Section 6 (Texas Traditional) Report Review

Form emailed to FWS S6 coordinator (mm/dd/yyyy): 1/3/2012

TPWD signature date on report: 9/21/2011

Project Title: Survey of Texas Hornshell Populations in Texas

Final or Interim Report? Final

Grant #: TX E-132-R-1

Reviewer Station: Austin ESFO

Lead station concurs with the following comments: NA (reviewer from lead station)

Interim Report (check one):

- ☐ Acceptable (no comments)
- ☐ Needs revision prior to final report (see comments below)
- ☐ Incomplete (see comments below)

Final Report (check one):

- ☒ Acceptable (no comments)
- ☐ Needs revision (see comments below)
- ☐ Incomplete (see comments below)

Comments:

FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-132-R-1

Endangered and Threatened Species Conservation

Survey of Texas Hornshell Populations in Texas

Prepared by:

Drs. Lyubov Burlakova and Alexander Karatayev

Carter Smith
Executive Director

Clay Brewer, Acting
Division Director, Wildlife

21 September 2011

FINAL REPORT

STATE: ____Texas_______________ **GRANT NUMBER:** ___E – 132-R-1____

GRANT TITLE: Status of Newly Discovered Cave and Spring Salamanders (Eurycea) in Southern Travis and Northern Hays Counties

REPORTING PERIOD: ____1 Oct 10 to 30 Sep 11

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:

Task 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, and determine habitat requirements;

Task 2. Establish population monitoring using mark-and-recapture methods;

Task 3. Use the microsatellite genetic tools developed by our New Mexico partners to enable further understanding of population processes;

Task 4. Evaluate growth, survivorship and population viability;

Task 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.

Significant Deviation: None.

Summary Of Progress: Please see Attachment A.

Location: Travis and Hays County, TX

Cost: ___Costs were not available at time of this report.__

Prepared by: _Craig Farquhar_____________ **Date:** _21 September 2011

Approved by: ______________________________ **Date:**___21 September 2011

C. Craig Farquhar

ATTACHMENT A

TEXAS PARKS AND WILDLIFE DEPARTMENT

MULTISTATE TRADITIONAL SECTION 6

Joint Project with New Mexico Department of Game and Fish

PERFORMANCE REPORT - FINAL

State: Texas **TPWD Contract#:** 407348

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

Data collection period: February 23 - August 31, 2011

State Contract Period: 23 February 2011 **To:** 30 September 2011

Principal Investigators: Lyubov E. Burlakova, Alexander Y. Karatayev

This joint Section 6 project is collaboration between the U.S. Fish and Wildlife Service (USFWS), Texas Parks and Wildlife Department (TPWD), Buffalo State College (BSC) and New Mexico Department of Game and Fish (NMDGF). It is coordinated between PIs Lyubov Burlakova and Alexander Karatayev (BSC), agency biologists Brian Lang (NMDGF) and Marsha May (TPWD).

1. 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.

2. Problem and Need

The Rio Grande/Rio Bravo River is one of the longest rivers in North America. 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 (Dahm et al., 2005; Wong et al., 2007). Due to water over-extraction for the increasing irrigation and domestic consumption, the riverbed between El Paso and Presidio frequently lies 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). Many additional factors contributed to the recent status of the Rio Grande, including persistent drought, increase in border population, and declines in the water quantity and quality (Dahm et al., 2005; Wong et al., 2007; Douglas, 2009).

The Rio Grande is a globally important river for freshwater biodiversity, supporting numerous endemic fish, birds, and molluscs (Grommbridge and Jenkins, 1998; Revenga et al., 1998; Johnson, 1999; Revenga et al., 2000). Many of these species have already become extinct; others are facing a sharp decrease in their population density or range fragmentation. As a result, the Rio Grande is considered the most endangered river system in the North American continent and one of the world's top 10 rivers at risk (Wong et al., 2007).

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 drainage represent a unique assemblage and are distinct from the rest of Texas (Neck, 1982; Neck and Metcalf, 1988; Burlakova et al., 2011a; Burlakova et al., 2011b). The first data on unionid bivalves of Rio Grande and its tributaries were published at the turn of the 19th century (Singley, 1893; Simpson, 1900; Simpson, 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.

Popenaias popeii, Texas hornshell, is a regional endemic 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. Nature Serve ranks the Texas hornshell as critically imperiled across its range (NatureServe, 2009). This species has been recently 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), Devils River (Singley, 1893; Neck, 1984), Pecos River (Metcalf, 1982) and from two distinct areas in Rio Grande (Metcalf, 1982; Neck and Metcalf, 1988) (Table 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 living population of *P. popeii*. Extensive surveys along 48 km of Las Moras Creek in 1971, 1973, and 1975 yielded no living *P. popeii* (Murray, 1975). This population is believed to be extirpated 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 (Murray, 1975; Howells *et al.*, 1996). Pecos River sites were flooded by Amistad Reservoir and *P. popeii* was extirpated. In Devils River *P. popeii* survived much longer and "localized concentrations of living specimens" were reported from the Devil's River, Val Verde County, by Neck (1984). No live *P. popeii* were found in the Rio Grande since mid-1970s (Howells, 2001). In 2008 during our state-wide survey of freshwater molluscs in Texas funded by the State Wildlife Grant Program (Burlakova and Kararayev, 2010), we found live *P. popeii* in the Rio Grande at two sites: Terrell County (n = 1) and Webb County (n = 9). Two more live Texas hornshell were found by T. Miller (Laredo Community College) in the Devils River (Val Verde County) in 2008.

2. Methods

To assess the diversity, distributions, and long-term changes in unionid assemblages of the Rio Grande system within Texas, we used both field studies and historic data (Karatayev et al. In Review, Appendix). To analyze the historical data, we created a database containing information of unionid species name, waterbody name, location, recorded date, and collector's name using all available data including published records, museum collections, and web-based searches.

To assess the current distribution of unionids, mussels were surveyed in the Rio Grande in March 2011 at 25 sites near Laredo (Webb County) and at 5 sites in the Devils River (Val Verde County). In July 2011 we surveyed four more sites on the Pecos River, Pecos County: at CR 290 (southeast of Sheffield), at Olson Road and US-190 (near of Iraan), and at FM 305 (these data were received after the manuscript submission and thus are not included in the manuscript and analysis). In addition, we used our data collected in 2008 (Burlakova & Karatayev, 2010) and data collected by Tom Miller from 2001-2011 (Laredo Community College, Appendix). These 162 sample sites (subsites) were pooled into 28 larger sites within the Rio Grande system during 2001– 2011 (Appendix). Fifteen of these sites were sampled once, while 13 sites were sampled from 2 to 25 times. Due to the prevalence of private land in Texas, where only 2% of the lands remain in public ownership (Texas Parks and Wildlife Department, 1974), survey sites were often selected within state parks, near public boat ramps, or based on accessibility from roads that either crossed or approached a waterbody. The work was carried out with an appropriate Scientific Research Permit issued by the Texas Parks and Wildlife Department.

Sampling was completed via hand collection of both live and dead mussels, by wading in low water and by snorkeling or diving. Reconnaissance sampling (timed searches) was used on some sites to reveal the presence of mussels and species diversity (Strayer *et al.*, 1997; Vaughn *et al.*, 1997). All mussels collected (live and dead) were taxonomically identified, counted, and measured with calipers to the nearest mm. 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.

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 March 2011 using methods described by Lang (2001) and Villella *et al*. (2004). The access to the site was gained from the US Border Patrol boat ramp. Following recommendations by Villella *et al*. (2004), we sampled three consecutive days to estimate capture probabilities using closed population models. 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, 1998). 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. To estimate population density at our mark-and-recapture site we used the Schnabel method, an extension of Petersen method to a series of samples (Krebs, 1999). To evaluate the total size of the *P. popeii* population we used the average density in the mark-and-recapture site and our estimation of available habitat area in La Bota.

Appendix *(manuscript by Karatayev, Miller and Burlakova submitted to Aquatic Conservation: Marine and Freshwater Ecosystems) describes detailed Methods and Data Analysis.*

3. Results and Benefits

In early March 2011, together with Thomas Miller (Environmental Science Center, Laredo Community College), Brian Lang (NMDGF), Dr. David J. Berg and Kentaro Inoue (Miami University, Oxford, OH), we sampled the Devils River at 5 sites, and found 1 live *P. popeii* during 30-person hours of search effort. Only one fragment of *P. popeii* valve (relatively recently dead) was found in our surveys of the Pecos River in July 2011 (at Olson Road near Iraan, Pecos County).

During ten years of surveys, in addition to the one live *P. popeii* found in the Rio Grande River in Terrell County (John's Marina) in 2008, a total of seven live mussels was found in the Devils River, and 604 live *P. popeii* were found in Laredo. Most live mussels were found at the La Bota mark-and-recapture site (Figure 1) which had an abundance of low-flow refuges occurring under large boulders, where sand and clay seams provide substrata for mussels. At this mark-and-recapture site (area sampled ca. 1,000 m^2) we found a total of 406 live *P. popeii*. We conducted three closed-population censuses over a 3-day period, spending ca. 200 man hours at the site. Dr. Yixin Zhang and Trey Noble (Texas State University, San Marcos) helped us in this work, as well as in some of the following surveys. We marked 297 *P. popeii*, but due to lack of tags, we were only able to record additional new captures (n = 109). 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, total population estimation may be near 1,500 *P. popeii* at the site, with density of ~1.5 m^{-2}. Shell lengths varied from 33.2 to 87 mm (mean 63 mm ± 1, 95% confidence interval). Over a third of the mussels measured were < 60 mm, and 12 individuals were < 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 4,700 individuals of this species may be in this area. At three other subsites located ca. 1 mile downstream from this mark-and-recapture locality (Figure 2), we found a total of 182 live *P. popeii* in 3 person-hours of timed searches. These subsites were located along a 280 m river stretch, and may contain up to 4,000 more mussels. Therefore, the total population of *P. popeii* in the La Bota area may contain up to 8,700 mussels.

This population consisted of multiple age-classes, including small mussels suggesting the successful recruitment of juvenile mussels (Figure 3). This implies a healthy reproducing population of *P. popeii*. This also suggests that a healthy host fish population occurs in this reach of the river, which is very important for unionid reproduction, and future population survival.

Data provided by this mark-recapture study indicate that the "La Bota" population of *P. popeii* is very robust, not only based on the number of unmarked mussels recovered over a 3-day period, but also based on shell morphometrics.

Figure 1. Tagged Texas hornshell (upper picture) and the mark-recapture site in La Bota area (lower). Note the specific habitat (bedrock, boulders and ledges) where the mussels were found.

Figure 2. Texas hornshell collected during 1.2 man hour time search (4 people x 18 min) downstream of the La Bota mark-and-recapture study site, and upstream from the World Trade Bridge, Laredo, Texas.

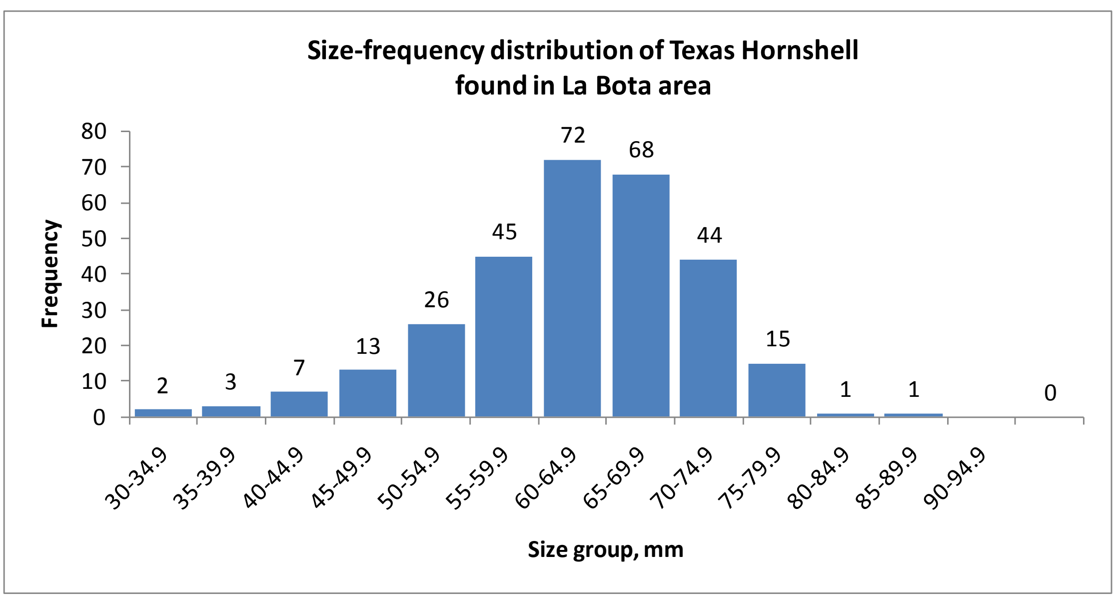


Figure 3. Size-frequency distribution of Texas hornshell at the mark-recapture site in La Bota, Laredo, Texas. Figures above the bars indicate the number of mussels in each size group.

Another important finding was identification of suitable habitat for *P. popeii* in Rio Grande. We found two distinct unionid assemblages depending on the substrate type were found in the Rio Grande above Laredo (Karatayev et al., In Review, Appendix). On soft, unconsolidated sediments (silt, sand, small gravel, and combination of these) unionid assemblages were dominated by *Quadrula apiculata,* and *Cyrtonaias tampicoensis*, additional species were *Megolonaias nervosa* and *Truncilla cognata.* On bedrock and boulders the dominant species was *P. popeii.* This species was most commonly found in crevices under flat boulder resting on the bedrock. We often found up to 10 individuals under one rock. Very few other unionids were found in this habitat including *Lampsilis teres, Q. apiculata,* and *T. cognata.* This habitat 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 substrata 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* (Appendix).

In addition to this large population of *P. popeii*, in northern Laredo (from San Isabel Creek to the railroad bridge, in total over 20 miles), we found another regional endemic, the *Truncilla cognata* (Mexican fawnsfoot) (Figure 4, 5). This species is considered Endangered by the American Fisheries Society, and was recently added to the state's list of threatened species (Texas Register 35, 2010). *Truncilla cognata* is currently under consideration for federal listing by the U. S. Fish and Wildlife Service (74 FR 66261, December 15, 2009). During our 2011 surveys, we found 12 live *T. cognata* in the Laredo area (Figure 5), often at the same sites were we found *P. popeii*.

Based on our conservative estimations, in March 2011 we found the largest known population of *P. popeii*. The population in Laredo, Texas, is healthy and reproducing, and we confirmed that the same area contains the only known population of another extremely rare regional endemic, *T. cognata*.

Figure 4. Regional endemic *Truncilla cognata* (Mexican fawnsfoot) found in the Rio Grande, Laredo, Webb County, Texas.

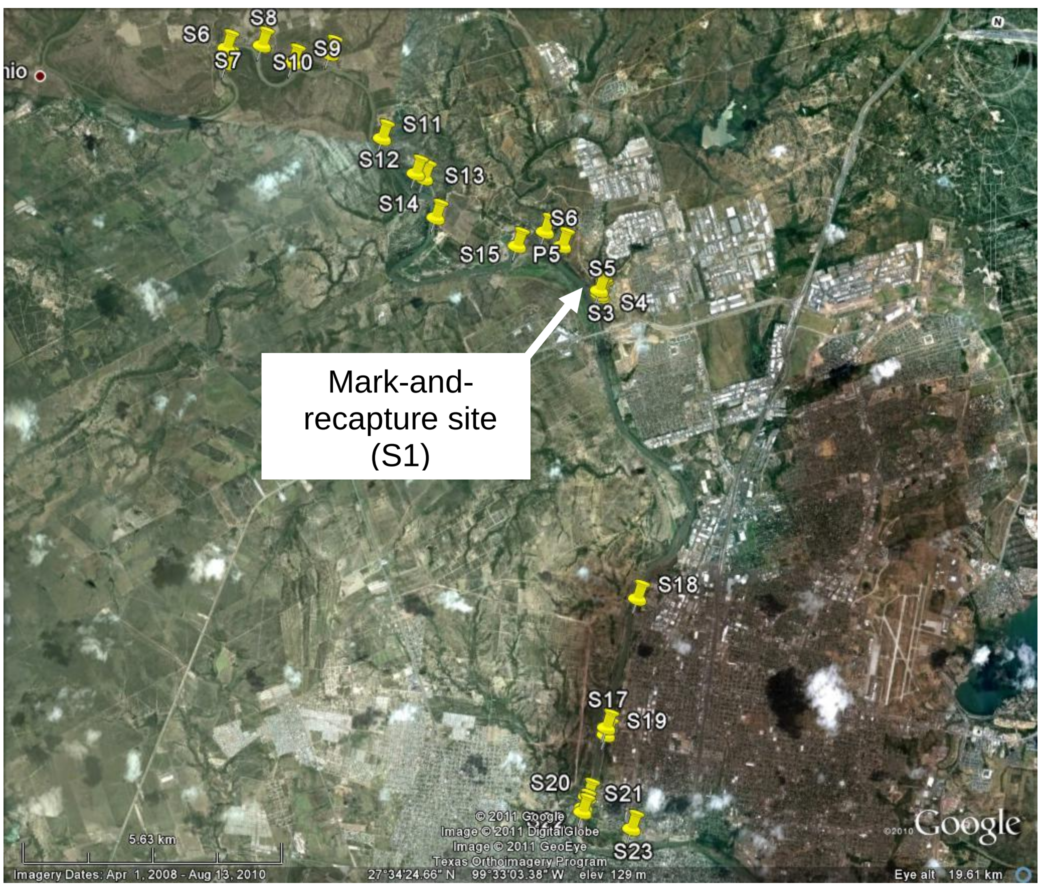


Figure 5. Map of Laredo (Webb County, Texas) area with sampling sites surveyed in March 2011. Live *Popenaias popeii* were found at sites 1-5, 15, 16, 21, and *Truncilla cognata* - at sites 1, 7, 9, 18, and 22.

Long-term Changes in Distribution Range

We found that the unionid assemblage of the Rio Grande Drainage changed dramatically over the last century (Karatayev et al. In Review, Appendix). 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 last 40 years (Karatayev et al. In Review, Appendix).

Several tributaries of the Rio Grande Drainage have lost all or a significant number of unionid species. In 1892, 8 species of unionids were found in Las Moras Creek in Fort Clark (Brackettville, Kinney County), including the endemic *Q. couchiana* and *P. popeii* (Taylor, 1967). Repeated reexamination of this site since 1971 failed to reveal any live or dead unionids (Murray, 1975; Howells, 1997a; authors unpublished data). Similarly, the Devils River used to support 5 species of unionids, including *Q. couchiana* and *P. popeii* (Singley, 1893; Strecker, 1931; Johnson, 1999). Repeated sampling of this river in recent years revealed only 6 live individuals of *P. popeii,* and one relatively recently dead shell of *Utterbackia imbecillis.* Five species of unionids historically were reported from the Pecos River, including endemic *Potamilus metnecktayi* and *T. cognata* (Singley, 1893; Metcalf, 1982; Johnson, 1999). Most of the species were found at the mouth of the Pecos River, which is now inundated by Lake Amistad (Metcalf, 1982). No live native unionids were found in this river since the 1980-s (Howells, 2001; Lang, 2001).

Along with the local extirpation of rare and endemic species from the Rio Grande drainage the unionid assemblage was reshaped by the introduction of common species (*Q. apiculata, Potamilus purpuratus,* and *Pyganodon grandis*) nonnative to this drainage (Metcalf and Smart, 1972; Metcalf, 1982; Johnson, 1999). *Quadrula apiculata* became a very common species in the 20th century in Rio Grande and its tributaries. Previous research noted a lack of fossil *Q. apiculata* (Metcalf, 1982), and no fossil specimens were found during our study. Our data indicate a slow, upstream range extension of *Q. apiculata* with a greater abundance in Casa Blanca and Falcon reservoirs. *Potamilus purpuratus* has been recorded in the Amistad Reservoir in 1994, 1995 and 1998 (Howells, 1997b; Howells, 1999). Another introduced species, *P. grandis,* was reported from Grandjeno Lake in 1892 (Singley, 1893) and canals in Hidalgo County (Ellis *et al.*, 1930), from El Toro Cement Agency Lake in El Paso in 1969 (Johnson, 1999), and in Topaz Power Plant cooling pond, Laredo in 2006 (T. Miller unpublished data).

We documented long-term changes in the distribution of *P.popeii* in Texas including range fragmentation and local extirpations. This species 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 the Rio Grande (Figure 6). Our discovery of seven live *P. popeii* in the Devils River in 2008 – 2011, and 43 live *P. popeii* in 2002-2008 in the Rio Grande River confirmed that the species was still present in Texas. However, more significant was our 2011 discovery of a large population (602 live specimens recorded) of *P. popeii* at Laredo. The conservative estimation of over 8,000 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 suggests that a healthy host fish population occurs in this reach of the river, which is very important for unionid reproduction, and future population survival.

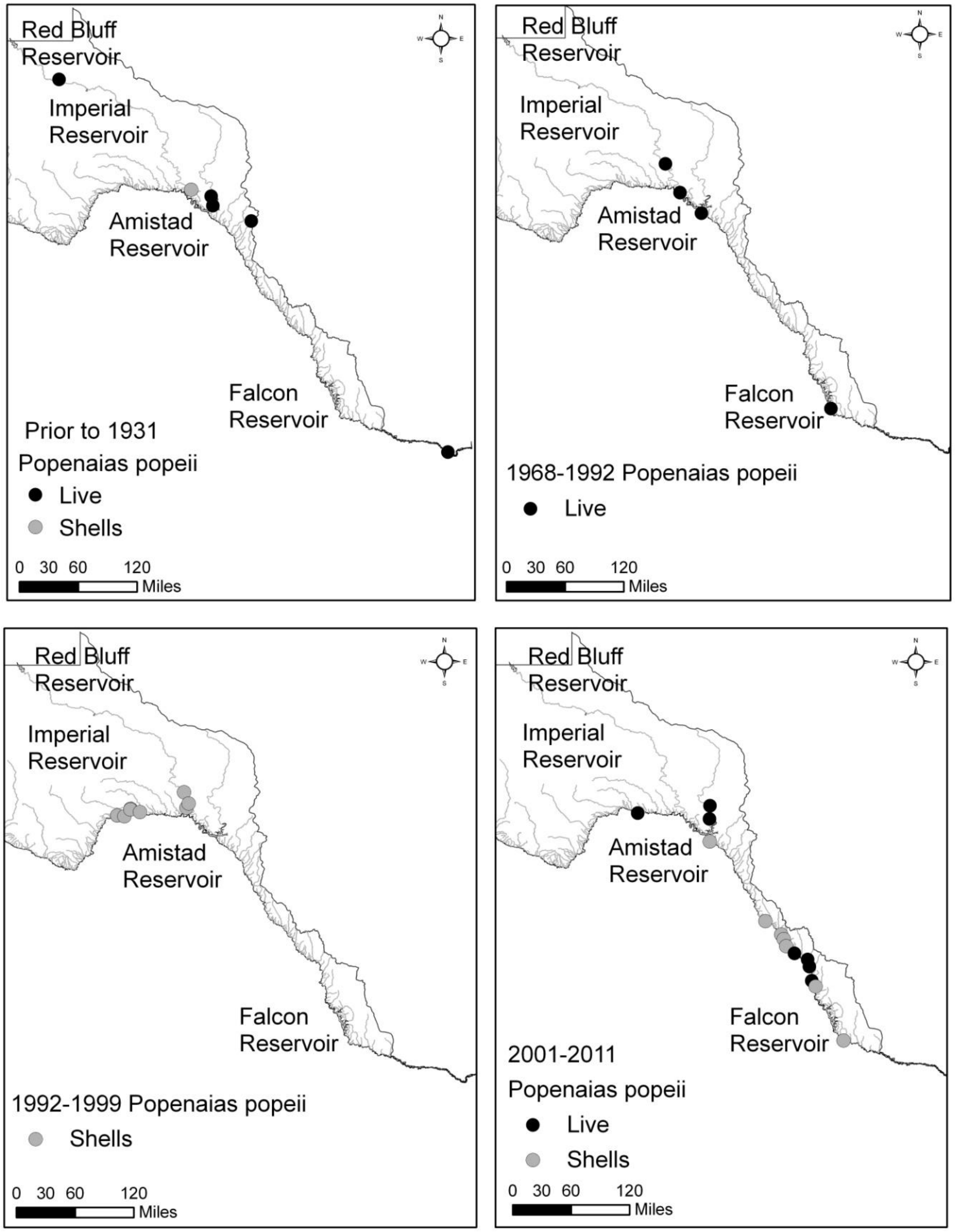


Figure 6. Map of the Rio Grande River watershed in Texas with sites where live and/or dead shells of *Popenaias popeii* were found prior to 1931 (based on data from Singley, 1893; Ellis *et al.*, 1930; Strecker, 1931; Taylor, 1967); in 1968-1992 (Metcalf, 1974, 1982;

Murray, 1975, Neck and Howells, 1984; Neck, 1987; Neck and Metcalf, 1988); in 1992 – 1999 (Howells 1994, 1996a, 1966b, 1997a, 1998, 1999, 2000); and from 2001 to 2011 (authors data).

These particular refuges in upper Laredo may be vulnerable to excess water fluctuations including periods of low water and floodings. During a low flow period (22.6 cms) in December, 2002, snowy egrets (*Egreta thula*) were observed feeding on *P. popeii* (Tom Miller, personal communication). Another site on Zacate Creek (Las Palmas Park, a TPWD mussel sanctuary) where > 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 (Tom Miller, personal communication). *Popenaias popeii* seem to have survived poorly after Zacate Creek (Las Palmas Park, Laredo) with only one live mussel and 2 shells found during numerous shore surveys of the 50 miles downriver to Falcon Lake.

Current and Potential Threats

The Rio Grande is presently one of the most impacted 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 the most destructive for unionid assemblages were impoundments, habitat degradation, salinization, pollution, and over extraction of water (Appendix, Table 5).

Water diversion from the middle 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). 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 border agricultural economic activity and regional maquiladoras (manufacturing or export assembly plants in border northern Mexico that produce parts and products for the United States) resulted in an over 5-fold loss of lower Rio Grande stream flow between 1905-1934 and 1951-1980 (reviewed in Douglas, 2009). Population in the basin was about 13 million inhabitants of 1990, and increased along the Texas border by 27% between 1980 and 1990, and by 26% on 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). Water-quality problems include elevated salinity, 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), and fragmentation of river channels by dams, diversions and depletions has eliminated the natural flood pulse, reducing productivity and structure of the riparian ecosystems.

Salinity concentrations in the Rio Grande are the result of both human activities and natural conditions. The naturally saline 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 the confluence with the Pecos River. Laboratory studies indicate that *P. popeii* show behavioral signs of physiological stress, followed by death, at a salinity of 7.0 ppt (Lang, 2001). Salinity in the area occupied by *P. popeii* in the Black River is around 0.9 ppt, increases significantly downstream to 2.8 ppt, and in the Pecos River, downstream of the Black River confluence range from 6.0-7.0 ppt (Lang, 2001). This increased salinity may have precluded populations in the main stem of the Pecos River even prior to its impoundment.

Some of the living species may now be extinct in the Pecos 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 mainstem (Taylor, 1967). 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 hydrologic 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 impact *P. popeii*.

Our discovery of the large extant population of *P. popeii* at Laredo, and analysis of existing and potential threats stress the necessity of conservation actions to protect this population and its habitat. We would advise the U.S. Fish and Wildlife Service, Texas Parks and Wildlife Department, Texas Commission on Environmental Quality, and local authorities to be alerted to possible threats that could extirpate this significant population of *P. popeii* in Texas, including the only known habitat of another extremely rare endemic, *T. cognata*. The most important measures to preserve these remaining populations in the Rio Grande at La Bota would be to ensure a constant stream flow from reservoirs upstream, and to prevent any damming of the river at this and adjacent sites, as well as to prevent any other activity that can increase streambed sedimentation, and suspended sediment and nutrient loading in the Rio Grande.

Past reports (Neck and Metcalf, 1988; Lang, 2001) have demonstrated that *P. popeii*, as well as other endemic species (Burlakova et al. 2011a), do not tolerate habitat inundation—these unionids require lotic habitat conditions (flowing water) rather than more lentic (a ponded, reservoir) hydrologic regimes. Impoundment of this area may destroy the habitat for two rare endangered regional endemics and may likewise affect the resident native fish population, which could in turn have adverse effects on the reproductive capacity of these native mussels that have an early life stage as obligate parasites on fishes.

4. Conclusions and Future Work

This joint Section 6 project between the New Mexico Department of Game and Fish and the Texas Parks and Wildlife Department determined the current distribution, documented long-term changes in distribution range, located existing population in need of protection, recorded habitat requirements of *P. popeii* in Texas, and established sites to monitor this population in the future. We discovered a largest extant population of *P. popeii* in the lower Rio Grande River in Laredo, Texas ever reported from Texas, New Mexico or Mexico. A species database with abundance and habitat data was provided to TPWD' Texas Natural Diversity Database, making all data readily available for conservation, monitoring and decision making. *Popenaias popeii*'s preference for low-flow refugia in shallow water and undercut banks of both the Black River and Rio Grande River implies that *P. popeii* is sensitive to habitat perturbations resulting from reduced stream discharge, large-volume pulse flows during seasonal rain events (Lang 2001, 2010), and habitat inundation. Our future studies in 2012-2014 will concentrate on population monitoring, reproductive biology, including identification of local fish hosts, and genetic structure of *P. popeii*. The La Bota population of *P. popeii* in the Rio Grande will be monitored to: (1) continue mark-and-recapture studies of mussels tagged in 2011; (2) document population status; and (3) assess habitat condition. As a result of this project we will suggest recommendations on sensitivity to disturbance and management options, and develop a common recovery plan and management options for *P. popeii* in the USA.

5. Status

Popenaias popeii is listed as New Mexico's Endangered (NMAC 1996), Texas' Threatened (Texas Register 35, 2010) and is a candidate for listing (priority 2) under the federal Endangered Species Act (Federal Register 2001). Live *P. popeii* have been reported and studied in New Mexico where it currently occupies approximately 12% of its historic range. It also has been reported from the Rio Grande below Big Bend National Park (Terrell Co.). A large (602 live specimens recorded) extant population of *P. popeii* in the lower Rio Grande River in Laredo, Texas was found in March 2011. The conservative estimation of over 8,000 individuals made this population by far the largest ever reported from Texas, New Mexico or Mexico.

6. Appendix

Manuscript by Karatayev, A. Y., Miller, T. D., and L. E. Burlakova "Long-term changes in unionid assemblages in Rio Grande, one of the World's top 10 Rivers at Risk" submitted to *Aquatic Conservation: Marine and Freshwater Ecosystems* in July 2011.

This appendix details 2011 activities accomplished under Objectives 1-4.

Acknowledgements

This survey was funded by the U.S. Fish and Wildlife Service (US FWS), Texas Parks and Wildlife Department (TPWD), and New Mexico Department of Game and Fish (NMDGF) (Joint Traditional Section 6 Project to LEB, AYK, B. Lang (NMDGF) and M. May (TPWD)). This study would not be successful without assistance of B. Lang (NMDGF), D. J. Berg and K. Inoue (Miami University, Oxford, OH), Y. Zhang and T. Noble (Texas State University, San Marcos). LEB was also supported by the Research Foundation of SUNY. We are very thankful to Mary F. Perrelli (Buffalo State College, Geography and Planning Department) for preparing GIS maps. We are very grateful to Ms. Carol A. Darstein, Ms. Carol A. Julian, Lisa A. Gatti (Research Foundation of SUNY), Ms. Cathleen Nasca (Great Lakes Center) and other staff of the Research Foundation and Buffalo State College for their help during the entire course of this project.

Prepared by: Lyubov E. Burlakova **Approved by:** ___________

Alexander Karatayev

Lyubov Burlakova, and Alexander Karatayev
BSC, Principal Investigators

Date: September 21, 2011

References

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

Burlakova LE, and Karatayev, AY. 2010. State-Wide Assessment of Unionid Diversity in Texas. Final Report to State Wildlife Grants Program. Federal Aid Grant T-43.

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. *Conservation Biology* **144**: 155-165.

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

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

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.

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.

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 74 (December 15, 2009) 66261.

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

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. 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. *The 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.

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* 1-65.

Karatayev AY, Miller TD, Burlakova LE. In Review. Long-term changes in unionid assemblages in Rio Grande, one of the World's top 10 Rivers at Risk. Submitted to *Aquatic Conservation: Marine and Freshwater Ecosystems* July 2011.

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. 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. Decsription 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.

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.

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.

Molles MC, Jr. , 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, Inc* **1975**: 43.

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

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. *The Texas Journal of Science* **40**: 259-268.

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. *The Texas Journal of Science* **39**: 177-182.

Federal Register 2009. 74 FR 66261 (December 15, 2009). **74**: 66261-66271.

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

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

Richter, B. D., D. P. Braun, M. A. Mendelson, and Master, L. L. 1997. Threats to imperiled freshwater fauna. Conservation Biology 11 (5): 1081-1093.

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

Strayer DL, Smith DR. 2003. *A guide to sampling freshwater mussel populations*. Bethesda, Maryland.

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 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]

USFWS (2010) *Species Reports for Texas*. Available at: http://ecos.fws.gov/tess_public/pub/stateListingIndividual.jsp?state=TX&status=listed.

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(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.

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

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

ALEXANDER Y. KARATAYEV[1*], THOMAS D. MILLER[2], and LYUBOV E. BURLAKOVA[1,3]

[1] Great Lakes Center, Buffalo State College, 1300 Elmwood Avenue, Buffalo, NY 14222, USA

[2] Environmental Science Center, Laredo Community College, West End Washington Street Laredo, TX 78040, USA

[3] The Research Foundation of SUNY, Buffalo State College, Office of Sponsored Programs, 1300 Elmwood Avenue, Buffalo, NY 14222-1095, USA

*Correspondence to: Alexander Y. Karatayev, Great Lakes Center, Buffalo State College, 1300 Elmwood Avenue, Buffalo, NY 14222, USA. E-mail: karataay@buffalostate.edu, Phone: (1) 716-878-5423, Fax: (1)-716-878-6644

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

ABSTRACT

1. The Rio Grande River 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 drainage used to be represented by unique assemblage, including four endemic species (*Truncilla cognata*, *Potamilis metnecktayi*, *Popenais popeii*, and *Quadrula conchiana*), however surveys in 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 authors in 2001-2011 on the Rio Grande and its tributaries recovered live *T. cognata*, *P. metnecktayi*, and the largest population of *P. popeii* ever reported. Overall the unionid assemblage of the Rio Grande drainage changed dramatically during the last century.
3. We documented decline in species diversity, range fragmentation, local extirpations, and introduction of widespread common species. Two species (*Q. conchiana* and *Quincuncina mitchelli*) are already extinct from Texas part of the Rio Grande basin. *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 Pecos River and Las Moras Creek along with the reduction and fragmentation of its range in Devils River and Rio Grande.
4. Among the environmental factors responsible for the degradation of unionid assemblages the most important are impoundments, habitat degradation, salinization, pollution, and water over extraction.

INTRODUCTION

The loss of biodiversity makes ecosystems vulnerable, and this may be particularly true for invertebrate taxa, which play an important role in ecosystem functioning (Tilman *et al.*, 1994; Palmer, 1997; Covich *et al.*, 1999). Species loss is especially large in freshwaters, where many species are far more imperiled than their marine or terrestrial counterparts (Jackson *et al.*, 2001; Strayer and Dudgeon, 2010). The large loss of diversity in freshwaters is due to widespread habitat degradation, pollution, flow regulation, and water extraction, and these activities are predicted to increase in the future (Naiman and Turner, 2000; Jackson *et al.*, 2001; Strayer and Dudgeon, 2010). The opportunity to conserve much of the remaining biodiversity in freshwaters may vanish if trends in human demands for freshwater remain unaltered and species losses continue at current rates (Dudgeon *et al.*, 2006).

The Rio Grande/Rio Bravo River is one of the longest rivers in North America. 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 (Dahm *et al.*, 2005; Wong *et al.*, 2007). Due to water over-extraction for the increasing irrigation and domestic consumption, the riverbed between El Paso and Presidio frequently lies 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). Many additional factors contributed to the recent status of the Rio Grande, including persistent drought, increase in border population, and declines in the water quantity and quality (Dahm *et al.*, 2005; Wong *et al.*, 2007; Douglas, 2009).

In addition to being a political border, the Rio Grande is a globally important river for freshwater biodiversity, supporting numerous endemic fish, birds, and molluscs (Grommbridge and Jenkins, 1998; Revenga *et al.*, 1998; Johnson, 1999; Revenga *et al.*, 2000). Many of these species have already become extinct; others are facing a sharp decrease in their population density or range fragmentation. As the result the Rio Grande is considered the most endangered river system in the North American continent, and one of the world's top 10 rivers at risk (Wong *et al.*, 2007).

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 drainage represent a unique assemblage and are distinct from the rest of Texas (Neck, 1982; Neck and Metcalf, 1988; Burlakova *et al.*, 2011a; Burlakova *et al.*, 2011b). The first data on unionid bivalves of Rio Grande and its tributaries were published at the turn of the 19th century (Singley, 1893; Simpson, 1900; Simpson, 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 authors in 2001-2011 recovered live *Truncilla cognata*, *Potamilis metnecktayi*, and the largest population of *Popenais popeii* ever reported, proving that at least three endemic unionid species are still present in the river. The goals of this paper are to describe over a hundred years of changes in the unionid assemblage of the Rio Grande River in Texas with the special focus on endemic species, and to discuss major factors affecting unionid diversity and distribution.

METHODS

Study area

The Rio Grande (length: 2,830 km, watershed area: 870,000 km^2) is the one of the longest rivers in the North America (Dahm *et al.*, 2005). With its headwaters high in the San Juan Mountains of Colorado, it flows through New Mexico in a generally southeastern direction and then forms the shared border between Texas and Mexico before it empties into the Gulf of Mexico near Brownsville, Texas (Dahm *et al.*, 2005). The Rio Grande traverses seven physiographic provinces with a variety of habitats, from mountain forests and high mountain deserts to chaparral and lowland brush country. A large portion of the watershed is arid or semiarid, and only about half of the total basin area actually contributes to the river's overall flow. Climate changes markedly from the headwaters to the mouth, and in Texas average temperatures change from 6.0°C in January to 27.9°C in July in El Paso, Texas, to 14.7^{o}C - 29.8°C near the mouth in McAllen. Average annual precipitation changes from 21.8 cm in the west to 57.7 cm in the east, with peaks in May and September and a minimum in March. Land use in the Rio Grande basin includes forest (14%), cropland (5%), shrub land (43%), grassland (31 %), and urban (7%) (Dahm *et al.*, 2005). Most of the basin is either desert shrub land or desert grassland. 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 United States. Over 10 million people live in the Rio Grande basin, and urban areas are growing fast, particularly in border towns between the United

States and Mexico. By 2060 the area from Eagle Pass to Brownsville is projected to almost triple in population (Texas Water Development Board, 2007).

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 3% - 5% of the agricultural consumption. The Rio Conchos, the Pecos River and the Devils River historically contributed the main flow of the Rio Grande in the stretch between the confluence and Amistad Reservoir, although these flows have been reduced substantially and are stored at Amistad International Reservoir. Irrigated agriculture is the primary use of the Rio Grande surface flow throughout the basin that accounts for > 80% of all water taken from the river (Dahm *et al.*, 2005).

Data collection

To assess the diversity, distributions, and long-term changes in unionid assemblages of the Rio Grande system within Texas, we used both field studies and historic data. To analyze the historical data we created a database containing information of unionid species name, waterbody name, location, recorded date, and a collector's name using all available data including published records, museum collections, and web-based searches. Published records included Singley (1893), Ellis *et al*. (1930), Strecker (1931), Taylor (1967), Metcalf (1974; 1982), Murray (1975), Neck (1984; 1987), Neck and Metcalf (1988), Howells (1994; 1996b; 1996a; 1997a; 1998; 1999; 2000), Johnson (1999), and Strenth *et al*. (2004). Based on the available data we analyzed unionid assemblages in Rio Grande system using the following time periods: (1) initial reports (before 1931), including collections made by the United States and Mexico Boundary Surveys (mostly done in 1892, Taylor, 1967), Singley (1893), Ellis *et al*. (1930), and Strecker (1931) data; (2) 1968-1990s based mostly on Metcalf and Neck data (Metcalf, 1974; Murray, 1975; Metcalf, 1982; Neck and Howells, 1984; Neck, 1987; Neck and Metcalf, 1988); (3) 1992 – 1999s based on Howells data (Howells 1994; 1996b; 1996a; 1997a; 1998; 1999; 2000); and (4) 2001 – 2011 based on our data.

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 to 25 times. Due to the prevalence of private land in Texas, where only 2% of the lands remain in public ownership (Texas Parks and Wildlife Department, 1974), survey sites were often selected within state parks, near public boat ramps, or based on accessibility from roads that either crossed or approached a waterbody. In addition, many days and nights were spent on the river, accessing numerous sites by canoe or kayak. When the survey was done 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 via hand collection of both live and dead mussels, by wading in low water and by snorkeling or diving. Reconnaissance sampling (timed searches) was used at some sites to reveal the presence of mussels and species diversity (Strayer *et al.*, 1997; Vaughn *et al.*, 1997). If mussel assemblages were present, quantitative methods (randomly placed 0.25 m^2 quadrats, 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 mm. Live mussels after measurements were carefully bedded into the sediment from which they were taken. Shell condition of dead mussels was recorded for each specimen. A mark-and-recapture-census was conducted at the La Bota Ranch site in Northern Laredo in March 2011 using methods described by Lang (2001) and Villella *et al*. (2004). Following recommendations by Villella *et al*. (2004), we sampled three consecutive days to estimate capture probabilities using closed population models. 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, to one valve. Specimens were identified using published taxonomic keys and descriptions (Howells *et al*., 1996; Johnson, 1998). 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, species name, name of person who collected and identified the specimen, date of collection, and detailed site information.

Data analysis

Several assumptions were made in our analysis. If the status of recorded unionid was not reported in the paper used for historical analysis, we assumed that the specimen was found alive; 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).

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

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 per each species found per time search effort at each sampling site (mussels per man per hour, mussels mh^{-1}). 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, we used Non-metric Multi-Dimensional Scaling (NMDS), 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 $p < 0.05$.

RESULTS

Long-term changes in unionid diversity

We found that Rio Grande still supports most of the unionid species ever reported from this river, including regional endemics *Potamilus metnecktayi*, *Popenaias popeii*, and *Truncilla cognata* (Table 1). Two species of unionids (*Quadrula apiculata* and *Potamilus purpuratus*) were introduced into the Rio Grande during 20th century (Johnson, 1999). Historical records of *P. purpuratus* from the Rio Grande drainage (Singley, 1893) have been shown to be misidentified specimens of *Cyrtonaias tampicoensis* (Neck and Metcalf, 1988; Johnson, 1999). The current status of another introduced species to Rio Grande drainage (*Pyganodon grandis*) is unknown.

During our study the most common unionid species were *C. tampicoensis* and *Q. apiculata* found live at 28.6% of sites sampled (Table 2). The percentage of sites where live molluscs were found compared to 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* that were found mostly in tributaries (Table 2). The highest diversity of unionids was found at about a 15 mile stretch of the Rio Grande above Laredo (Figure 2-4). 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 (Fig. 5, R = 0.942, p = 0.001, one-way ANOSIM). On soft and unconsolidated sediments (silt, sand, small gravel, and combination 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 boulder resting on the bedrock. We often found up to 10 individuals 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 Rio Grande on the John's Marina site, South of Dryden, Terrell County in 2003-2008. Mussels were generally found along the shores, in soft sediments (sometimes 20 cm deep in a mixture of silt and clay) at 0.5 - 1.2 m depth (at low water levels > 30cms). Their size varied from 63 to 124 mm averaging 87.1 mm (±17.6 standard deviation). Dead shells of *P. metnecktayi* were found at 7 more sites (Figure 2). *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 fifteen sites below Lake Amistad, only 50 long dead or sub-fossil valves were found, possibly indicating a once widespread population.

Truncilla cognata

We found a total of 19 live *T. cognata* 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 the small size, it was difficult to distinguish *T. cognata* from gravel, adding to the hardship of detecting this cryptic species. Many excavations were done below the Water Treatment Plant in Laredo, but no live mussels were found there. In 2011 a total of 12 *T. cognata* were found on 5 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 on a total of 12 sites (Figure 3). Very recently dead specimens (i.e. shells with flesh, to 51 mm) were found at four subsites below Laredo into Zapata County. Based on our results, it is likely to find additional specimens at Pinto Valle and Dolores Creek (Webb County). All of the 19 live *T. cognata* from our recent surveys have been found at confluences of Santa Isabel, Sombrerito, and Zacate Creeks above Laredo. Their presumed habitat preference of small gravel/sand/silt mixed substrates is also well known by sand and gravel operations as each of these areas has or had a material excavation site nearby.

Popenaias popeii

During ten years of our surveys, we found one live *P. popeii* in Rio Grande River in Terrell County (John's Marina), seven in Devils River, and 604 live *P. popeii* were found in Laredo. In total, live mussels were found at 7 sites, and dead shells were found at a total of 5 more sites (Figure 4). 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 substrata for mussels. At this mark-recapture site (area sampled ca. 1,000 m^2) we found a total of 406 live *P. popeii*. 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, total population estimation may be near 1,500 *P. popeii* at the site, with density of ~1.5 m^{-2}. This population consisted of multiple age-classes, from 33.2 to 87 mm (mean 63 mm (± 1, 95% confidence interval). Over a third of the mussels measured were < 60 mm, and 12 individuals were < 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 4,700 individuals of this species may be in this area. At three other subsites located ca. 1 mile downstream from this mark-and-recapture locality, we found a total of 182 live Texas hornshell in 3 person-hours of timed searches. These subsites were located along a 280 m river stretch, and may contain up to 4,000 more mussels. Therefore, the total population of *P. popeii* in the La Bota area may contain up to 8,700 mussels.

DISCUSSION

Long-term changes in unionid diversity

Unionid assemblage of the Rio Grande Drainage changed dramatically over the last century (Tables 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 conchiana* and *Quincuncina mitchelli*) are already extinct from the Texas part of the Rio Grande basin. The most drastic changes were recorded during last 40 years (Table 3).

Several streams and rivers of the Rio Grande Drainage have lost all or a significant number of unionid species. In 1892, 8 species of unionids were found in Las Moras Creek in Fort Clark (Brackettville, Kinney County), including endemic *Q. couchiana* and *P. popeii* (Taylor, 1967). Repeated reexamination of this site since 1971 failed to reveal any live or dead unionids (Murray, 1975; Howells, 1997a; authors unpublished data). Similarly, Devils River used to support 5 species of unionids, including *Q. couchiana* and *P. popeii* (Singley, 1893; Strecker, 1931; Johnson, 1999). Repeated detailed sampling of this river in recent years revealed only 6 live individuals of *P. popeii*, and one relatively recently dead shell of *U. imbecillis.* Five species of unionids historically were reported from the Pecos River, including endemic *P. metnecktayi* and *T. cognata* (Singley, 1893; Metcalf, 1982; Johnson, 1999). Most of the species were found at the mouth of the Pecos River, which is now inundated by the Lake Amistad (Metcalf, 1982). No live native unionids were found in this river since 1980-s (Howells, 2001; Lang, 2001).

Along with the local extirpation of rare and endemic species from the Rio Grande drainage the unionid assemblage was reshaped by the introduction of common species (*Q. apiculata, P. purpuratus*, and *P. grandis*) nonnative to this drainage (Metcalf and Smart, 1972; Metcalf, 1982;

Johnson, 1999). *Quadrula apiculata* became very common species in the 20th century in Rio Grande and its tributaries. Previous research noted a lack of fossil *Q. apiculata* (Metcalf, 1982), and no fossil specimens were found during our study. Our 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; Howells, 1999). Another introduced species, *P. grandis*, was reported from Grandjeno Lake in 1892 (Singley, 1893) and canals in Hidalgo County (Ellis *et al.*, 1930), from El Toro Cement Agency Lake in El Paso in 1969 (Johnson, 1999), and in Topaz Power Plant cooling pond, Laredo in 2006 (T. Miller unpublished data).

Endemic species account

Potamilus metnecktayi

This regional endemic was reported to be extremely rare in the Rio Grande in Texas (Neck and Metcalf, 1988) and uncommon even at the fossil localities sampled in New Mexico and Mexico (Metcalf, 1982). Live specimens in the US were collected in Texas only by Metcalf on 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). This last area is now flooded by Amistad Reservoir and it is very likely that the population of *P. metnecktayi* is already extinct in the Pecos River. No live or dead *P. metnecktayi* were found in Del Rio area during our sampling in 2008. Only dead shells of *P. metnecktayi* were found in Texas since mid 1970s, including specimens from Rio Grande in Terrell County in 1992, Brewster and Terrell counties in 1998, and another one in Terrell county in 1999 (Howells, 1994; Howells *et al.*, 1997; Howells, 1999, 2000). There is no available data on the status of *P. metnecktayi* in

Mexico, where the species has been reported from the Rio Salado Drainage (Johnson, 1999). 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 it's distribution range has been significantly reduced during 20th century. Additional studies are urgently needed to estimate the current distribution and population size of *P. metnecktayi* in the Rio Grande, and to develop appropriate measures for the species conservation.

Truncilla cognata

Truncilla cognata is another regional endemic that was described from the Devil's River, Texas, and Rio Salado, Nuevo Leon, Mexico (Lea, 1857; Johnson, 1999). This species which has a Nature Serve rounded global status critically imperiled (NatureServe, 2009) and is considered endangered by the American Fisheries Society, has been recently added to the state's list of threatened species (Texas Register 35, 2010). *Truncilla cognata* is currently under consideration for federal listing by the U. S. Fish and Wildlife Service (74 FR 66261, December 15, 2009). In US *T. cognata* was reported only from few sites in Texas (Table 4). Living or freshly dead specimens of *T. cognata* were reported from the same two sites as *P. metnecktayi,* in Rio Grande near Del Rio in 1972 and at mouth of the Pecos River (now inundated) by the former US Hwy 90 crossing in 1968 (Metcalf, 1982). No living or dead specimens were collected there since 1972 (Howells *et al.*, 1997; Howells, 2001). Again it is likely that the Pecos River population of *T. cognata* is already extinct and the 19 live specimens that we 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. Nature Serve ranks the Texas hornshell as critically imperiled across its range (NatureServe, 2009). This species has been recently 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), Devils River (Singley, 1893), Pecos River (Metcalf, 1982) and from two distinct areas in Rio Grande (Metcalf, 1982; Neck and Metcalf, 1988) (Table 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 living population of *P. popeii.*

Extensive surveys along 48 km of Las Moras Creek in 1971, 1973, and 1975 yielded no living *P. popeii* (Murray, 1975). This population is believed to be extirpated 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 (Murray, 1975; Howells *et al.*, 1996). Pecos River sites were flooded by Amistad Reservoir and *P. popeii* was extirpated. In Devils River *P. popeii* survived much longer and “localized concentrations of living specimens” were reported from the Devil’s River, Val Verde County by Neck (1984). No live *P. popeii* were found in Rio Grande since mid-1970s (Howells, 2001). Our discovery of seven live *P. popeii* in the Devils River in 2008 – 2011, and 43 live *P. popeii* in 2002-2008 in Rio Grande River confirmed that the species was still present in Texas. However, more significant was our discovery of a large population (602 live specimens recorded) of *P. popeii* in 2011 in Laredo. The conservative estimation of over 8,000 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 suggests 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 floodings. During a low flow period (22.6 cms) in December, 2002, snowy egrets (*Egreta thula*) were observed feeding upon *P. popeii*. Another site on Zacate Creek (Las Palmas Park, a TPWD mussel sanctuary) where > 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. *Popenaias popeii* seem to survive poorly after Zacate Creek (Las Palmas Park, Laredo): only one live mussel and 2 shells have been found in numerous shore surveys of the 50 mile downriver to Falcon Lake.

Another important finding was identification of suitable habitat for *P. popeii* in Rio Grande. This habitat is similar to the preferred habitat for this species in Black River: low-flow refuges 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 such as *C. tampicoensis*, *T. cognata*, *M. nervosa*, and *Q. apiculata* (Figure 5).

Environmental factors affecting unionids

The Rio Grande presently is one of the most impacted 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 were impoundments, habitat degradation, salinization, pollution, and water over extraction (Table 5).

Impoundments. Some of the living species may now be extinct in the Pecos 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 mainstem (Taylor, 1967). 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 hydrologic 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 impact the Texas hornshell.

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 Texas hornshell distribution in Pecos River and in Rio Grande below the confluence with Pecos River. Laboratory studies indicate that Texas hornshell show behavioral signs of physiological stress, followed by death, at a salinity of 7.0 ppt (Lang, 2001). Salinity in the area occupied by Texas hornshell in the Black River is around 0.9 ppt, increases significantly downstream to 2.8 ppt, and in the Pecos River, downstream of the Black River confluence range from 6.0-7.0 ppt (Lang, 2001). This increased salinity may have precluded populations in the main stem of the Pecos River even prior to its impoundment.

Water Over extraction, Habitat destruction, and Pollution. Water diversion from middle 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). 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 border agricultural economic activity and regional maquiladoras (manufacturing or export assembly plants in border northern Mexico that produce parts and products for the United States) resulted in an over 5-fold loss of lower Rio Grande stream flow between 1905-1934 and 1951-1980 (reviewed in Douglas, 2009). Population in the basin was about 13 million inhabitants of 1990, and increased along the Texas border by 27% between 1980 and 1990, and by 26% on 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). Water-quality problems include elevated salinity, 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), and fragmentation of river channels by dams, diversions and depletions has eliminated the natural flood pulse, reducing productivity and structure of the riparian ecosystems.

Conclusions

Although the Rio Grande still supports the majority of unionid species ever reported alive from this river, including endemic *P. popeii, P. metnecktayi,* and *T. cognata*, its unionid assemblage has changed dramatically during the last century. We documented decline in species diversity, range fragmentation, local extirpations, and introduction of widespread common species. Two species (*Q. conchiana* and *Q. mitchelli*) are already extinct from Texas part of the Rio Grande basin. *Potamilus metnecktayi* and *T. cognata* have been extirpated from the Pecos River and their ranges in the Rio Grande have been reduced to only one small location in Terrell County for *P. metnecktayi* and very limited area near Laredo for *T. cognata. Popenaias popeii* has been extirpated from Pecos River and Las Moras Creek along with the reduction and fragmentation of its range in Devils River and Rio Grande. The largest ever known population of *P. popeii* was found near Laredo in 2011. Numerous streams and rivers of the Rio Grande Drainage lost all or significant number of unionid species. Among the environmental factors responsible for the degradation of unionid assemblages the most important are impoundments, habitat degradation, salinization, pollution, and water over extraction.

ACKNOWLEDGEMENTS

Surveys were conducted in conjunction with the US Geological Survey. Many kayak and canoe trips surveys were accomplished with the assistance of Dr. Tom Vaughan (Texas A&M International). This study was funded in part by the U.S. Fish and Wildlife Service (US FWS), Texas Parks and Wildlife Department (TPWD), and New Mexico Department of Game and Fish (NMDGF) (Joint Traditional Section 6 Project to LEB, AYK, B. Lang (NMDGF) and M. May

(TPWD) and by the US FWS' State Wildlife Grant Program through the TPWD (PIs LEB, AYK, M. E. May, M. D. Warriner, and B. Gottfried, TPWD). LEB was also supported by the Research Foundation of SUNY. We are very thankful to Mary F. Perrelli (Buffalo State College, Geography and Planning Department) for help with GIS. This study would not be successful without assistance of B. Lang (NMDGF), D. J. Berg and K. Inoue (Miami University, Oxford, OH), Y. Zhang and T. Noble (Texas State University - San Marcos).

REFERENCES

Bogan AE. 1993. Freshwater bivalve extinctions (Molusca: 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. *Conservation Biology* **144**: 155-165.

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

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.

Covich AP, Palmer MA, Crowl TA. 1999. The role of benthic invertebrate species in freshwater ecosystems - Zoobenthic species influence energy flows and nutrient cycling. *Bioscience* **49**: 119-127.

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.

Federal Register 74 (December 15, 2009) 66261.

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.

Grommbridge 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. *The 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 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.

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* 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. New Mexico Department of Game and Fish, Albuquerque, NM.

Lea I. 1857. Decsription 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.

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.

Molles MC, Jr. , 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, Inc* **1975**: 43.

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

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. *The 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. *The Texas Journal of Science* **40**: 259-268.

Palmer MA. 1997. Biodiversity and ecosystem processes in freshwater sediments. *AMBIO: A Journal of the Human Environment* **26**: 571-577.

Register F. 2009. 74 FR 66261 (December 15, 2009). **74**: 66261-66271.

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). U.S. Fish and Wildlife Service: Twin Cities, Minnesota; 5-18.

Strayer DL, Smith DR. 2003. *A guide to sampling freshwater mussel populations*. Bethesda, Maryland.

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 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.

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 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 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]

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]

Tilman D, May RM, Lehman CL, Nowak MA. 1994. Habitat destruction and the extinction debt. *Nature* **371**: 65-66.

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(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.

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

Species	Before 1931		1968 - 1990		1994 – 1999		2000 – 2011	
	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.	n. r.	n. r.
Quadrula apiculata[b]	n. r.	n. r.	L	L	L	L	L	L
Quadrula conchiana[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 (1)	5 (4)	5	8

[a] Regional endemics

[b] Introduced species

*Only fossil and greatly weathered specimens are known from Texas part of Rio Grande drainage

Table 2. Occurrence of unionid species in Rio Grande drainage, and separately in the river main steam and its tributaries based on 2001-2011 surveys. A total of 28 pooled sites were studied in the drainage, including 21 sites in Rio Grande River (excluding reservoirs) and 7 sites on tributaries. Species occurrence was calculated as a number of sites where the species was found, and percent occurrence was calculated as the percent of sites where the species was found from total. Single valves were counted as ½ of a shell.

Species	Rio Grande Drainage			Rio Grande River			Tributaries only		
	Total found	Occurrence (# pooled sites)	% occurrence	Total	Occurrence (# pooled sites)	% occurrence	Total	Occurrence (# pooled sites)	% 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	0.0%	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%**

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 conchiana* 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*

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

Waterbody	Historical collections	Current Status
Potamilus metnecktayi		
Rio Grande, 9.7 km West of Del Rio in 1972	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 authors in 2003-2008
Pecos River, 1.28 km above its mouth at the former US Hwy 90 crossing	1968 (Metcalf, 1982)	Flooded by Amistad Reservoir
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 authors at 2 sites in 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)	Population extirpated (Murray, 1975)
Devils River, Val Verde County	1892 (Singley, 1893)	7 live mussels were found by authors in 2008-2011
Pecos River, Val Verde County	1903, 1968, 1972, 1973 (Metcalf, 1982)	Flooded by Amistad Reservoir, population extirpated
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 authors in 2002-2011
Rio Grande, Johnson Marina, Terrell County	No historical records from this location	1 live specimen was collected by authors in 2008

Table 5. Environmental factors associated with the human activities impacted unionid assemblages in the Texas part of Rio Grande Drainage

Environmental factor	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
Water over extraction	• Decreased or fragmented range of all unionids, including *P. popeii*, *P. metnecktayi*, and *T. cognata* in the Rio Grande

Figure Legends

Figure 1. Map of the Rio Grande watershed in Texas with sampling sites. Unionid mussels were surveyed during 2001– 2011 at total 162 sample sites (subsites) pooled into 28 larger sites. Texas counties, major cities (in italic) and reservoirs are indicated.

Figure 2. Map of the Rio Grande River watershed in Texas with sites where live and/or dead shells of *Potamilus metnecktayi* were found in 1968-1992 (Metcalf, 1974, 1982; Murray, 1975; Neck and Howells, 1984; Neck, 1987; Neck and Metcalf, 1988); in 1992 – 1999 (Howells 1994, 1996a, 1966b, 1997a, 1998, 1999, 2000); and from 2001 to 2011 (authors data).

Figure 3. Map of the Rio Grande River watershed in Texas with sites where live and/or dead shells of *Truncilla cognata* were found in 1968-1992 (data from Metcalf, 1974, 1982; Murray, 1975, Neck and Howells, 1984; Neck, 1987; Neck and Metcalf, 1988), and from 2001 to 2011 (authors data).

Figure 4. Map of the Rio Grande River watershed in Texas with sites where live and/or dead shells of *Popenaias popeii* were found prior to 1931 (based on data from Singley, 1893; Ellis *et al.*, 1930; Strecker, 1931; Taylor, 1967); in 1968-1992 (Metcalf, 1974, 1982; Murray, 1975, Neck and Howells, 1984; Neck, 1987; Neck and Metcalf, 1988); in 1992 – 1999 (Howells 1994, 1996a, 1966b, 1997a, 1998, 1999, 2000); and from 2001 to 2011 (authors data).

Figure 5. NMDS ordination plot of the unionid assemblages in Rio Grande near Laredo found on sand, silt and gravel and under rocks. Relative density data (mussels mh^{-1}) for live molluscs collected at all sampled sites (excluding sites where less than two species were collected) were square-root transformed and converted to similarity matrix using

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).

Figure 1

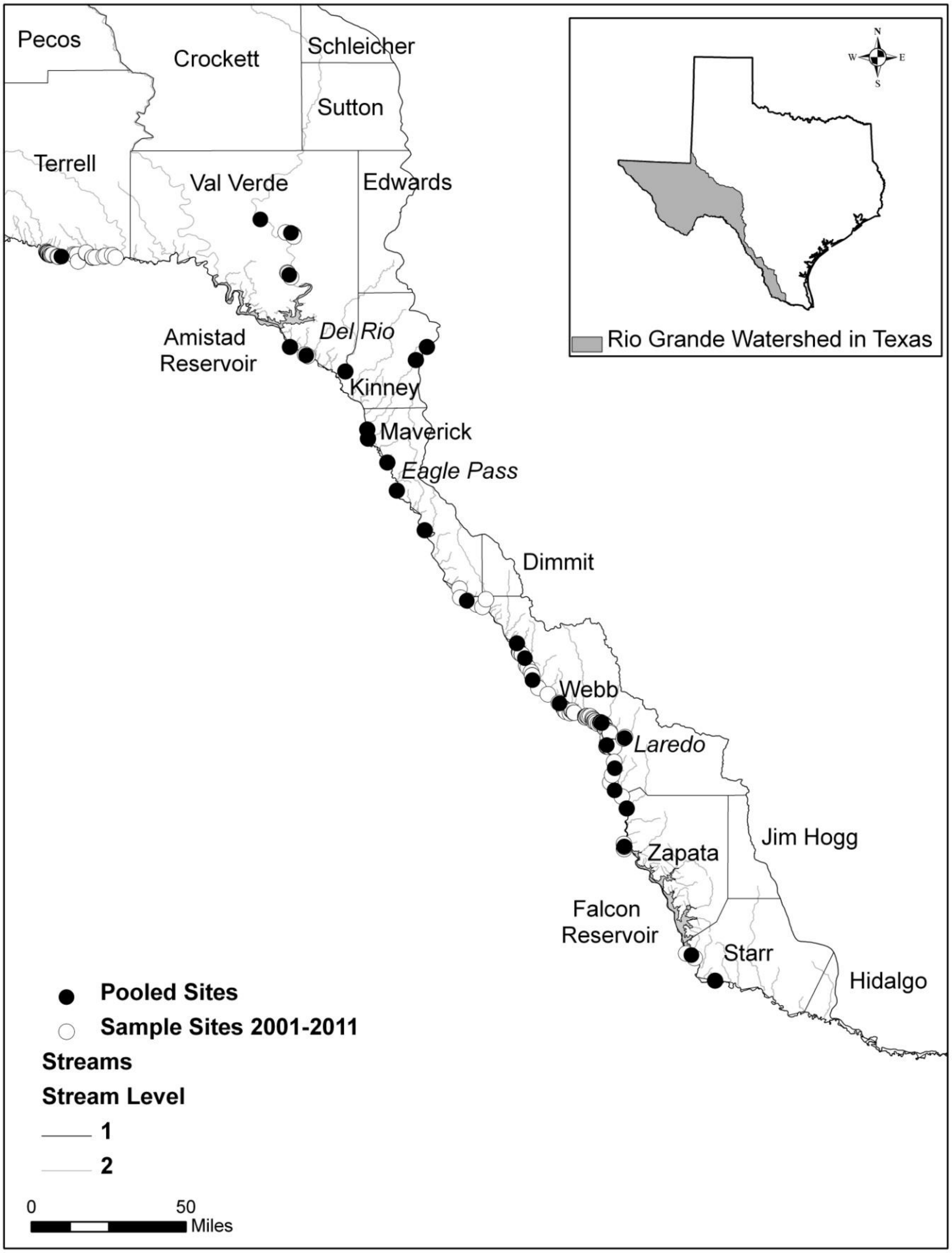

Pecos
Crockett
Schleicher
Sutton
Terrell
Val Verde
Edwards
Amistad Reservoir
Del Rio
Kinney
Maverick
Eagle Pass
Dimmit
Webb
Laredo
Jim Hogg
Zapata
Falcon Reservoir
Starr
Hidalgo
Rio Grande Watershed in Texas
N
W
E
S
Pooled Sites
Sample Sites 2001-2011
Streams
Stream Level
1
2
0
50
Miles

Figure 2

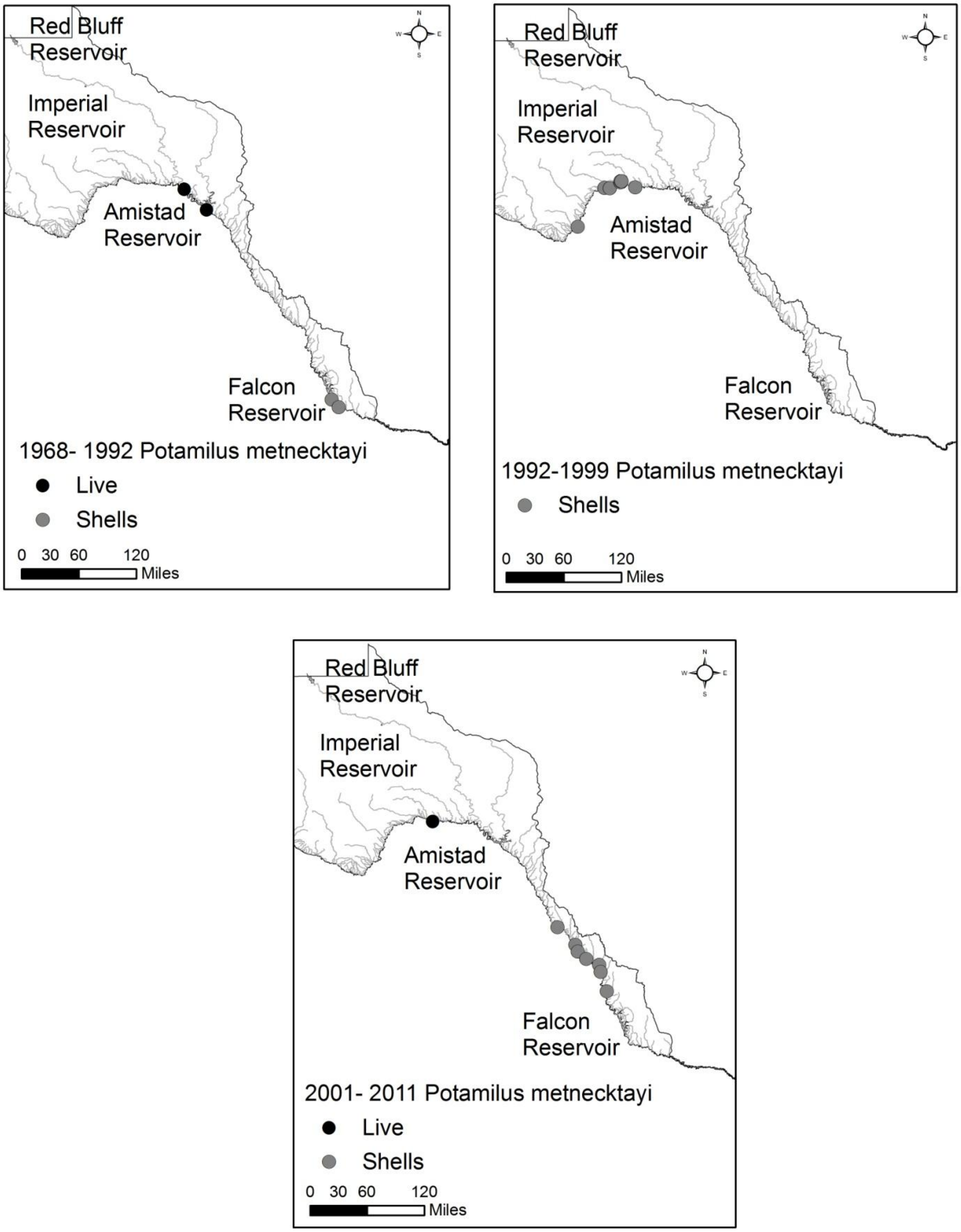

Red Bluff Reservoir
Imperial Reservoir
Amistad Reservoir
Falcon Reservoir
1968- 1992 Potamilus metnecktayi
Live
Shells
0 30 60 120
Miles
Red Bluff Reservoir
Imperial Reservoir
Amistad Reservoir
Falcon Reservoir
1992-1999 Potamilus metnecktayi
Shells
0 30 60 120
Miles
Red Bluff Reservoir
Imperial Reservoir
Amistad Reservoir
Falcon Reservoir
2001- 2011 Potamilus metnecktayi
Live
Shells
0 30 60 120
Miles

Figure 3

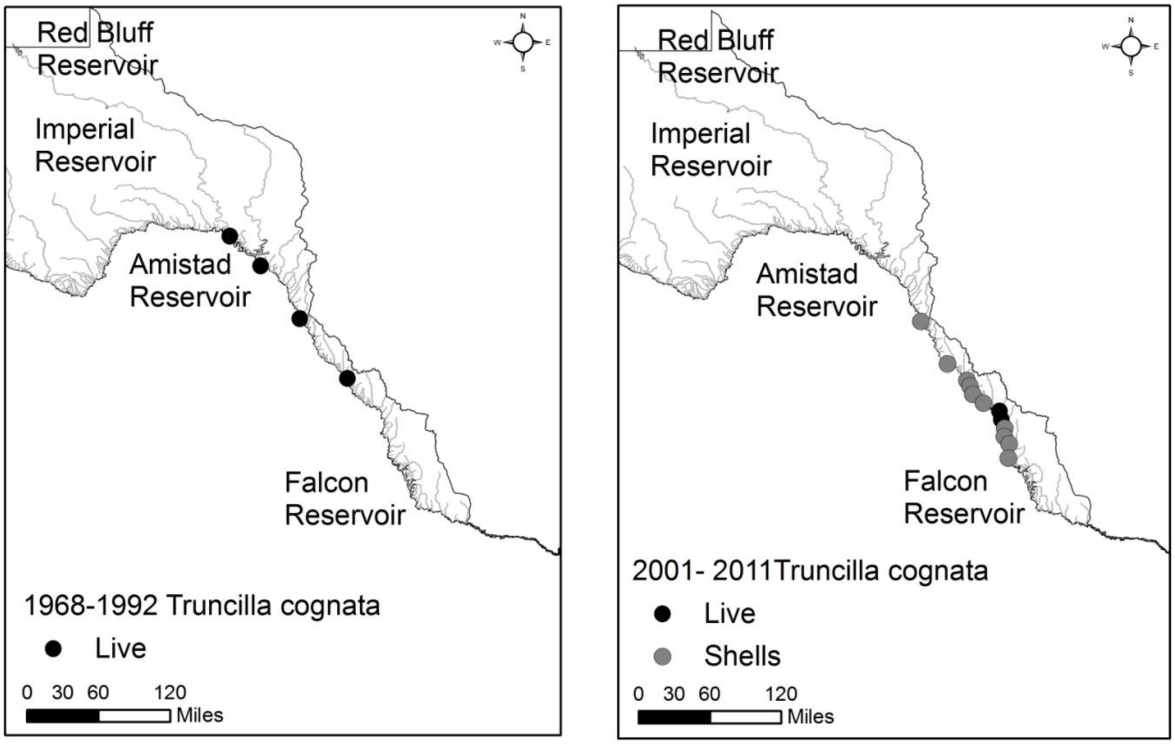

Red Bluff
Reservoir
Imperial
Reservoir
Amistad
Reservoir
Falcon
Reservoir
1968-1992 Truncilla cognata
Live
0 30 60 120
Miles
Red Bluff
Reservoir
Imperial
Reservoir
Amistad
Reservoir
Falcon
Reservoir
2001- 2011Truncilla cognata
Live
Shells
0 30 60 120
Miles

Figure 4

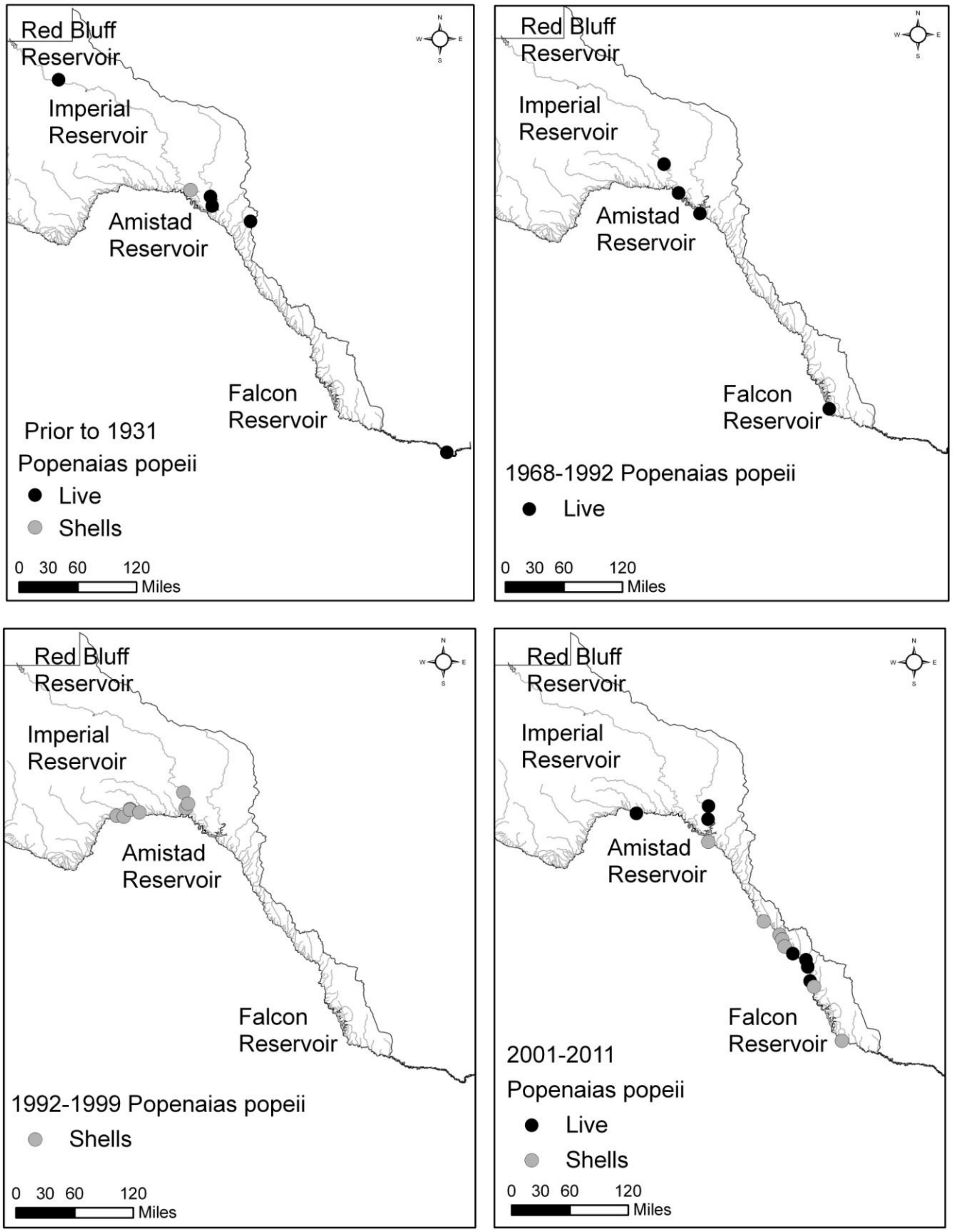
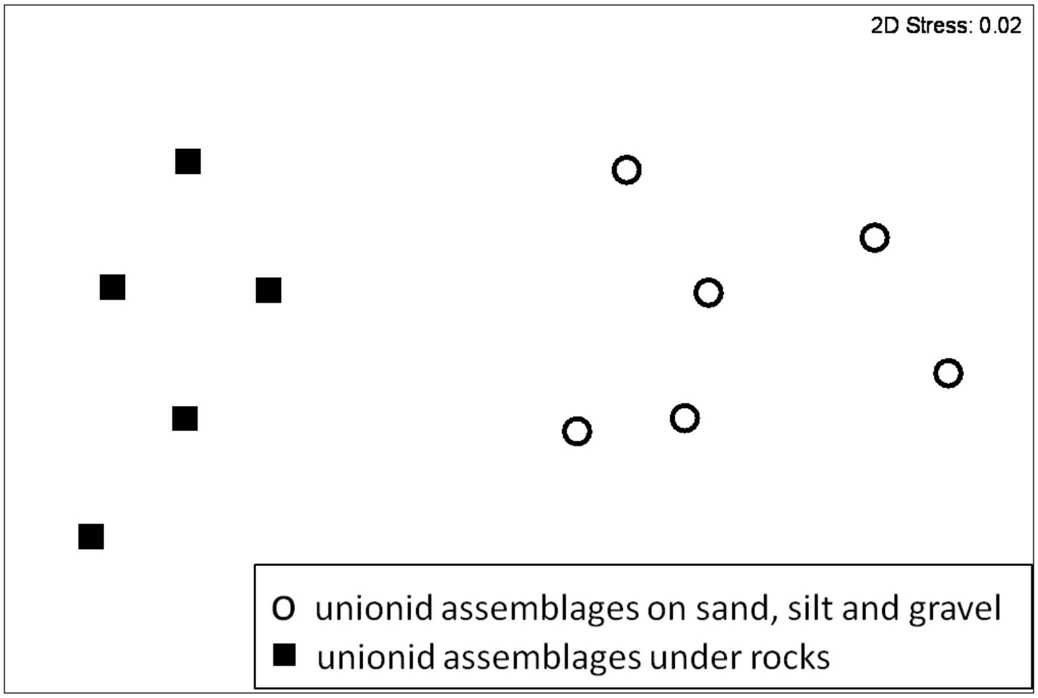

Red Bluff Reservoir
Imperial Reservoir
Amistad Reservoir
Falcon Reservoir
Prior to 1931
Popenaias popeii
Live
Shells
0 30 60 120
Miles
Red Bluff Reservoir
Imperial Reservoir
Amistad Reservoir
Falcon Reservoir
1968-1992 Popenaias popeii
Live
0 30 60 120
Miles
Red Bluff Reservoir
Imperial Reservoir
Amistad Reservoir
Falcon Reservoir
1992-1999 Popenaias popeii
Shells
0 30 60 120
Miles
Red Bluff Reservoir
Imperial Reservoir
Amistad Reservoir
Falcon Reservoir
2001-2011
Popenaias popeii
Live
Shells
0 30 60 120
Miles

Figure 5


2D Stress: 0.02
o unionid assemblages on sand, silt and gravel
■ unionid assemblages under rocks