Contemporary Status of the Rio Grande Cooter (Testudines: Emydidae: *Pseudemys gorzugi*) in Texas: Phylogenetic, Ecological and Conservation Considerations

LINDLEY BAILEY[1], MICHAEL R. J. FORSTNER[1*], JAMES R. DIXON[2], AND RICK HUDSON[3]

[1]*Department of Biology, Texas State University, San Marcos, TX 78666, USA*

[2] *Department of Wildlife and Fisheries Science, Texas A&M University, College Station, Texas 77843, USA*

[3] *Conservation Biology, Ft. Worth Zoo, Ft. Worth Texas, 76110, USA*

* Correspondent*: (MF@txstate.edu)*

ABSTRACT—Among the turtles in Texas most in need of immediate conservation work is *Pseudemys gorzugi*, the Rio Grande cooter. This species is a unique component of clear water spring and river systems on the edge of the Trans-Pecos in Texas; these habitats are among the most threatened by human changes to the drainages and groundwater. Extensive surveys of the historical and current distribution in Texas revealed low population densities, a paucity of juveniles in the population, and evidence of several threats commonly affecting chelonians worldwide, acting upon the remaining population in Texas. Monophyly without detectable range-wide genetic structure was found using mitochondrial DNA sequence data. Individuals have been captured, marked and released at numerous locations; behavioral and phenotypic characteristics were measured and compared with the Texas river cooter (*Pseudemys texana*). The survey work and analyses continue, but our results provide other researchers and management authorities with information on the species and the issues it faces in Texas.

INTRODUCTION—Three species of *Pseudemys*, the cooter turtles, occur in Texas (Dixon 2000). *P. concinna metteri* can be found from south-central Missouri and adjacent south-east Kansas, south through eastern Oklahoma, western Arkansas, extreme north-west Louisiana, and eastern Texas through the Gulf of Mexico (Conant and Collins 1998). *P. texana* can be found throughout most of central Texas, from San Antonio Bay and Galveston on the Gulf of Mexico west in the Colorado, Brazos, Guadalupe, and San Antonio river drainages. One of the least known of the Texas species is the Rio Grande cooter (*Pseudemys gorzugi*). This species is usually characterized as a locally abundant, but uncommon turtle in three Texas river systems. The taxon extends northward into New Mexico where it is a state protected wildlife species.

Pseudemys gorzugi—Pseudemys gorzugi (Ward 1984), the Rio Grande cooter, had been formerly considered a subspecies of *P. concinna* (Le Conte) (Ernst 1990; Collins 1991). Ernst (1990) elevated the individuals found in the Rio Grande and Pecos river drainages to full species status and justified his position based on the lack of genetic exchange between these specimens and other *Pseudemys concinna* populations. The distinction was also supported by the examination of morphological characters; the turtle does not have the usual "C" markings on the plastron, but instead black and yellow concentric circles (Degenhardt et al. 1996).

The Rio Grande cooter is a large freshwater turtle (Stout et al. 2005). The carapace is an elongate oval, not highly domed, with an intricate pattern of green, yellow, and black markings. Older males may become melanistic obscuring much of the carapace design with vermiculations of black on a reddish or gray background (Bailey et al. 2005b). The upper and lower jaws have well-developed denticulations; the upper jaw bears a medial notch bordered by tooth-like cusps (Degenhardt et al. 1996). Sexual dimorphism is pronounced. Males have a broader tail and may have a slightly concave plastron. Males also have long, straight foreclaws. Females tend to grow larger than males (Degenhardt et al. 1996).

The Rio Grande cooter occurs in the Rio Grande, Pecos, and Devils river system in the South Texas Plains and Edwards Plateau regions of Texas (Iverson 1992). There is also a disjunct population in the Pecos River basin at the base of the Guadalupe Mountains in New Mexico (Degenhardt et al. 1996). *Pseudemys gorzugi* are primarily found in high flow areas, with deep pools; aquatic vegetation is preferred for foraging and protection but the lack of vegetation will not preclude the species from an area. Muddy, sandy, rocky areas, or areas of algae-covered limestone may serve as suitable substrate for the species (Degenhardt et al. 1996).

Anthropogenic Changes to Texas Rivers—Modification to the river flow rates in Texas has drastic impacts on the environment and consequences to the organisms found in these areas. Flood-control practices, and the construction of dams, channels, and water diversions have caused the Rio Grande to become increasingly intermittent (USDOI 1998). These practices have significantly degraded the water quality and often result in little to no surface water flow on the river (USDOI 1998), and in 2003, the conservation group American Rivers listed the Rio Grande as one of the top ten most endangered rivers in America (American Rivers 2003). Sufficient stream flow is necessary to support aquatic and riparian habitats as well as to meet current levels of human consumption (USDOI 1998).

Before 1915, the lower Rio Grande flow was virtually unimpeded. The Rio Grande was impounded at Amistad Dam in 1969; the Pecos and Devils rivers contribute flow directly into the reservoir at Amistad Dam. Although the Pecos River is larger than the Devils River, the Devils River mean annual flow is twice that of the Pecos. The lower flow rate in the Pecos River is due to the arid environment, high amounts of alluvial deposits, and a significant number of water diversions for irrigation (USDOI 1998).

Untreated sewage inflows, runoff from agriculture and mining activities, and atmospheric deposits are some of the point and nonpoint sources that contribute to the declining water quality of the Rio Grande drainage system (USDOI 1998). Elevated levels of arsenic, cadmium, chromium, copper, lead, mercury, phosphorus, selenium, silver, zinc, DDD, DDE, DDT, dieldrin, endrin, hexachlorobenzene, PCBs, and total PHAs have been recorded in the drainage (Texas Water Commission 1992; Texas Natural Resources Conservation Commission 1994a, 1994b). The declining water quality of the Rio Grande has been shown to cause decrease in fish density and diversity (Bestgen and Platania 1988).

Current human changes to the landscape of Texas are dramatic and are increasing. Among those are impacts to water, groundwater resources, and instream flow rates. Little work has been done using turtles to monitor the health of streams and rivers despite considerable evidence (USFWS 1984, 1987) that turtles provide remarkable value as biological indicators (Gibbons 1990). Instream water reductions coupled with increased harvest pressure on turtle species (IUCN/SSC 1991) may be placing many Texas species of turtles at considerable risk. The goal of this research is to provide information on the current status of the *Pseudemys gorzugi* population in Texas in order to evaluate trend data where possible, and to provide information that will be specifically useful in determining appropriate non-game harvest rates and warning signs of population decline or extirpation. Preliminary work indicated troubling declines and a lack of recruitment. Thus the research sought to determine the abundance of this turtle in Texas, evaluate genetic monophyly of the taxon, and then collect ecological data useful to informed management or conservation decisions ensuring self-sustaining populations of the species within the state.

Methods—*Field Surveys*—Field surveys were conducted throughout the range of *Pseudemys gorzugi* in the Rio Grande drainage system in Texas. Since *P. gorzugi* is often collected for the pet trade, the specific locations of our sample sites have been intentionally removed from this report; upon request by qualified individuals this information may be made available.

Specimens were collected from thirteen specific locations, within four study regions. Individuals were collected using seining, hoop, or basking log traps, as well as physical collection using a snorkel or SCUBA apparatus (Anderson 1965). Once the turtles were captured, a small aliquot of blood was drawn (approximately 1cc) from the femoral vein and then placed into blood storage buffer (100mM Tris: 100mM EDTA: 2% SDS) while in the field. Additionally, the turtles were marked (Cagle 1939) so that recaptured individuals could be identified. The specimens were measured (carapace length, carapace width, plastron length, plastron width, and body depth), weighed and the data was recorded. The morphological data was compared to similar data on *P. texana* using a two-tailed *t*-test.

Data were also collected for 14 *Pseudemys gorzugi* hatchlings born in captivity. The specimens were measured (carapace length, carapace width, plastron length, plastron width, and body depth; cm), weighed (g), and the data was recorded. The data were used to compare the sizes of *P. gorzugi* and *P. texana* hatchlings, using a two-tailed *t*-test, to determine if differences existed between *P. texana* and *P. gorzugi* at hatching.
Data were recorded in an attempt to determine the abundance of *Pseudemys gorzugi* in the Pecos and Devils rivers. A 97-km stretch of the Pecos River and a 35-km stretch of the Devils River were surveyed and the numbers of turtles observed in each species present were recorded. For comparison, the number of *Pseudemys texana* per river km was recorded for the San Marcos River using the same methods. A 9-km stretch of the San Marcos River was surveyed in late May and late September in each of two years. It should be noted that the data collected in late September of the first year were collected directly following a large flood. The number of specimens recorded per river km was used in comparison with similar data collected for *P. gorzugi*.

Laboratory Work—A fragment of the mitochondrial ND4 gene was sequenced to determine the amount of variation among *Pseudemys gorzugi* from the sample locations as well as to compare the sequence with other *Pseudemys* found in Texas. Genomic DNA was extracted from blood samples using the Qiagen Dneasy Tissue Extraction Kit in accordance with the manufacturers specifications. The samples were then amplified via the polymerase chain reaction (PCR) using primers previously found to be successful in our laboratory (Forstner et al. 1995). The hot start PCR method was used with the following thermal cycling parameters: 1 cycle of 95° C for 5 minutes, then 40° cycles at 95° C for 30 seconds, 50° C for 1 minute, 72° C for 1 minute, followed by one cycle of 72° C for 5 minutes. The PCR products were purified using the Promega Wizard SV Gel and PCR Clean-up System in accordance with the manufacturers specifications. The purified PCR products were cycle sequenced using the following thermal cycling

parameters: 25 cycles at 96° C for 10 seconds, 50° C for 5 seconds, and 60° C for 1 minute. The cycle sequence products were purified using Princeton Separations Centri-Sep Columns in accordance with the manufacturers specifications. The purified products were then directly scored using the ABI 377 Automated DNA Sequencer.

Analyses using both parsimony and distance-based methods were used to construct a phylogenetic hypothesis for the mitochondrial sequences (Hillis et al. 1996). The mitochondrial DNA sequences were aligned using Sequencher 4.2. The dataset was analyzed using PAUP* 4.0b10 (Swofford 2003). *Trachemys scripta elegans* was set as the outgroup and the tree was rooted so that the outgroup and ingroup were monophyletic. The optimality criterion was set to parsimony and a heuristic search was conducted using TBR branch swapping. Starting trees were obtained using stepwise addition from a random addition sequence; 1000 replicates were performed and one tree was held at each step. A strict consensus tree of the two most parsimonious trees was produced. The CI and RI were recorded. A bootstrap analysis was performed using 100 replicates and the percentage of support for each branch was recorded. Using the same outgroup, the optimality criterion was set to distance and the Neighbor-joining algorithm was employed to produce a phylogram base upon Jukes-Cantor corrected distances.

Thermocron Data—The Thermocron iButton, manufactured by Dallas Semiconductors (Dallas, Texas, USA), was used to record temperature at user-specified intervals. The thermocrons are currently being employed in behavioral and physiological ecology as an inexpensive and accurate alternative to other systems (Angilletta and Krochmal 2003). Each thermocron is 5.9 mm thick with a diameter of 17.4 mm and weight of 3.1 g. The thermocrons have 512 bytes of memory and use an iButton reader (Blue Dot Receptor, Model DS1402D-DR8) in conjunction with software (32-Bit iButton-TMEX Runtime Environment) to display the temperature records.

Within one of the study populations, 20 *Pseudemys gorzugi* were tagged with thermocrons. They were attached to the rear carapace of the individuals using marine epoxy; the data loggers were positioned so as not to interfere with copulation. The thermocrons were deployed in three subsets where one group of loggers was set to record temperatures for three months (every hour), one group for six months (every two hours) and the last group for one year (every four hours). The individuals tagged with the thermocrons were recaptured and the thermocrons removed in order to retrieve the data. During the study period a Thermocron iButton was also deployed in the river itself to record the environmental fluctuations in the river at a depth of one meter.

Results—In total, approximately 250 specimens were observed and 166 were collected, sampled, and measured (Table 1). An additional group of approximately nine dead individuals, with complete or partial skeletal remains, were located during 2003 and 2004. The population meristics of the recorded individuals, as well as comparative data for *Pseudemys texana*, is summarized in Table 2. A two-sample *t* test (two-tailed version) was performed. The test shows a statistically significant size difference between the two species for the carapace length, carapace width, and plastron length (Table 2). The population meristics for captive-born *Pseudemys gorzugi* and *Pseudemys texana* hatchlings are summarized in Table 3. A two-sample *t* test (two-tailed version) was performed and the results show a statistically significant difference in the carapace length between the two species (Table 2).

The number of *Pseudemys gorzugi* per river kilometer was recorded for the Pecos and the Devils rivers (Table 4). A 97-km stretch of the Pecos River was surveyed. Over the entire stretch, 123 adults were recorded (1.3 per river km), and 3 juveniles were recorded (0.03 per river km). A 35-km stretch of the Devils River was surveyed. Over the entire stretch, 68 adults were recorded (1.94 per river km), and 3 juveniles

Table 1—Number of *Pseudemys gorzugi* samples collected from each of the thirteen sample sites.

Study Site Location	Males	Females	Juveniles	Total Number of Individuals
Eddy County 1				8
Kinney County 1	3	1		5
Kinney County 2	1	2		3
Maverick County 1	2		2	4
Maverick County 2	1	1		2
Terrell County 1	12	10		23
Terrell County 2				2
Val Verde County 1		1		15
Val Verde County 2		1		2
Val Verde County 3	62	24	1	97
Val Verde County 4		2		2
Val Verde County 5		1		1
Val Verde County 6				2
Total	81	43	3	166

Table 2—Population meristics of the *Pseudemys gorzugi* sample collected in Texas. The mean population meristics for *Pseudemys texana* are also shown.

	Carapace Length (mm)	Carapace Width (mm)	Plastron Length (mm)	Plastron Width (mm)	Body Depth (mm)	Weight (g)
Males	19.8	14.7	17.0	11.2	7.1	957
Females	24.3	28.9	32.8	14.0	9.5	1956
Juveniles	8.4	7.6	8.0	5.9	4.4	105
Mean (Adults)	22.05	21.8	24.9	12.6	8.3	1456.5
Minimum	8.3	7.6	8.1	5.9	4.4	105
Maximum	37.2	25.0	33.5	20.4	15.0	5600
Mean for *P. texana* (n=300)	19.81	14.95	17.92			1162.2

were recorded (0.08 per river km). For comparison, the number of *Pseudemys texana* per river km was recorded for the San Marcos River (Table 4). A 9-km stretch of the river was surveyed in late May and late September over the course of two years. It should be noted that the data collected in late September of the first year was collected directly following a large flood. On average, 85.8 adults were recorded over the entire stretch (9.5 adults per river km); 18.7 juveniles were recorded over the entire stretch (2.1 juveniles per river km).

Analysis of the mitochondrial DNA sequence revealed one polymorphic site resolving two haplotypes. The parsimony analysis of the data in PAUP (Swofford 2003) produced two equally parsimonious trees. High levels of bootstrap support resulted for the bifurcations on the trees (Fig. 1). The CI was 0.988 and the RI was 0.975 revealing minimal amounts of homoplasy within the dataset. The phylogram from the neighbor-joining analysis using the Jukes-Cantor distance correction method is shown in Fig. 2. The topologies for the parsimony and distance trees were identical.

The Thermocron iButton data were collected from four specimens as well as from the environmental sampling thermocron placed at a depth of 1 m in the river then compared to the temperature data from the environmental sampling thermocron; the temperature peak occurs at hour 16, or 4 p.m. (Fig. 3).

Table 3—Measurements taken from *Pseudemys gorzugi* hatchlings bred in captivity. The mean population meristics for *Pseudemys texana* are also shown.

Individual	Carapace Length (mm)	Carapace Width (mm)	Plastron Length (mm)	Plastron Width (mm)	Body Depth (mm)	Weight (g)
1	41.5	39.4	39.0	26.1	21.4	16.0
2	41.4	38.9	37.9	30.3	20.6	16.0
3	40.0	36.8	37.5	30.6	21.4	15.0
4	40.6	36.4	38.0	27.5	19.4	15.0
5	41.7	38.2	38.4	31.3	22.0	16.0
6	41.1	38.4	38.0	30.6	20.9	16.0
7	38.4	36.6	36.5	30.1	21.1	13.0
8	42.4	39.7	39.5	32.6	21.0	18.0
9	40.1	37.8	38.1	29.8	21.1	16.0
10	40.2	37.5	37.1	29.5	21.4	15.0
11	39.9	37.9	37.3	29.7	21.1	15.0
12	39.1	35.5	36.3	28.2	19.1	12.0
13	34.6	33.2	32.6	24.4	16.3	8.0
14	36.6	34.7	35.0	29.0	17.3	10.0
Mean	36.9	37.2	37.2	29.3	20.3	13.4
Mean for *P. texana* (n=89)	39.0	37.2	37.3			14.0

Table 4—Density per river mile of *Pseudemys gorzugi* in the Devils and Pecos rivers and density per river mile of *Pseudemys texana* in the San Marcos River

Species	River (mi.)	Adults	Juveniles	Total	Individuals per river mile
P. gorzugi	Pecos (60)	123	3	126	2.10
	Devils (22)	68	3	71	1.18
P. texana	San Marcos (5.5)	56	16	72	13.09
	San Marcos (5.5)	77	17	94	17.09
	San Marcos (5.5)	109	19	128	23.27

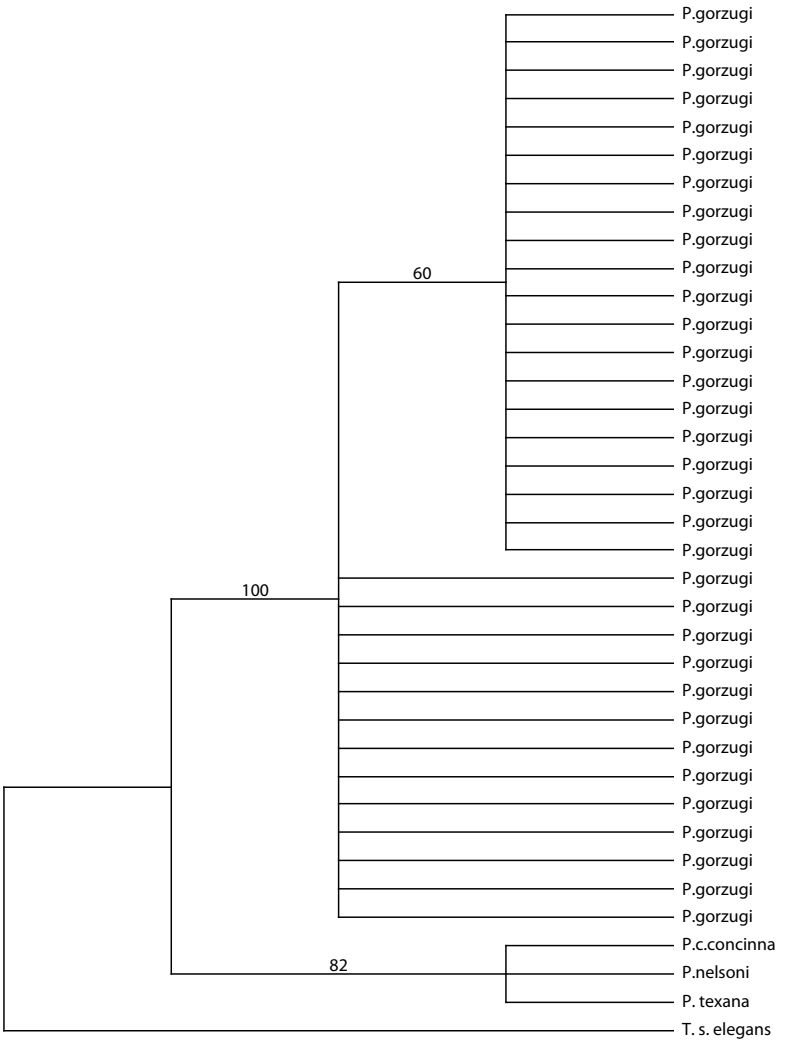

Fig. 1—The figure shows the most parsimonious tree discovered using PAUP for *P. gorzugi*. The bootstrap values are shown on the branches. The tree is based on 876 base pairs of ND4 mtDNA sequence data. *Trachemys scripta elegans* was used as the outgroup. The tree required 83 steps (CI=0.988, RI=0.975).

(turtles were recaptured with one of the five iButtons not working at recovery). The temperature data retrieved was recorded at multiple time intervals depending on whether the thermocron was originally set to record temperatures every hour, every two hours, or every four hours. Because of these scale differences, daily temperature recordings at four-hour intervals were used for subsequent analyses. The daily temperature data were then averaged across the six-week period in which these measurements were taken. The temperature data from the turtle's thermocrons were then compared to the temperature

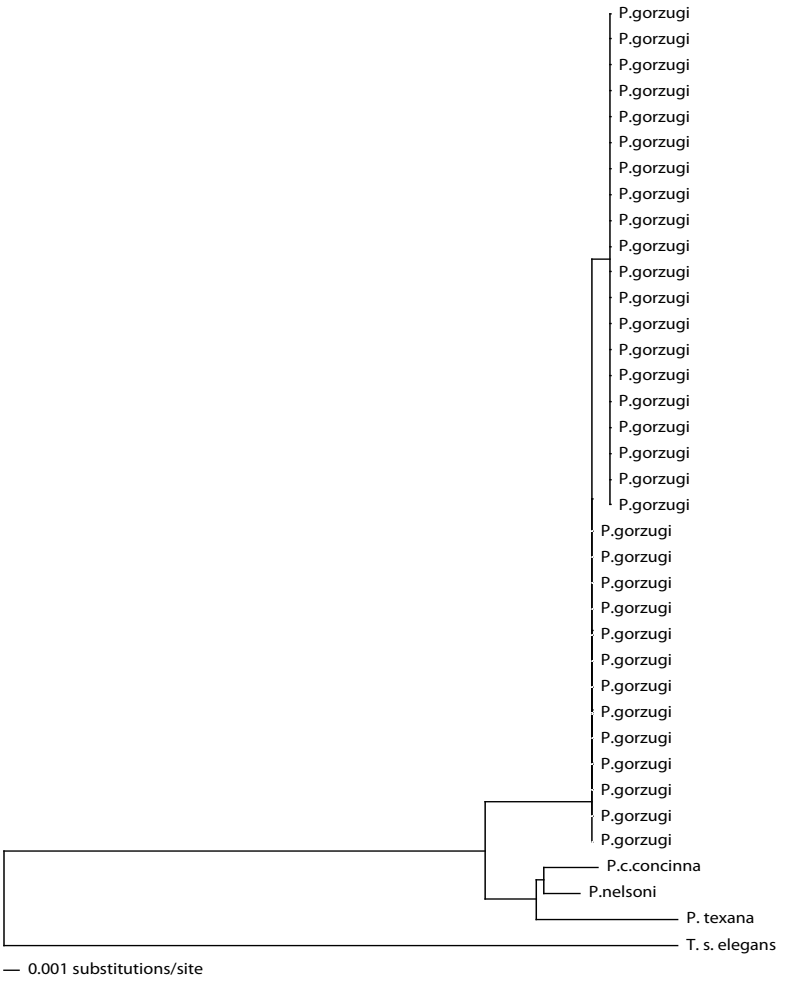

Fig. 2—The figure shows the neighbor-joining phylogram for *P. gorzugi* mtDNA (876 bp) using the Jukes-Cantor distance correction method. *Trachemys scripta elegans* was used as the outgroup.

data from the environmental sampling thermocron; the temperature peak occurs at hour 16, or 4 p.m. (Fig. 3).

Discussion—The most important benefit of this research is in providing a current depiction of the distribution and characteristics of *Pseudemys gorzugi* in all of the Texas river systems in which it occurs. Multiple factors appear to be threatening the remaining Texas *P. gorzugi* populations. An overall low population density, the lack of evidence of significant recruitment, anthropogenic changes to the habitat, toxins and the novel presence of imported fire ants (*Solenopsis invicta*) represent factors that are actually or likely impacting *P. gorzugi* in Texas. It appears that very little genetic variation is present

among the populations of *Pseudemys gorzugi* in Texas, which could limit the species ability to adapt to environmental changes.

The historical range of *Pseudemys gorzugi* spans, much if not all of the length of the Rio Grande in Texas, and the majority of the Pecos and Devils river drainages. However, extant populations of *P. gorzugi* are no longer found throughout much of the potential range. We were able to locate two new distributional records for the species during the study (Bailey and Forstner 2005; Bailey et al. 2005a). A comparatively very low population density of *Pseudemys gorzugi* relative to *Pseudemys texana* was seen for the rivers sampled within Texas (Table 4). The low population density, coupled with existing threats and direct anthropogenic mortality, would appear to pose a considerable threat to the persistence of this species in Texas. The results from both general survey long-term studies (1988 to 2002), and the relatively intense data collected over the last few years, show a conspicuous lack of juveniles throughout the range (Table 1).

Juvenile turtles are notoriously difficult to locate in the wild, however the sampling efforts directed towards locating *Pseudemys gorzugi* were unsuccessful whereas the same sampling efforts were successfully employed to locate juvenile *Pseudemys texana* (Table

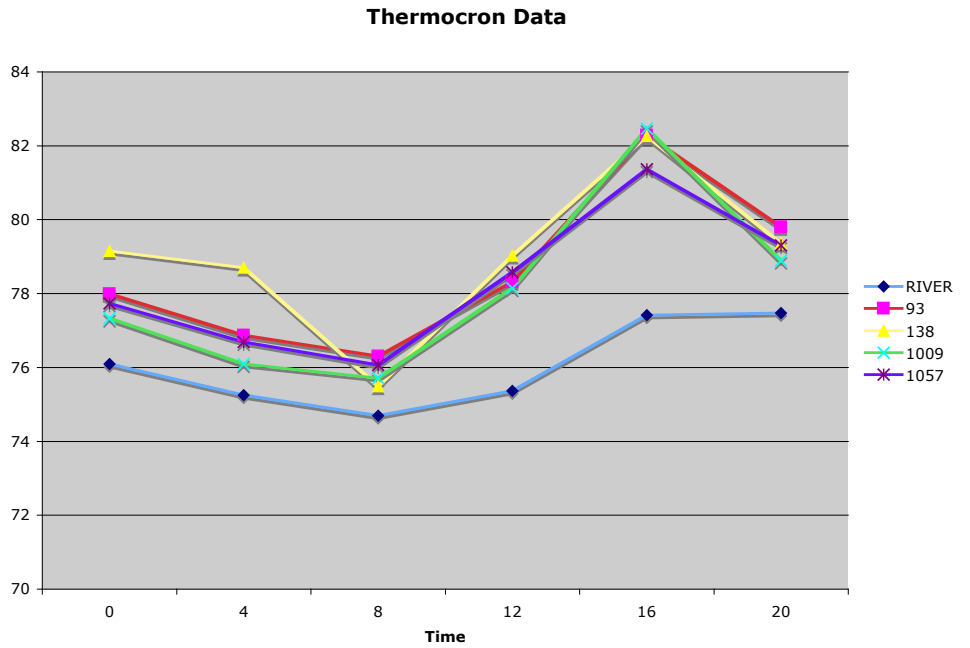

Fig. 3— Summary of the Thermocron iButton temperature data obtained from four *Pseudemys gorzugi* (individuals marked 93, 138, 1009, and 1057) and the environmental sampling thermocron temperature data for the logger placed at a depth of 1 m in the river. Each line on the graph represents a summary of the temperatures logged over three months during the summer of 2004. Error bars have been removed for presentation. For any given individual the daily cycle varied, but all individuals followed the same general trend across the study period.

4). The surveys record nearly an order of magnitude difference in the number of juveniles observed per river mile between *P. gorzugi* and *P. texana* (Table 4). The values provided for the Pecos and Devils rivers are likely overestimates if extrapolated to the currently occupied *P. gorzugi* habitat in each of the rivers. Densities of the species in significantly compromised areas of the Pecos River, or within the Rio Grande itself, are so low that collection of individuals in those areas was unsuccessful. It has been shown that low recruitment success alone will not drastically impact turtle populations since the organisms are long lived and usually have a large population size (Congdon et al. 1993, 1994), but low juvenile density in combination with threats to the adult population, like habitat degradation and over-collection for the pet trade, could pose a serious threat to the stability of the population in the future.

The mitochondrial DNA analyses indicate that *Pseudemys gorzugi* is a monophyletic group distinct from the *Pseudemys* included in the analyses (Fig. 1). The data supports previous morphological analyses in grouping these individuals as one species on its own unique evolutionary trajectory. The bootstrap values also support the monophyly of the species (Fig. 1). The high CI and RI values indicate minimal amounts of homoplasy within the dataset revealing high levels of structure within the data (Fig. 1). However, there is no evidence of population structure across the sample of geographically distributed *P. gorzugi*.

The thermocron results provide preliminary data regarding the basking habits of *P. gorzugi* in west Texas. For the late spring and summer months logged, the average temperature of the turtles is always well above the mean temperature of the river and that temperature spikes in late afternoon when the highest probability of substrate basking occurs (Fig. 3). The partitioning of different basking behaviors is important in this study, as we believe the results support our use of observational (sight) surveys in the determination of the density of turtles in these rivers. The environmental monitoring indicates that these turtles are actively basking during the day, allowing for a higher probability of visual encounters during our field surveys.

Anthropogenic changes to west Texas river systems have degraded the habitat to the point where some local populations of *Pseudemys gorzugi* have been extirpated and others are likely at considerable and continuing risk of extirpation. Surveys indicate low population densities in all localities, especially in the number of juvenile and sub-adult turtles, and thus the populations may not be able to persist without management intervention. Decreased river flow rates and diminishing water quality throughout the entire range of this species makes habitat protection and restoration a vital portion of any effective management strategy for the protection of *Pseudemys gorzugi*. The

continuing research on the life history, ecology, and abiotic factors affecting *Pseudemys gorzugi* will provide other researchers and state management authorities with up to date information on the species and the issues it faces in the desert rivers of Texas. The assembled information will eventually aid in the conservation of this unique Texas reptile.

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