Data Series* ***105***. Texas Parks and Wildlife Department: Austin, TX.

Howells RG. 1996a. Distributional surveys of freshwater bivalves in Texas: progress report for 1994. *Management Data Series* ***120***. Texas Parks and Wildlife Department: Austin, TX.

Howells RG. 1996b. Distributional surveys of freshwater bivalves in Texas: progress report for 1995. *Management Data Series* ***125***. Texas Parks and Wildlife Department: Austin, TX.

Howells RG. 1997a. Distributional surveys of freshwater bivalves in Texas: progress report for 1996. *Management Data Series* ***144***. Texas Parks and Wildlife Department: Austin, TX.

Howells RG. 1997b. Range extention of the freshwater mussel *Potamilus purpuratus* (Bivalvia: Unionidae) in Texas. *Texas Journal of Science* **49**: 79–82.

Howells RG. 1998. Distributional surveys of freshwater bivalves in Texas: progress report for 1997. *Management Data Series* ***147***. Texas Parks and Wildlife Department: Austin, TX.

Howells RG. 1999. Distributional surveys of freshwater bivalves in Texas: progress report for 1998. *Management Data Series* ***161***. Texas Parks and Wildlife Department: Austin, TX.

Howells RG. 2000. Distributional surveys of freshwater bivalves in Texas: progress report for 1999. *Management Data Series* ***170***. Texas Parks and Wildlife Department: Austin, TX.

Howells RG. 2001. *Status of freshwater mussels of the Rio Grande, with some comments on other bivalves*. Texas Parks and Wildlife Department: Austin, TX.

Howells RG, Neck RW, Murray HD. 1996. *Freshwater Mussels of Texas*. Texas Parks and Wildlife Press: Austin, TX.

Howells RG, Mather CM, Bergmann JAM. 1997. Conservation status of selected freshwater mussels in Texas. In *Conservation and Management of Freshwater Mussels II (Initiatives for the future): Proceedings of a UMRCC Symposium*, Cummings KS, Buchanan AC, Mayer CA, Naimo TJ (eds). Upper Mississippi River Conservation Committee: Rock Island, IL; 117–129.

Jackson RB, Carpenter SR, Dahm CN, McKnight DM, Naiman RJ, Postel SL, Running SW. 2001. Water in a changing world. *Ecological Applications* **11**: 1027–1045.

Johnson RI. 1998. A new mussel, *Potamilus metnecktayi* (Bivalvia: Unionidae) from the Rio Grande system, Mexico and Texas with notes on Mexican *Disconaias*. *Occasional Papers on Mollusks* **5**: 427–455.

Johnson RI. 1999. The Unionidae of the Rio Grande (Rio Bravo del Norte) system of Texas and Mexico. *Occasional Papers on Mollusks* **6**: 1–65.

Krebs CJ. 1999. *Ecological Methodology*. Addison-Welsey Educational Publishers, Inc.: Menlo Park, CA.

Lang BK. 2001. Status of the Texas Hornshell and Native Freshwater Mussels (Unionoidea) in the Rio Grande and Pecos River of New Mexico and Texas. Completion Report (E-38) to the Division of Federal Aid, U.S. Fish and Wildlife Service, Region 2. New Mexico Department of Game and Fish, Albuquerque, NM.

Lang BK. 2010. Texas Hornshell, *Popenaias popeii* (Bivalvia: Unionidae), Population Monitoring. Interim Performance Report to the Division of Federal Aid, U.S. Fish and Wildlife Service, Region 2. New Mexico Department of Game and Fish, Albuquerque, NM.

Lea I. 1857. Description of six new species of fresh water and land shells of Texas and Tamaulipas, from the collection of the Smithsonian Institution. *Proceedings of the Academy of Natural Sciences of Philadelphia* **9**: 101–102.

Lydeard C, Clark SA, Perez KE, Cowie RH, Ponder WF, Bogan AE, Bouchet P, Gargominy O, Cummings KS, Frest TJ, *et al.* 2004. The Global Decline of Nonmarine Mollusks. *Bioscience* **54**: 321–330.

McKinney ML, Lockwood JL. 1999. Biotic homogenization: a few winners replacing many losers in the next mass extinction. *Trends in Ecology & Evolution* **14**: 450–453.

Metcalf AL. 1974. Fossil and living freshwater mussels (Unionacea) from the Pecos River, New Mexico and Texas. *Bulletin of the American Malacalogical Union* **33**: 47–48.

Metcalf AL. 1982. Fossil unionacean bivalves from three tributaries of the Rio Grande. In *Symposium on Recent Benthological Investigations in Texas and Adjacent States*, Davis JR (ed.). Texas Academy of Science: Austin, Texas; 43–59.

Metcalf AL, Smart RA. 1972. Records of introduced mollusks: New Mexico and Western Texas. *Nautilus* **85**: 144–145.

Metcalf AL, Stern EM. 1976. Notes on unionacean mollusks of the Rio Grande system, United States and Mexico. *Bulletin of the American Malacological Union* **1976**: 42–43.

Molles MC, Crawford CS, Ellis LM, Valett HM, Dahm CN. 1998. Managed flooding for riparian ecosystem restoration: managed flooding reorganizes riparian forest ecosystems along the middle Rio Grande in New Mexico. *Bioscience* **48**: 749–756.

Murray HD. 1975. *Melanoides tuberculata* (Müller), Las Moras Creek, Bracketville, Texas. *Bulletin of the American Malacological Union* **1975**: 43.

Naiman RJ, Turner MG. 2000. A future perspective on North America's freshwater ecosystems. *Ecological Applications* **10**: 958–970.

National Parks and Recreation Act of 1978, Public Law 95–625. 10 November 1978. United States Statutes at Large 92: 3522. http://www.fs.fed.us/cdt/pdf_documents/pl_95-625.pdf [15 April 2011]

NatureServe. 2009. http://www.natureserve.org/explorer [8 May 2010]

Neck RW. 1982. Preliminary analysis of the ecological zoogeography of the freshwater mussels of Texas. In *Symposium on Recent Benthological Investigations in Texas and Adjacent States*, Davis JR (ed.). Texas Academy of Science: Austin, Texas; 33–42.

Neck RW. 1984. Restricted and declining nonmarine molluscs of Texas. *Technical Series 34*. Texas Parks and Wildlife Department: Austin, Texas.

Neck RW. 1987. Freshwater bivalves of the Baffin Bay drainage basin, southern Texas. *Texas Journal of Science* **39**: 177–182.

Neck RW, Howells RG. 1984. *Status survey of Texas heelsplitter, Potamilus amphichaenus* (Frierson, 1898). Resource Protection Division and Inland Fisheries Division: Texas Parks and Wildlife Department, Austin, TX.

Neck RW, Metcalf AL. 1988. Freshwater bivalves of the Lower Rio Grande, Texas. *Texas Journal of Science* **40**: 259–268.

Revenga C, Murray S, Abramovitz J, Hammond A. 1998. *Watersheds of the World: Ecological Value and Vulnerability*. World Resources Institute, Washington, DC.

Revenga C, Brunner J, Henninger N, Kassem K, Payne R. 2000. *Pilot Analysis of Global Ecosystems (PAGE): Freshwater Systems*. World Resources Institute, Washington, DC.

Simpson CT. 1900. Synopsis of the naiades, or pearly fresh-water mussels. *Proceedings of the United States National Museum* **22**: 501–1044.

Simpson CT. 1914. *A Descriptive Catalogue of the Naiades, or Pearly Fresh-Water Mussels*. B. Walker: Detroit, Michigan.

Singley JA. 1893. Contributions to the Natural History of Texas. Part I. Texas Mollusca. A preliminary list of the land, fresh water, and marine Mollusca of Texas. Department of Agriculture, Insurance, Statistics, and History, Austin, TX.

Stansbery DH. 1971. Rare and endangered mollusks in the eastern United States. In *Rare and Endangered Mollusks (Naiads) of the United States*, Jorgenson SE, Sharp RW (eds). US Fish and Wildlife Service: Twin Cities, Minnesota; 5–18.

Strayer DL, Smith DR. 2003. A guide to sampling freshwater mussel populations. *American Fisheries Society Monograph* **8**: 1–103.

Strayer DL, Dudgeon D. 2010. Freshwater biodiversity conservation: recent progress and future challenges. *Journal of the North American Benthological Society* **29**: 344–358.

Strayer DL, Claypool S, Sprague SJ. 1997. Assessing unionid populations with quadrats and timed searches. In *Conservation and Management of Freshwater Mussels II (Initiatives for the future): Proceedings of an Upper Mississippi River Conservation Committee (UMRCC) Symposium*, Cummings KS, Buchanan AC, Mayer CA, Naimo TJ (eds). Upper Mississippi River Conservation Committee: Rock Island, IL. 163–169.

Strecker JK. 1931. The distribution of the naiades or pearly freshwater mussels of Texas. *Baylor University Museum Bulletin* **2**: 69.

Strenth NE, Howells RG, Correa-Sandoval A. 2004. New records of the Texas hornshell *Popenaias popeii* (Bivalvia: Unionidae) from Texas and northern Mexico. *Texas Journal of Science* **56**: 223–230.

Taylor DW. 1967. Freshwater mollusks collected by the United States and Mexican Boundary surveys. *Veliger* **10**: 152–158.

Texas Parks and Wildlife Department. 1974. An Analysis of Texas Waterways: a Report on the Physical Characteristics of Rivers, Streams and Bayous in Texas. The Texas Agricultural Extension Service, Texas A&M University System. http://www.tpwd.state.tx.us/publications/pwdpubs/pwd_rp_t3200_1047/ [12 November 2009]

Texas Register 31. 2006. Natural resources and conservation. Part 2. Texas Parks and Wildlife Department. Chapter 57. Fisheries §57.157, Proposed Rules concerning Mussels and Clams. June 9, 2006: 4699–4704. Texas Secretary of State. http://www.sos.state.tx.us/texreg/pdf/backview/0108/0108adop.pdf [15 April 2011]

Texas Register 35. 2010. Threatened and endangered nongame species. Chapter 65. Wildlife Subchapter G. 31 TAC § 65.175. Adopted rules. January 8, 2010: 249–251. Texas Secretary of State. http://www.sos.state.tx.us/texreg/pdf/backview/0108/0108adop.pdf [15 February 2010]

Texas Water Development Board. 2007. Water for Texas. 2007 State Water Plan. Chapter 4. Population and Water Demand Projections. GP-8-1. http://www.twdb.state.tx.us/publications/reports/State_Water_Plan/2007/2007StateWaterPlan/CHAPTER%204_Final_112806.pdf [7 March 2011]

Vaughn CC, Taylor CM, Eberhard KJ. 1997. A comparison of the effectiveness of timed searches vs. quadrat sampling in mussel surveys. In *Proceedings of an Upper Mississippi River Conservation Committee (UMRCC) Symposium, 16–18 October 1995*, Cummings KS, Buchanan AC, Mayer CA, Naim TJ (eds). Upper Mississippi River Conservation Committee: Rock Island, IL; 157–162.

Villella RF, Smith DR, Lemarie DP. 2004. Estimating survival and recruitment in a freshwater mussel population using mark-recapture techniques. *American Midland Naturalist* **151**: 114–133.

Williams JD, Warren ML Jr., Cummings KS, Harris JL, Neves RJ. 1993. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* **18**: 6–22.

Wong CM, Williams CE, Pittock J, Collier U, Schelle P. 2007. *World's top 10 rivers at risk*. Executive Summary: WWF International, Gland, Switzerland.