2019 Final Report

Surveys for Western River Cooter (*Pseudemys gorzugi*) in the Pecos River Drainage and its Tributaries

Submitted to:

Share with Wildlife Program

New Mexico Department of Game and Fish

1 Wildlife Way

Santa Fe, NM 87507

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13 March 2019

INTRODUCTION

Western River Cooter (*Pseudemys gorzugi*) is a relatively large riverine turtle native to New Mexico and Texas within the United States of America with its range extending to Tamaulipas, Nuevo Leon, and Coahuila in Mexico (Ernst and Lovich 2009). Western River Cooter is listed as threatened in Mexico and New Mexico (Secretaríade Medio Ambiente y Recursos Naturales [SEMARNAT] 2010, New Mexico Department of Game and Fish [NMDGF] 2016) and a species of greatest conservation need in Texas (Texas Parks and Wildlife Department [TPWD] 2012). Recently, a United States Fish and Wildlife Service (USFWS) review for inclusion of *P. gorzugi* as a threatened or endangered species under the United States Endangered Species Act (Adkins Giese et al. 2012) has begun, with listing findings scheduled for 2023. Furthermore, the species is designated a near threatened by the International Union for Conservation of Nature (IUCN) mainly due to habitat degradation and pet trade collection.

P. gorzugi range is restricted to the Rio Grande watershed, from the Lower Rio Grande Valley of Texas northward to the Big Bend north of Del Rio, and in the Pecos River drainage of northwestern Texas and Southeastern New Mexico (Ernst and Lovich 2009). In New Mexico, the species primarily occurs in the lower Pecos River drainage downstream from Brantley Reservoir (Degenhardt et al. 1996). Current research suggests *P. gorzugi* generally prefer sections of river with deep clear pools (Degenhardt et al. 1996), but the species has also been found in nearby lentic water bodies (see review by Pierce et al. 2016). Although *P. gorzugi* have been found to be locally abundant at a few locations (Dixon 2013), a low range-wide population density may be a natural characteristic for this species (Bailey et al. 2008). In the United States of America, it is believed that *P. gorzugi* habitat is declining due to pollution and human alterations of river flow such as dam and canal development (Bailey et al. 2008). Main threats to the species include not only habitat alteration but also direct take from the wild for commercial trade (Bailey et al. 2008; Dixon 2013; Mali et al. 2014).

Unfortunately, very little is known about the biology of this species. Evaluation based on number of citations and amount of text written about freshwater turtle species places *Pseudemys gorzugi* as the second least studied turtle species of United States of America and Canada, being surpassed only by Arizona mud turtle (*Kinosternon arizonense*; Ennen and Lovich 2013). Very few studies have assessed population status and trends across the range of *P. gorzugi*, and systematic studies of the ecology and behavior of this species are needed (Ernst and Lovich 2009). This is especially important given the species is currently under review by the USFWS for potential federal listing.

In 2016, we began surveys of Western River Cooter along the Black River drainage in New Mexico, with a goal of establishing a long-term monitoring program for this species of conservation concern. Through the Share with Wildlife Program, we were able to shed new light on appropriate survey methods and provide abundance estimates (Mali et al. 2018) and data on hatchling movement (Curtis et al. 2017) and diet (Letter et al. *in review*), among other aspects of their biology. The 2018 field season represents the third year of long-term data collection at already established sites on the Black River. In addition to continued monitoring and building a larger dataset, we were studying reproductive status of females and prevalence of fish hook ingestion in all adult turtles. We were also able to expand our surveys to Chaves County, NM, in order to assess whether the species occurs at Bitter Lake National Wildlife Refuge (BLNWR) and the surrounding area. This was especially important given previous accounts of *P. gorzugi* shells at BLNWR.

METHODS

Sites

The surveys in 2018 occurred on the Black River (Eddy County), BLNWR (Chaves County), and Berrendo Creek (Chaves County). Five sites on the Black River have been surveyed since 2016 (Sites 1, 2, 4, 5, and 6). Two upstream stretches of the Black River, Sites 1 and 2, within the Bureau of Land Management (BLM) were surveyed from June 5 to June 11 (Site 1) and June 11 to June 17 (Site 2; Table 1). These two stretches are directly adjacent to each other. Three downstream stretches, Sites 4–6, are located downstream within private properties and are also directly adjacent to each other. Site 5 was surveyed from May 15 to May 21, Site 6 was surveyed from May 21 to May 27, and Site 4 was surveyed from July 21 to July 27. Site 7 was surveyed from July 14 to July 20. Site 3 is the only site on the Black River we were able to trap on multiple occasions throughout the season: from May 17 to May 25 and from August 11 to August 17. We began monitoring this site in the summer of 2017. This site is located several miles upstream from Site 4. We opportunistically surveyed turtles at other locations via snorkeling, where the water clarity was high. Those sites included Cottonwood Day Use Area on the Black River, stretches near the headwaters of the Black River (i.e., upstream from Site 1), and a private pond (Table 2). The Cottonwood Day Use Area and the Pond site were surveyed via snorkeling in 2017.

In Chaves County, we trapped the oxbow lake at BLNWR as well as a ~500 m segment of Berrendo Creek located within a private property. The BLNWR was surveyed from July 29 to August 3 and Berrendo Creek from July 28 to August 3 (Table 3).

Surveys

Sites 1–7 on the Black River as well as BLNWR and Berrendo creek sites were trapped with traditional turtle hoop net traps. Traps are 76.2 diameter fiberglass single-throated wide-mouth hoop nets with a 2.54-cm mesh size and four hoops per net (Memphis Net and Twine Co., Memphis, TN). The nets were stretched by homemade wooden poles and a floating device was placed inside the trap to prevent drowning. We primarily used canned sardines as bait as they have proven to be cost effective and successful in capturing all size classes of *P. gorzugi* (Mirabal et al. 2018). The bait was placed in non-consumable containers with drilled holes for scent dispersal. We occasionally added romaine lettuce in the traps to see whether that would improve capture rates of large females. The traps were placed in the river with the mouth facing downstream and tied to nearby available vegetation for safety. We checked the traps once a day and the bait was replaced every two days. Snorkel surveys were conducted by actively pursuing the turtles under water.

Table 1. Summary of survey sites, dates, and trap days on the Black River, NM.

Site #	Ownership	Start Date	End Date	Trap Days
1	BLM	6/5/2018	6/11/2018	300
2	BLM	6/11/2018	6/17/2018	300
3	Private	5/17/2018	5/25/2018	126
3	Private	8/11/2018	8/17/2018	182
4	Private	7/21/2018	7/27/2018	180
5	Private	5/15/2018	5/21/2018	300
6	Private	5/21/2018	5/27/2019	300
7	Private	7/14/2018	7/20/2018	341
Pond	Private	7/17/2018	7/24/2018	13
Pond	Private	8/15/2018	8/17/2018	10

Table 2. Summary of survey sites, dates, and person-hours for the sites on the Black River that were surveyed via snorkeling in 2018.

Site #	Date	# of hours	# of surveyors	Person hours
Pond	5/15/2018	1	1	1
Pond	5/18/2018	1	1	1
Cottonwood Day Use Area	6/8/2018	1	3	3
Cottonwood Day Use Area	6/9/2018	1	3	3
Pond	6/10/2018	1	3	3
Pond	6/12/2018	4	1	4
Pond	6/16/2018	0.5	1	0.5
Black River Headwaters1	6/20/2018	1.5	2	3
Black River Headwaters2	6/20/2018	2	2	4
Pond	6/21/2018	2	2	4
Cottonwood Day Use Area	6/21/2018	1	2	2
Pond	6/22/2018	5	2	10
Cottonwood Day Use Area	7/24/2018	1	3	3

Table 3. Summary of survey sites, dates, and trap days at Bitter Lake National Wildlife Refuge (BLNWR) and Berrendo Creek in Chaves County, NM.

Site	Start Date	End Date	Trap Days
BLNWR	7/29/2018	8/3/2018	69
Berrendo Creek	7/28/2018	8/3/2018	220

For all captures, we took standard measurements: straight line carapace length (CL), carapace width (CW), plastron length (PL), plastron width (PW), body depth (BD), and weight. Length measurements were taken using Haglof® tree calipers and weight measurements were taken using Pesola® precision scales. Sex was determined using secondary sexual characteristics. Adult males have elongated foreclaws and the pre-cloacal portion of the tail lies beyond the edge of the carapace. Small juvenile turtles were not sexed unless it was clear that they were male. All hard-shell turtles were marked with at least one of the following methods depending on the size of the turtle: 1. Injecting a passive integrated transponder (PIT) tag into the body cavity in the

anterior inguinal region parallel to the spine (Buhlmann and Tuberville 1998), 2. Drilling marginal scutes using a portable rotary tool (Cagle 1939), and 3. Toe clipping (hatchlings only). Softshell turtles were marked by engraving individual numbers on the posterior end of the carapace using a portable rotary tool. Not all softshell turtles were marked at BLNWR due to time constraints.

All turtles caught on the Black River that were larger than110 mm CL were taken to the Desert Willow Wildlife Rehab Center where they were X-rayed to determine female reproductive status (i.e., presence of shelled eggs) and presence of ingested fish hooks in males and females. With the veterinarian's assistance, we used a portable MinXray 308 (MinXray Inc., Northbrook, Illinois) at 70kV peak, 20 mA, and 0.06 sec for individuals with a body depth of less than 65 mm; individuals with a body depth of more than 65 mm were x-rayed at 70kV peak, 20 mA, and 0.16 sec. We placed a turtle on a foam cushion made out of a pool noodle on the X-ray cassette to prevent the turtle from moving. Images were examined for the presence of shelled eggs, ingested fish hooks, or metal pieces such as bullet fragments. If shelled-eggs were present on an X-ray, we counted the number of eggs directly from the digital radiograph images. We measured egg widths to the nearest 0.01 mm using VetView software (University of Georgia, Athens, Georgia). If shelled-eggs were absent from female x-ray images, and the turtle was large enough (PL > 173 mm), we then examined the turtles using Mindray Digi Prince DP-6600 ultrasound (Mindray Medical International Ltd., Shenzhen, China) to assess the development of egg follicles. To ultrasound a turtle, we pulled turtle's hind leg and placed the ultrasound probe in the inguinal region (Kuchling 1989; Shelby et al. 2000). We examined both the left and right sides of the turtle's inguinal region. Ultrasound transmission gel was used to amplify the ultrasonic signal. Following examination, all turtles were immediately returned to the site of capture.

RESULTS

BLACK RIVER

Pseudemys gorzugi.— In total, 2052 trap days and 41.5 person-hours (i.e., snorkeling the Pond and Cottonwood Day Use Area) yielded 665 *P. gorzugi* captures, of which 446 were unique individuals (Table 4). Of 446 unique individuals: 132 were males, 165 were females, and 149 were juveniles. The overall mean capture per unit effort (CPUE) for *P. gorzugi* was: 0.26 (Site 1), 0.48 (Site 2), 0.32 (Site 3), 0.29 (Site 4), 0.21 (Site 5), 0.34 (Site 6), and 0.21 (Site 7). On the upstream stretch of the river (Site 1, Site 2, and Cottonwood), we captured a total of 248 *P. gorzugi*, including all within and among years recaptures. Of those, we caught and marked 99 new unique individuals. We were successful in recapturing individuals we marked in 2016 and 2017 (N = 84). We were unsuccessful in capturing any *P. gorzugi* at the stretches near the Black River headwaters. Three-person hours were spent near the headwaters. Moreover, we failed to observe any *P. gorzugi* basking or under water. At the lower stretch of the river (Sites 3–7), we captured a total of 386 *P. gorzugi*, including all recaptures. Of those, we caught and marked 163 new unique individuals. We were also successful in recapturing individuals we marked in 2016 and 2017 (N = 84). We spent a total of 23.5-person hours snorkeling turtles, as well as 23 trap days at the Pond, and caught a total of 31 turtles. Of those 16 were new unique individuals and we caught 5 turtles that were recaptures from previous years.

Other species.— We captured five additional species of turtles (41 total captures including recaptures): spiny softshell turtle (*Apalone spinifera*), common snapping turtle (*Chelydra serpentina*), painted turtle (*Chrysemys picta*), yellow mud turtle (*Kinosternon*

flavescens), and red eared slider (*Trachemys scripta*). While we caught snapping turtles, red eared sliders, and yellow mud turtles at upper and lower stretches, painted turtles and softshell turtles were only caught at the lower stretches of the river (Table 4).

Table 4. Summary of all captures on the Black River and the Pond in 2018 broken out by site, species, and sex. The data were further divided based on: 1. new captures in 2018, where total includes within year (2018) recaptures, and 2. recaptures that were marked in 2016/2017 where total includes within year (2018) recaptures.

		Male				female				Juvenile			
Site	species	new from 2018		recapture		new from 2018		recapture		new from 2018		recapture	
		total	unique	total	unique	total	unique	total	unique	total	unique	total	unique
Site 1	*P gorzugi*	10	8	14	13	15	13	3	3	20	12	16	13
Site 1	*T. scripta*	2	2	-	-	-	-	-	-	-	-	-	-
Site 2	*P. gozugi*	19	12	34	24	25	20	23	17	28	17	16	8
Site 2	*T. scripta*	-	-	1	1	-	-	-	-	-	-	-	-
Site 2	*C. serpentina*	-	-	1	1	-	-	-	-	-	-	-	-
Site 3	*P. gozugi*	22	10	9	4	26	19	7	4	24	16	11	6
Site 3	*T. scripta*	1	1	-	-	-	-	-	-	-	-	-	-
Site 3	*A. spinifera*	1	1	-	-	-	-	-	-	-	-	-	-
Site 4	*P. gozugi*	9	4	4	2	14	7	6	5	11	6	9	7
Site 4	*T. scripta*	-	-	-	-	-	-	-	-	3	1	-	-
Site 5	*P. gozugi*	12	5	11	7	10	8	5	4	4	4	21	13
Site 5	*C. picta*	-	-	3	1	-	-	-	-	-	-	-	-
Site 5	*T. scripta*	4	3	1	1	-	-	3	2	5	3	-	-
Site 6	*K. flavescens*	-	-	2	1	-	-	-	-	-	-	-	-
Site 6	*P. gozugi*	22	12	16	6	15	11	10	6	22	10	16	12
Site 6	*T. scripta*	-	-	-	-	-	-	-	-	2	1	-	-
Site 7	*P. gozugi*	17	14	3	2	22	21	3	3	21	16	4	3
Site 7	*A. spinifera*	-	-	2	1	-	-	-	-	1	1	-	-
Site 7	*T. scripta*	4	3	2	2	1	1	1	1	1	1	-	-
Cottonwood	*P. gozugi*	-	-	3	3	17	11	3	2	1	1	1	1
Headwaters	*K. flavescens*	-	-	-	-	1	1	-	-	-	-	-	-
Pond	*P. gozugi*	5	5	2	1	11	7	8	4	5	4	-	-

***Pseudemys gorzugi* reproductive status.—** We x-rayed a total of 152 unique female *P. gorzugi*. We were able to x-ray 3 individuals twice because we recaptured them more than a month after the original capture (i.e., turtles from the Pond). Sixty nine females were too small to ultrasound as the ultrasound probe could not fit between the carapace and plastron. Therefore, their reproductive status could not be determined with certainty. Of all females examined using both radiograph and ultrasound (N = 86), 16 contained shelled-eggs (196–251 mm PL; Figure 1), 28 contained egg follicles (185–244 mm PL; Figure 2), and 42 did not contain any shelled egg or

egg follicle development structures (173–248 mm PL; Table 5). The smallest turtle containing egg follicles was 185 mm PL and the smallest turtle containing shelled eggs was 196 mm PL. Clutch size ranged from 5 to 14 eggs, with a mean of 9.3 eggs (N = 15; Table 5). We could not obtain a complete count of clutch size from one individual with shelled-eggs due to a thick layer of tissue, which was impenetrable by the X-ray. Egg width ranged from 24.8 to 34.3 mm, with a mean of 30.1 mm (N = 144). The first female with shelled eggs was captured on May 15, the first day of our trapping season. The last female containing shelled eggs was captured on June 21. The females with egg follicles were found throughout the length of the study, with the highest frequency in May and June. We recaptured three individuals with PL of 230, 231, and 237 mm containing follicles when recaptured at the Pond site. These individuals were first captured in May and, at first capture, contained shelled eggs, providing evidence of multiple clutching during a single season.

Table 5. Reproductive output and body size of X-rayed female *Pseudemys gorzugi* in different reproductive statuses (i.e., non-gravid, gravid with egg follicles, and gravid with shelled-eggs) captured on the Black River, New Mexico from May to August 2018. We considered the individuals producing the second clutch as new individuals (N = 3).

	n	**Mean ± SD**	**Range**
PL of all x-rayed females (mm)	155	182.47 ± 35.57	118–251
PL of females that were too small for ultrasound (mm)	69	148 ± 17.41	118–228
PL of females gravid with shelled-eggs (mm)	16	216 ± 18.44	196–251
PL of females gravid with oviductal follicles (mm)	28	212.75 ± 16.60	185–244
PL of non-gravid females (mm)	42	206.12 ± 17.47	173–248
Clutch size	15	9.33 ± 2.89	5–14
Egg Width (mm)	144	30.07±1.72	24.8–34.3

Figure 1. An X-radiograph image of a female *Pseudemys gorzugi* containing 14 shelled-eggs.

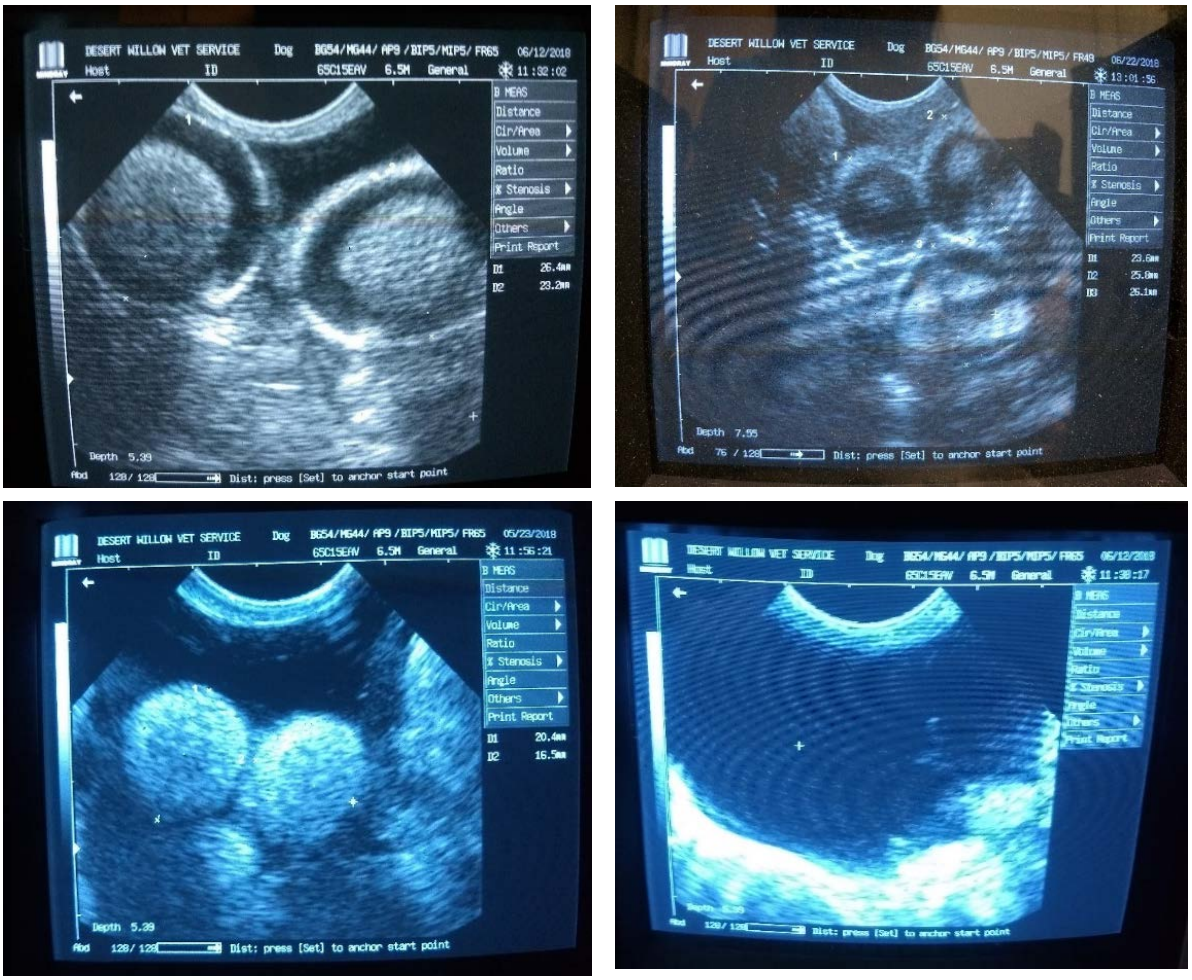

Figure 2. Ultrasound images of different stages of egg development observed during the study. Top left: an image of shelled-eggs; at this stage, shelled-eggs can be detected by the X-radiograph. Top right: an image of nearly-shelled eggs; at this stage, the eggs cannot be detected by the X-radiograph. Bottom left: an image of egg follicles; the smallest follicle that we could confirm was 7.57 mm in diameter. Bottom right: an image of a turtle that did not contain any shelled eggs or egg follicles.

Prevalence of fish hook ingestion.— Of 288 X-rayed individual turtles, we found 2 female *T. scripta* and 1 female *P. gorzugi* with fish hook in the mouth (Figure 3). Hooks appeared to be J hooks, with the size ranging from 8.9–31 mm long. We also found 3 female *P. gorzugi* (PL > 150 mm) with a bullet in the hind leg, bullet fragments in the front leg (Figure 4), and a metal piece in the throat region. Individuals shot appeared to only have a minor scar on their legs. Surprisingly, we did not observe any individuals with bullet scars on the carapace.

Figure 3. Female *Trachemys scripta elegans* with fish hook inside the mouth caught on the Black River (downstream) in the summer 2018.

Figure 4. X-radiograph image of *Pseudemys gorzugi* with bullet fragments on the left front leg (left) and an image of the bullet scar (right). The turtle was caught on the Black River (downstream) in the summer 2018.

BITTER LAKE NWR AND BERRENDO CREEK

Bitter Lake NWR.— After consultation with the BLNWR wildlife biologist, we focused on trapping the oxbow lake, given that a *P. gorzugi* shell was found on land near this water body. We failed to capture any *P. gorzugi* after 69 trap days. The most dominant species at the lake was *Apalone spinifera* (70 total captures: 42 females, 27 males, and 1 juvenile). *Chelydra serpentina* (3 total captures, unknown sex), and *Trachemys scripta* (5 total captures: 2 females and 3 males) were also present. The habitat itself did not seem optimal for *P. gorzugi* (Figure 5). The water was relatively shallow (~1–1.5 m) and we were having trouble finding vegetation to tie our traps to.

Figure 5. Oxbow lake at the Bitter Lake National Wildlife Refuge trapped in the summer 2018.

Berrendo Creek.— Prior to trapping, we were contacted by a local fisherman who sent us a photo of a turtle on a fishing line that had been caught at Berrendo Creek. We were able to confirm with certainty that the turtle was indeed *P. gorzugi*. To our knowledge, this was the first report of live *P. gorzugi* in the wild in Chaves County. Our trapping location was only ~30 m downstream from where the fisherman previously caught *P. gorzugi* (Figure 6). We put a total effort of 220 trap days and caught a total, including recaptures, of: 14 *Apalone spinifera*, 2 *Chelydra serpentina*, 7 *P. gorzugi*, and 43 *Trachemys scripta* (Table 6). Of 14 *A. spinifera* captures, all were unique individuals: 2 males, 11 females, and one newly hatched individual that was caught walking toward the water. Of 43 *T. scripta* captures, 38 were unique individuals (14 females and 24 males) and 5 were recaptures. Among *P. gorzugi*, we caught 3 unique females and 2 unique males (one was an old melanistic male), with one male recaptured 2 times. We took blood samples from all captured *P. gorzugi*. Female sizes ranged from 252–274 mm CL while male sizes were 179 and 212 mm CL.

Figure 6. A portion of Berrendo Creek near by fishing areas trapped in the summer 2018.

Table 6. A summary of turtle captures at Berrendo Creek, Chaves County, NM in summer 2018.

Species	Total	Unique
A. spinifera	14	14
C. serpentina	2	2
P. gorzugi	7	5
T. scripta	43	38

DISCUSSION

BLACK RIVER

Our work in the summer 2018 primarily focused on building a larger long-term dataset in order to gather enough *P. gorzugi* capture-recapture information to evaluate the size of the population on the Black River. We continued to have success at all localities surveyed since 2016 (Sites 1, 2, 4, 5, and 6). *P. gorzugi* continues to dominate the Black River drainage system in comparison to other turtle species. We also continue to capture *P. gorzugi* of all size classes and were able to recognize recaptured juveniles using Wild.ID software (Suriyamongkol and Mali 2018). Our overall capture rates increased in comparison to 2016 and 2017, but we have also increased our trapping effort and survey area since 2016. With a multi-year monitoring effort of at least seven more seasons, we believe we will be able to estimate survivorship of *P. gorzugi* of all age classes and obtain somatic growth rate estimates. Interestingly, we failed to detect any *P. gorzugi* near the headwaters of the Black River during snorkel surveys while Painter caught multiple captures in that area in the early 1990s. With the data to date, we started developing somatic growth curves for males and females, but the data are highly preliminary. Since we continue to capture and recapture juveniles, which is usually rare for freshwater turtle populations, this is a unique opportunity to study somatic growth rates. However, freshwater turtles are long lived and late maturing, which means years or even decades of monitoring may be needed to enable estimation of these important life history parameters.

Since this was our third year of surveys, it is worth pointing out observed changes in the habitat. In 2017, we noted that the BLM portion of the Black River appeared drastically different in comparison to the 2016 field season. Specifically, the river was at lower levels than in 2016 and there was an obvious dry line on the reeds that reflected a river drop (Figure 7). Also, water was distinctly more turbid in 2017 than 2016. In 2018, water levels at the BLM sites are at similar levels as in 2016 (Figure 7). We do not yet know the exact reasons for these inter-annual oscillations nor how it affects *P. gorzugi* populations. At the downstream sites, we did not observe any drastic changes to the river itself, but we did notice intensified traffic in the area related to oil drilling operations.

Figure 7. A side-by-side comparison of the Bureau of Land Management (BLM) portion of the Black River in June 2018 (bottom), June 2017 (middle) and June 2016 (top), showing annual oscillations in water levels and its effects on riparian vegetation.

Our 2018 field season also represents the first comprehensive study of *P. gorzugi* reproductive biology. Previous records on *P. gorzugi* reproduction in New Mexico were restricted to three reports of gravid females. Letter et al. (2017) reported the largest previously documented clutch size of 12 eggs for a female caught on June 13 at the Pond near the Black River headwaters. Lovich et al. (2016) x-rayed six female *P. gorzugi* on the Black River; one

was gravid containing 10 eggs on June 13. Via X-ray and injection of oxytocin, Degenhardt et al. (1996) reported a female on May 23 with a clutch size of 9 eggs. We found the first gravid female with shelled eggs on May 15. Therefore, the ovulation cycle likely started between late April and the beginning of May, but it is possible that the cycle begins even earlier given that we did not assess reproductive status prior to mid-May. The last female with shelled-eggs was found on June 21. Interestingly, six females carrying egg follicles were still found in July and August; the observation of these individuals further into the season suggests that nesting can extend into August, given that Letter et al. (2017) reported a gravid female *P. gorzugi* that laid eggs 26 days after the first detection of shelled-eggs on the X-ray. We observed three turtles at the Pond site developing egg follicles in June and July, after the detection of their first clutch (i.e., shelled eggs) in May. Of the three females, one individual was identified as having nearly-shelled eggs with a mean diameter of 25.43 mm on July 2. We could safely assume that *P. gorzugi* produce multiple clutches per reproductive season and it is possible that all *P. gorzugi* with egg follicles at the end of June and July might be developing their second clutches.

We also report the smallest and the largest clutch size for *P. gorzugi* to date: 5 and 14 eggs, respectively. The smallest female containing egg follicles was 185 mm in PL (207 mm CL), with all captured females containing follicles or shelled eggs ranging from 185–251 mm PL. The largest female caught in our study had a PL of 251 mm (278 mm CL). In previous studies, the sizes of gravid females were 237 mm PL (Letter et al. 2017), 242 mm CL (Lovich et al. 2016), and 240 mm CL (Degenhardt et al 1996). Out of all females captured (N = 165), only 48.5% were considered sexually mature by our (potentially) conservative estimate (PL > 185 mm). An absence of follicles in any female we examined with ultrasound could be due to females not being sexually mature (i.e., follicles being too small or < 4–5 mm in diameter, which is beyond our limit of detection) or a female having just laid eggs prior to the scanning (Kuchling 1989). Therefore, our estimates of size at maturity are conservative given we were not able to confirm whether the individuals with PL less than 185 mm were non-gravid or had not reached sexual maturity.

We remain unsuccessful in observing nesting females or finding *P. gorzugi* nests but capturing small juvenile turtles provides evidence of reproduction. Future studies should focus on locating nesting sites and monitoring nesting success of the species. In the meantime, with the current knowledge on egg development and deposition of *P. gorzugi* presented here, protection of the riparian area along the Black River should be emphasized during May and June, but we caution that the nesting season may start even earlier (i.e., March and April). Given that reproductive outputs may vary among populations and geographic regions, comparing the reproductive ecology of different populations of *P. gorzugi* across its range will contribute to a deeper understanding of the species' natural history. This can inform appropriate conservation practices tailored for each population.

We found a relatively low proportion of turtles with ingested fish hooks on the Black River. In contrast, Steen et al. (2014) found approximately 5% of 438 freshwater turtles across four different species in Tennessee, and 3.5% of 170 *C. serpentina* in Virginia, contained fish hooks. Furthermore, the ingestion of fish hooks could be species and sex specific as the percentage of turtles with ingested fish hooks could be as high as 33% in female *C. serpentina* (Steen et al. 2014). We found only 2% of all turtles X-rayed had signs of ingested fish hook or bullet shots on the Black River, mainly from the downstream sites which were located within private properties. Although these individuals appeared to be in healthy condition, despite the injuries, carcasses of *P. gorzugi* and *T. scripta* have been found in New Mexico with evidence of

gunshots (Pierce et al. 2016). We were not able to confirm the potential threats of fish hook ingestion and recreational shooting to this population as the mortality due to these activities is unknown. However, further impact of these activities on turtles can potentially be minimized by educating anglers and recreationists about the presence of threatened freshwater turtle species.

BITTER LAKE NWR

Our trapping efforts at BLNWR did not result in successful capture of *P. gorzugi*. We focused on trapping the oxbow lake because the previously recovered *P. gorzugi* shells were found in close proximity to the lake. Based on what we know about the species' habitat requirements, the lake did not look optimal for the species. Water levels were relatively low (1–1.5 m) and we had a hard time finding live vegetation to tie our traps to. We attempted to conduct visual surveys, but the lack of basking structures prevented us from observing turtles' entire bodies. To the best of our knowledge, all turtles we visually observed were softshell turtles.

BERRENDO CREEK

In May 2018, we were able to visually (via photograph) confirm a new positive locality for the species, with adult *P. gorzugi* caught on a fishing line at Berrendo Creek. Berrendo Creek is a tributary of Rio Hondo that drains into the Pecos River. We trapped turtles ~30 m downstream from the location where *P. gorzugi* was caught on a fishing line and captured five adults. This site is located ~80 km north of the northernmost edge of the assumed species range. The site is also located near BLNWR, which leads us to speculate that recovered shells at the refuge could have been dragged there by turtles' natural predators (i.e., osprey; Beauchamp, BLNWR, pres. comm.). In the future, we are particularly interested in expanding our surveys to the rest of Berrendo Creek, as well as the surrounding water bodies (i.e., Rio Hondo and Pecos River), in order to understand the extent of species occurrence in the area. It is important to mention that a section of Berrendo Creek has been recently opened to the public and has become a very popular fishing area. We have now received multiple photographs of *P. gorzugi* captured via fishing lines. Furthermore, we have captured multiple softshell turtles with clear damage from fish hooks (i.e., damaged mouth and nose), suggesting that intense recreational fishing in this area impacts the freshwater turtle community. We recommend posting signs or educating the public on Berrendo Creek about the presence of a threatened freshwater turtle species and generally the importance of freshwater turtles in the ecosystem.

We have also collected blood samples from all 5 *P. gorzugi* captured at Berrendo Creek. Although *P. gorzugi* shows low genetic structure between populations in New Mexico and Texas (Bailey et al. 2008), the collected blood samples can be useful in further evaluating any genetic variation between different populations of *P. gorzugi* across the species' range. Our future efforts will focus on using stable isotope analyses to study diet, niche overlap, and species competition among freshwater turtle species found on the Black River and Berrendo Creek, with specific focus on the western river cooter and the red eared slider. This work will significantly contribute to our understanding of *P. gorzugi* habitat requirements and, therefore, further conservation measures for the species.

ACKNOWLEDGMENTS

We thank Korry Waldon and Alissa Kreikemeier for their help with turtle surveys. We thank Kelley Reid and Dustin Mudgett with BLM for working with us and allowing us to trap turtles. We also thank private land owners for continuing to work with us and allowing us access to their

properties. We thank Desert Willow Wildlife Rehab Center, especially Dr. Uhrig, for helping us with assessing reproduction and fish hook ingestion. All work was conducted under Scientific Research Permit Authorization No. 3621 issued to Ivana Mali and Michael R.J. Forstner, as well as Eastern New Mexico University IACUC approval number 03-02/2016. Research was supported by the Share with Wildlife program and U.S. Fish and Wildlife Service State Wildlife Grant T-32-5, activity 2.

LITERATURE CITED

Adkins Giese CL, Greenwald DN, Curry T. 2012. Before the secretary of the interior: petition to list 53 amphibians and reptiles in the United States as threatened or endangered species under the Endangered Species Act. Circle Pines, MN: Unpublished report, Center for Biological Diversity.

Bailey LA, Dixon JR, Hudson R, Forstner MRJ. 2008. Minimal genetic structure in the Rio Grande cooter (*Pseudemys gorzugi*). Southwestern Naturalist 53:406–411.

Buhlmann KA, Tuberville TD. 1998. Use of passive integrated transponder (PIT) tags for marking small freshwater turtles. Chelonian Conservation and Biology 3:102–104.

Cagle FR. 1939. A system of marking turtles for future identification. Copeia 1939:170–173.

Curtis J, Mali I, Forstner MRJ. 2017. *Pseudemys gorzugi* (Rio Grande cooter). Hatchling movement. Herpetological Review 48(2):426

Degenhardt WG, Painter CW, Price AH. 1996. Amphibians and reptiles of New Mexico. Albuquerque, New Mexico: University of New Mexico Press.

Dixon JR. 2013. Amphibians and Reptiles of Texas. 3rd edition. College Station, Texas: Texas A&M University Press.

Ernst CH, Lovich JE. 2009. Turtles of the United States and Canada. 2nd edition. Baltimore, Maryland: Johns Hopkins University Press.

Ennen JR, Lovich JE. 2013. A quantitative analysis of the state of knowledge of turtle of United States and Canada. Amphibia-Reptilia 34:11–23.

Kuchling G. 1989. Assessment of ovarian follicles and oviductal eggs by ultra-sound scanning in live freshwater turtles, *Chelodina oblonga*. Herpetologica 45(1):89–94.

Letter AW, Waldon KJ, Mali I, Reams RD. 2017. *Pseudemys gorzugi* (Rio Grande Cooter). Maximum clutch size. Herpetological Review 48(4):836–837

Letter AW, Pollock DA, Waldon, KJ, Mali I. *in review*. Dietary habits of Rio Grande cooter, *Pseudemys gorzugi*, within the Black River drainage, Eddy County, New Mexico. Journal of Herpetology.

Lovich JE, Agha M, Painter CW, Cole L, Fitzgerald A, Narum K, Jennings RD. 2016. Aspects of the reproductive ecology of female turtles in New Mexico. Western North American Naturalist 76(30):291–297.

Mali I, Vandewege MW, Davis SK, Forstner MRJ. 2014. Magnitude of the freshwater turtle exports from the US: long term trends and early effects of newly implemented harvest management regimes. PloS ONE 9(1):e86478.

Mali I, Duarte A, Forstner MRJ. 2018. Comparison of hoop-net trapping and visual surveys to monitor abundance of the Rio Grande cooter (Pseudemys gorzugi). PeerJ 6:e4677 https://doi.org/10.7717/peerj.4677

Mirabal J, Letter AW, Waldon KJ, Mali I. 2018. *Pseudemys gorzugi* (Rio Grande Cooter). Attractions to trap baits. Herpetological Review 49(2):323–324.

New Mexico Department of Game and Fish. 2016. The comprehensive wildlife conservation strategy for New Mexico. Santa Fe, New Mexico: New Mexico Department of Game and Fish.

Pierce, LJS, Stuart JN, Ward JP, Painter CW. 2016. *Pseudemys gorzugi* Ward 1984 – Rio Grande Cooter, Western River Cooter, Tortuga de Oreja Amarilla, Jicotéa del Río Bravo. In: Rhodin, A.G.J., Iverson, J.B., van Dijk, P.P., Saumure, R.A., Buhlmann, K.A., Pritchard, P.C.H., and Mittermeier, R.A. (Eds.). Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. Chelonian Research Monographs 5(9):100.1–12.

Shelby JA, Mendonça MT, Horne BD, Seigel RA. 2000. Seasonal variation in reproductive steroids of male and female yellow-blotched map turtles, *Graptemys flavimaculata*. General and Comparative Endocrinology 119(1):43–51.

SEMARNAT [Secretaríade Medio Ambiente y Recursos Naturales]. 2010. NORMA Oficial Mexicana NOM-059-SEMARNAT-2010, Protección ambiental—Especies nativas de México de flora y fauna silvestres—Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio—Lista de especies en riesgo. Diario Oficial de la Federación, 30 Diciembre 2010, No. 2, 77 pp.

Steen DA, Hopkins BC, Van Dyke JU, Hopkins WA. 2014. Prevalence of ingested fish hooks in freshwater turtles from five rivers in the southeastern United States. PloS one 9(13):e91368.

Suriyamongkol T, Mali I. 2018. Feasibility of using computer-assisted software for recognizing individual Rio Grande cooter (*Pseudemys gorzugi*). Copeia 106(4):646–651.

Texas Parks and Wildlife Department. 2012. Species account: the Rio Grande river cooter (*Pseudemys gorzugi*). Pages 1075–1076 in Bender S, Shelton S, Bender K, Kalmbach A, editors. Texas Comprehensive Wildlife Conservation Strategy 2005–2010. Austin, Texas: Texas Parks and Wildlife Department, Nongame Division.

APPENDIX. Field photos (taken by Korry Waldon)