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TROPHIC AND DIETARY OVERLAP STUDY BETWEEN THREATENED AND COMMON RIVERINE TURTLES IN SOUTHEAST NEW MEXICO USING STABLE ISOTOPE ANALYSES

NM WRRI Technical Completion Report No. 394

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Thanchira Suriyamongkol gathered environmental data on a pond near the Black River in Eddy County, NM, home to Rio Grande Cooters (*Pseudemys gorzugi*).

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ABSTRACT

Aquatic turtles represent important biotic components of freshwater ecosystems. The Pecos River watershed is inhabited by six turtle species, including the widespread *Trachemys scripta* (Red-eared Slider) and a species of conservation concern, *Pseudemys gorzugi* (Rio Grande Cooter). We assessed isotopic niche widths of the Rio Grande Cooter and niche overlap where it co-occurs with the Red-eared Slider in the Pecos River tributaries, New Mexico, USA. We used carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analyses of blood and claws. Our results showed niche partitioning among populations of *P. gorzugi* and among sex classes. At sites where both species occur, we documented niche overlap, especially for $\delta^{15}N$ values. Although stable isotopes showed niche overlap between *T. scripta* and *P. gorzugi* based on the ellipse area overlap (SEA_B), the distribution of prey items in the diets of *P. gorzugi* and *T. scripta* revealed the differences in resource selection. We observed that differences in *P. gorzugi* diets among populations correspond to resource availability, suggesting opportunistic foraging behavior of *P. gorzugi*. Our study aids in understanding the ecology and natural history of *P. gorzugi*. Moreover, our study provides insights into interspecific relations of *T. scripta* in their native range.

Keywords: Freshwater turtles, Pecos River, niche overlap, stable isotopes, Rio Grande Cooter

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INTRODUCTION

Riverine habitats are dynamic systems playing key roles in the maintenance of regional fauna and flora (Deacon and Minckley 1974; Ernst and Lovich 2009). In desert areas, characterized by scarcity of water and low precipitation regimes, high diversity and abundance of aquatic vertebrate species provide evidence of the importance of riverine ecosystems in these arid environments (Lovich and Meyer 2002; Free et al. 2013). Water bodies cover only 233.96 square miles out of 121,589.48 square miles total area of New Mexico. The Pecos River and its tributaries represents a major river system stretching across the northern Chihuahuan Desert. This river system has long been affected by anthropogenic activities (i.e., channelization, agriculture, oil extraction), causing profound effects on aquatic diversity (Hoagstrom 2003; Cheek and Taylor 2015). Ecosystems that are highly disturbed may lead to an alteration of resource diversity and availability, which could change the magnitude of inter- and intra-specific competitions (Chen and Lue 2009; Aresco 2010; Pearson et al. 2013). Furthermore, in recent years New Mexico has been under moderate to exceptional drought conditions affecting fragile riparian areas and posing new challenges to the sustainability of aquatic fauna. Approximately 40 federally designated threatened and endangered animal species occur in New Mexico; of those, over half are aquatic (New Mexico Department of Game and Fish 2016). Among the threatened/endangered taxa of desert rivers in New Mexico are freshwater turtles.

Aquatic turtles represent an integral component of freshwater ecosystems, having some of the largest biomass (e.g., 596 kg/ha) of all vertebrates, only surpassed by some fishes (Iverson 1982). They have significant effects on the surrounding aquatic environment including pH, sediment accumulation, and leaf litter decomposition (Lindsay et al. 2013). They are also key components in nutrient cycling and act as ecological bioindicators for the health of

ecosystems due to their longevity and slow growth rate (Sterrett et al. 2015). Overall, turtles play an important part in maintaining and supporting a successful ecosystem.

In the Pecos River system, the Rio Grande Cooter (*Pseudemys gorzugi*) is of particular concern. The Rio Grande Cooter 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). In New Mexico, the species primarily occurs in the lower Pecos River drainage, downstream of Brantley Reservoir, but the species has also been recently discovered 80 km north of their assumed range (Suriyamongkol et al. 2020). Currently, the species is under review by the United States Fish and Wildlife Service (USFWS) for potential federal protection with listing findings scheduled for 2023. Yet, the knowledge of this species to date is limited, which makes the decision-making process challenging.

Pseudemys gorzugi commonly co-occurs with the Red-eared Slider (*Trachemys scripta*; Ernst and Lovich 2009; Mali et al. 2019). *Trachemys scripta* is considered native to the eastern portion of New Mexico, including the Pecos River and its tributaries (New Mexico Department of Game and Fish 2016). While *P. gorzugi* is listed as near-threatened by the International Union for Conservation of Nature (IUCN) (van Dijk 2011), *T. scripta* is considered a species of Least Concern and is also included in the IUCN/SSC Invasive Species Specialist Group's 100 Worst Invasives List (van Dijk et al. 2011). Where introduced, *T. scripta* is known to compete for resources with native freshwater turtles (e.g., Arvy and Servan 1998; Cadi and Joly 2003; Cadi and Joly 2004; Polo-Cavia et al. 2008; Pearson et al. 2015; Balzani et al. 2016). However, little is known about the resource competition and niche overlap between native *T. scripta* and other sympatric species.

This research sought to expand ecological knowledge of *P. gorzugi* by studying species trophic level and its diet through the use of carbon and nitrogen stable isotope analyses of animal tissues, which in turn would provide information on the species habitat requirements and aid to management decision making. Specifically, we successfully obtained information on: 1) the isotopic signatures among *P. gorzugi* populations; 2) the extent of resource overlap between *P. gorzugi* and *T. scripta* within the same habitat; 3) ontogenic diet shift in *P. gorzugi*; 4) differences in isotopic values between tissue types (blood and claws); and 5) dietary preferences (i.e., prey items) of *P. gorzugi* and *T. scripta*. In addition, this study contributed to a long-term mark-recapture study initiated in 2016 by the Principle Investigator (PI). With continuous surveying efforts, this dataset has the potential to derive age-specific survivorship estimates and somatic growth rate estimates, both of which are unknown for the species.

METHODS

Study Sites

We surveyed freshwater turtles at five localities on three Pecos River tributaries in southeastern New Mexico (Figure 1): Berrendo Creek (Chaves County, New Mexico; Bck), Black River (Eddy County, New Mexico; Rup and Rdo), and Delaware River (Eddy County, New Mexico; D). We also surveyed turtles at a private pond located near Rup (P).

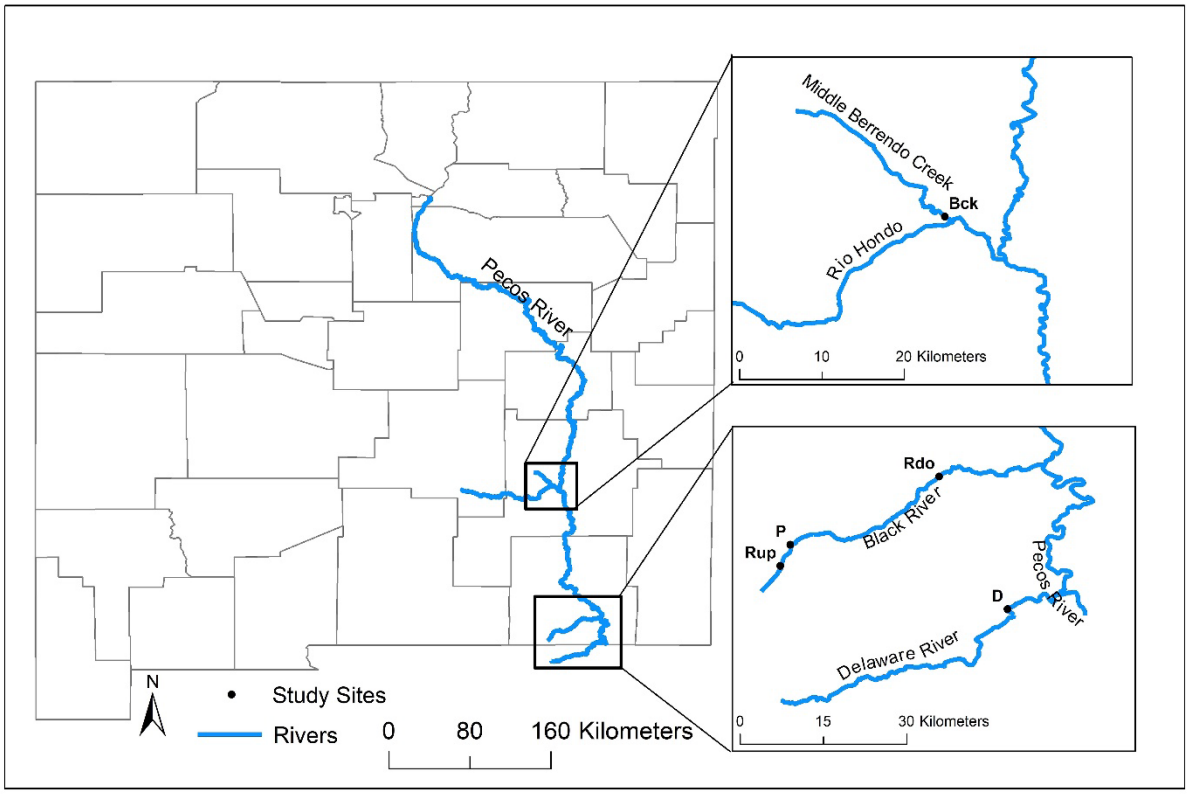

Figure 1. Map representing five sites surveyed for *Pseudemys gorzugi* and *Trachemys scripta* throughout the Pecos River tributaries in New Mexico, USA. We surveyed three sites in Eddy County in the summer of 2019: the upstream stretch of the Black River (Rup), the downstream stretch of the Black River (Rdo), and Delaware River (D). We surveyed one site in Chaves County in the summer of 2019: Berrendo Creek (Bck). Additionally, we surveyed a pond located near the Black River headwaters (P) in the summer of 2017.

Berrendo Creek, located in the city of Roswell, is a ~10 km long tributary of Rio Hondo, which feeds into the Pecos River. This is the newly discovered positive location for *P. gorzugi*

approximately 80 km north of their assumed range (Suriyamongkol et al. 2020). We surveyed a ~450 m stretch of Berrendo Creek located within private property (Figure 2). Although *P. gorzugi* have been detected in Bck, the site is heavily dominated by *T. scripta*, followed by Spiny Softshell Turtle (*Apalone spinifera*), and Common Snapping Turtle (*Chelydra serpentina*; Mahan et al. 2020). This site is also known for recreational fishing and additional Rio Grande Cooters have been caught on fishing lines by anglers. The Black River is a ~87 km tributary of the Pecos River, and is known to support populations of *P. gorzugi* along the entirety of its length (Degenhardt et al. 1996). Rup is ~1500 m long, located near the Black River headwaters and is managed by the Bureau of Land Management (BLM) for recreational purposes such as fishing (Figure 3). Rdo is ~2000 m long and is located approximately 30 km downstream from Rup and is surrounded by private properties, where irrigation and cattle ranching are the most common land uses (Figure 4). Historic and recent surveys show relatively high abundances of *P. gorzugi* on the Black River with fewer *T. scripta* and occasional *A. spinifera*, *C. serpentina*, Yellow Mud Turtle (*Kinosternon flavescens*) and Painted Turtle (*Chrysemys picta*; Degenhardt et al. 1996; Mali et al. 2019.). Delaware River is a ~112 km tributary of the Pecos River (Figure 5). Site D is ~300 m long stretch managed by the BLM, where *T. scripta* and *A. spinifera* are the most dominant species (Degenhardt et al. 1996). Although the Delaware River has been known to support *P. gorzugi*, recent surveys failed to detect the species (Pierce et al. 2016; East, M. 2019). Finally, the private pond (P) has an area of ~3,200 m² and is used frequently for recreational purposes (i.e., camping) and special events (i.e., weddings; Figure 6). The pond is inhabited exclusively by *P. gorzugi* and is also heavily occupied by filamentous algae on its bottom. In addition, the water in the pond has a remarkably low turbidity in comparison to more turbid riverine sites.

Figure 2. Photograph of Site Bck, a tributary of the Rio Hondo that connects to the Pecos River in Roswell, New Mexico, USA. This site was located within private land and surveyed for Rio Grande Cooters (*Pseudemys gorzugi*) and Red-eared Sliders (*Trachemys scripta*) during the summer of 2019. Turtle species found at this location were *T. scripta, P. gorzugi, Chelydra serpentina* (Common Snapping Turtle), and *Apalone spinifera* (Spiny Softshell Turtle).

Figure 3. Photograph of the upstream stretch of the Black River (Rup), a tributary of the Pecos River located in Eddy County, New Mexico, USA, managed by Bureau of Land Management and surveyed for Rio Grande Cooters (*Pseudemys gorzugi*) and Red-eared Sliders (*Trachemys scripta*) during the summer of 2019. The majority of turtles found at this location was *P. gorzugi* and occasional *T. scripta*.

Figure 4. Photograph of the downstream stretch of the Black River (Rdo), a tributary of the Pecos River located in Eddy County, New Mexico, USA, within private land and surveyed for Rio Grande Cooters (*Pseudemys gorzugi*) and Red-eared Sliders (*Trachemys scripta*) during the summer of 2019. Turtle species found at this location were *P. gorzugi*, *T. scripta* and occasional *Apalone spinifera* (Spiny Softshell Turtle), *Chelydra serpentina* (Common Snapping Turtle), *Kinosternon flavescens* (Yellow Mud Turtle), and *Chrysemys picta* (Painted Turtle).

Figure 5. Photograph of the surveyed stretch of the Delaware River (D), a tributary of the Pecos River located in Eddy County, New Mexico, USA, managed by the Bureau of Land Management and surveyed for Rio Grande Cooters (*Pseudemys gorzugi*) and Red-eared Sliders (*Trachemys scripta*) during the summer of 2019. The dominant species of turtles found at this location were *T. scripta* and *Apalone spinifera* (Spiny Softshell Turtle), and occasional *P. gorzugi.*

Figure 6. Photograph of the private pond (P), located nearby the upstream stretch of the Black River in Eddy County, New Mexico, USA, within private property. This location was occupied exclusively by the Rio Grande Cooters (*Pseudemys gorzugi*). The turtles were captured via snorkeling at this location in the summer of 2017.

Data Collection

All samples were collected from May to September 2019, with the exception of Site P samples, which were collected in the summer of 2017. Systematic surveys primarily relied on passive sampling techniques, such as trapping using traditional hoop-net traps. Fiberglass hoop-net traps were 50.8 cm-diameter single opening, single throated, wide-mouth hoop nets with 2.54 cm mesh-size and four hoops per net (Memphis Net and Twine Co., Memphis, TN). Traps were secured by tying them to the available live vegetation. A floating device was placed inside the traps to prevent drowning, and mouths of the traps were placed facing downstream (Figure 7). Traps were baited with canned sardines that were placed into small plastic containers drilled

with small holes to attract the turtles but prevent them from feeding on the bait (Mirabal et al. 2018). Traps were checked every day and rebaited every two days while checking for captured turtles. The density of traps varied between 0.07/m and 0.10/m depending on site accessibility. At Site P, however, we collected turtles via snorkeling.

Figure 7. Photograph of a deployed hoop-net trap at Site D, a section of the Delaware River, Eddy County, New Mexico, USA. The trap was stretched using two wooden poles. A flotation device (yellow pool noodle) was placed inside the trap to prevent turtles from drowning.

For every turtle captured, we took standard measurements (i.e., straight-line carapace length, carapace width, plastron length, plastron width, body depth, and weight; Figure 8) using a tree caliper, and marked new individuals by marginal scute notching using a portable rotary tool (Dremel, Racine, WI), PIT (Passive Integrated Transponder) tags or toe-clipping, according to the size of the turtle (Cagle 1939; Buhlmann and Tuberville 1998; Suriyamongkol and Mali 2018). For *P. gorzugi* and *T. scripta*, we clipped the terminal 0.5 cm of the medial toe claw on the hind foot (either right or left) using a sterilized veterinarian nail-clipper. From a subset of

these individuals (i.e., individual was large enough), we also drew approximately 0.2 ml of blood from the femoral vein using a sterile syringe. In the field, claws and blood were placed into 1.5 ml plastic microcentrifuge tubes and stored provisionally in -20 °C freezer. Within five to ten days, samples were transferred to a -80 °C freezer until preparation for analysis. On the Black River, we opportunistically collected invertebrate and fish that were found inside or on the hoop-net traps and hand captured overhanging riparian vegetations and algae samples that could constitute potential food sources for the turtles (Letter et al. 2019). Fish samples, invertebrates, and invertebrate larvae were stored in plastic vials and placed in the -80 °C freezer until preparation, and plants were kept in a plant press until complete dehydration (Hobson et al. 1997; Lara et al. 2012; Pearson et al. 2013).

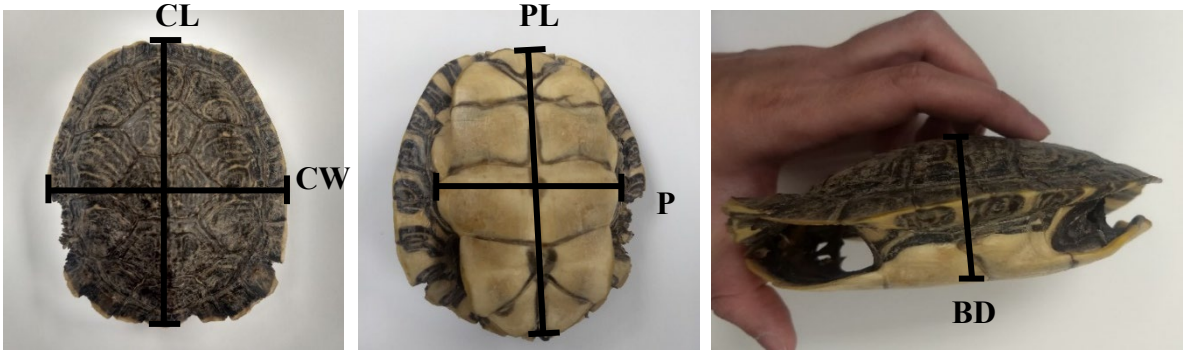

Figure 8. The standard morphometric measurements of turtles including straight-line carapace length (CL), carapace width (CW), plastron length (PL), plastron width (PW), and body depth (BD).

Sample Preparation

Claws, whole blood, vertebrate (muscle tissue), and invertebrate samples (whole body) were dried at 60 °C for 24 hours and ground into fine powder using a pestle and mortar. Approximately 1 mg (0.7–1.4 mg) of each material was packed into capsules. Claws were packed into silver capsules because the hardiness of claws even when ground tend to break regular tin capsules. All other samples were packed into regular tin capsules. Vegetation

samples (i.e., leaves, seeds, and flowers) that were dried in the plant press were ground into powder and placed into tin capsules. We made duplicates of 10% of our total sample number to account for potential variation. The duplicated samples provided similar results when compared to the original samples, with a mean standard deviation of ±0.036‰ for $\delta^{13}C$ and ±0.05‰ for $\delta^{15}N$ in blood, ±0.14‰ for $\delta^{13}C$ and ±0.28‰ for $\delta^{15}N$ in claws. No lipid extraction was performed due to the extremely low concentration of lipids in claws and whole blood of turtles (Chaikoff and Entenmen 1946; Pearson et al. 2013; Balzani et al. 2016).

Analyses

Samples were sent to the University of California at Davis Stable Isotope Facility for stable isotope analysis on carbon and nitrogen natural abundances. All samples were analyzed through combustion via a Europa 20-20 isotope mass spectrometer. Atmospheric air and Vienna PeeDeeBelemnite were used as standards for nitrogen and carbon, respectively. We compared carbon and nitrogen isotopic values between blood and claw samples from individuals for which both tissue types were collected at the time of the survey. We grouped the samples based on their locality. Datasets with a small sample size ($n<30$) were tested for normality using the Shapiro-Wilk test. For sample groups with a large sample size or normal distribution, the Student paired t-test was used. However, the *T. scripta* dataset from Rdo was not normally distributed; therefore, we used the Wilcoxon signed-rank test for non-parametric data.

We used the Stable Isotope Bayesian Ellipses (SIBER) package in R (Jackson et al. 2011; (R Core Team 2016) to estimate Bayesian Layman's isotope metrics: NR ($\delta^{15}N$ range) and CR ($\delta^{13}C$ range), TA (the area of the convex hull that encompasses all group members), and CD (the average Euclidian distance of each member to the centroid; Layman et al. 2007). We

also included MNND (mean nearest neighbor distance) and SDNND (standard deviation of the nearest neighbor distance) as a measure of trophic redundancy. We calculated the Bayesian standard ellipse areas (SEA_B) to obtain estimates of isotopic niche widths as well as standard ellipses corrected for the small samples size (SEA_c), as a measure of the mean core group isotopic niche for *P. gorzugi* across all habitats and *T. scripta* where it co-occurs with *P. gorzugi* (Jackson et al. 2011). Here, we used claw samples, as we collected the largest sample size of this tissue type, and considering that claws were the only tissue collected from site P.

In addition, we used a Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson et al. 2008) to assess the differences in isotopic niche position by comparing the location of the centroids (i.e., means of $\delta^{13}C$–$\delta^{15}N$) of (1) *P. gorzugi* among study sites (Rdo, Rup, Bck, and P), (2) *T. scripta* among study sites (Rdo and Bck), and (3) *P. gorzugi* among sex classes (males, females, and juveniles). Absolute values of $\delta^{13}C$ were used and all data (i.e., $\delta^{13}C$ and $\delta^{15}N$) were square root transformed and converted to a Euclidean distance matrix before the analyses. The differences in isotopic niche were considered significant when P-values were <0.05. PERMANOVA analyses were done using the function "adonis2" in the vegan package (Oksanen et al. 2020) in R (R Core Team 2016). To evaluate the accuracy of PERMANOVA results, we also performed analyses to test homogeneity of group dispersions using the function "betadisper" in the vegan package (Oksanen et al. 2020).

To evaluate the ontogenic diet shift, we used simple linear regression models with nitrogen isotopic values as the dependent variable and straight-line carapace length as an independent variable. Blood samples were used for the analyses to represent the most recent diet pertaining to their current age/size class. Only *P. gorzugi* samples from the Black River were included in the analyses because individuals from all size classes were captured at these

sites. Finally, we estimated the percentage of prey distribution in the diet of *P. gorzugi* and *T. scripta* on the Black River using the stable isotope mixing model in MixSIAR package in R (Stock et al. 2018). Blood samples were used in the models to represent the most recent diet and to most closely correspond to the season during which the prey items were collected. We focused only on prey items found in fecal samples (Letter et al. 2019) because mixing models lose discriminatory power as source number increases. To account for trophic fractionation of nitrogen isotopic values between preys and consumers, we used the discrimination factor of +2.2‰, calculated based on whole blood without lipid extraction (Seminoff et al. 2007). However, the trophic fractionation of carbon isotopic values for whole blood is unknown in freshwater turtles; therefore, we used the discrimination factor of +0.23‰, calculated based on claw samples (Aresco 2005). Letter et al. (2019) were able to identify several plants to species level in *P. gorzugi* fecal samples while other vegetation was classified as dicots. We followed this logic to separate plant species into groups. We separated animal prey into two groups: invertebrate (i.e., ants, beetles, and dragonfly larvae) and vertebrate (i.e., green sunfish). We would like to note that other fish species including common carp, channel catfish, and large-mouth bass were present in the area; however, we only observed that green sunfish were eaten in the traps with turtles. Filamentous algae were separated into two groups based on the microhabitat where the samples were obtained (i.e., benthic vs. floating).

DISCUSSION OF RESULTS AND THEIR SIGNIFICANCE

In 2019, our survey effort consisted of 2033 trap days, varying from 270 to 844 trap days per site. We captured 316 specimens of *P. gorzugi* and 133 specimens of *T. scripta*. Sites where *P. gorzugi* and *T. scripta* co-occurred were Bck, Rup, Rdo, and D. At Bck, we captured nine *P. gorzugi* and 95 *T. scripta*. We captured 155 *P. gorzugi* and one *T. scripta* at Rup and 151 *P. gorzugi* and 23 *T. scripta* at Rdo. At Site D, we captured a single *P. gorzugi* and 14 *T. scripta*. In 2017, we sampled seven *P. gorzugi* at site P. The absence of *T. scripta* at site P, and a single *T. scripta* captured at Rup restricted interspecific comparisons with *P. gorzugi*. In addition, the number of captures of both *P. gorzugi* and *T. scripta* at Site D were too small to allow further analyses of the data.

We collected a total of 161 claw and 145 blood samples from *P. gorzugi* and 76 claw and 78 blood samples from *T. scripta* (Table 1). Among all sites, mean $\delta^{13}C$ values for *P. gorzugi* ranged from -20.89‰ to -28.46‰ for claws and -21.71‰ to -30.05‰ for blood (Table 2). Meanwhile, mean $\delta^{15}N$ values ranged from 5.64‰ to 11.94‰ for claws and 8.42‰ to 10.92‰ for blood (Table 2). Based on the t-tests and a Wilcoxon sign-ranked test, the isotopic values of carbon and nitrogen between claw and blood samples showed a significant difference ($P<0.05$) for all groups except for the nitrogen values of *T. scripta* from Rdo (Table 2). Overall, we found lower isotopic values in blood, similarly to Balzani et al. (2016) who studied blood and claw samples of *T. scripta* and *Emys orbicularis*.

Table 1. A summary of straight-line carapace length (mm) ranges, along with the number of sample sizes of *Pseudemys gorzugi* (PG) and *Trachemys scripta* (TS) broken down by sex (M = male, F = female, J = juvenile) of which two different tissue types (claw and blood) were collected for $\delta^{13}C$ and $\delta^{15}N$ stable isotope analyses. The samples were collected from three tributaries of the Pecos River in New Mexico, USA: Black River (Rup and Rdo), Berrendo Creek (Bck), and Delaware River (D), as well as a pond located near the Black River headwaters (P). All samples were collected in the summer 2019 with the exception of turtles at site P, which were collected in the summer 2017. The numbers in parentheses represent the sample size of each tissue type.

Site	Species	M claw (*n*)	M blood (*n*)	F claw (*n*)	F blood (*n*)	J claw (*n*)	J blood (*n*)
Bck	PG	170–211 (4)	170–211 (4)	244–282 (4)	200–282 (5)	0	0
	TS	144–227 (33)	144-227 (35)	193–257 (19)	193–257 (17)	155 (1)	0
Rup	PG	116–190 (20)	116–190 (22)	118–230 (26)	118–230 (23)	85.1–131 (21)	85.1–117 (23)
	TS	161 (1)	0	0	0	0	0
Rdo	PG	125–203 (24)	125–203 (23)	123–258 (26)	123–258 (24)	72.4–138 (28)	72.4–138 (20)
	TS	188–195 (3)	160–195 (3)	159–244 (4)	159– 244 (4)	64.5–101 (5)	64.5–129 (8)
P	PG	232 (1)	0	134–266 (5)	0	112 (1)	0
D	PG	183 (1)	183 (1)	0	0	0	0
	TS	163–187 (4)	163–187(4)	148–243 (5)	148–243 (5)	107 (1)	107–115 (2)

Table 2. Mean $\delta^{13}C$ and $\delta^{15}N$ values (± SD) of claw and blood samples of *Pseudemys gorzugi* (PG) and *Trachemys scripta* (TS) collected at five surveyed locations in New Mexico, USA: Berrendo Creek (Bck), Black River (Rup and Rdo), Delaware River (D), and a pond (Site P) located near the Black River headwaters. All samples were collected in summer 2019, except the samples from site P, which were collected in summer 2017. The Student paired t-test was used to compare the carbon and nitrogen isotopic values between claw and blood samples for all samples, with the exception of the *T. scripta* dataset from Rdo. For this dataset, we used the Wilcoxon signed-rank test for non-parametric data because the data were not normally distributed.

Species	Site	$\delta^{13}C$ ‰					$\delta^{15}N$ ‰				
		Claw	Blood	t-statistics	df	p-value	Claw	Blood	t-statistics	df	p-value
PG	Bck	-20.89 (1.58)	-22.09 (1.09)	2.79	7	0.03	11.94 (0.92)	10.92 (1.28)	6.15	7	<0.01
	Rup	-27.61 (0.97)	-28.02 (0.87)	3.89	60	<0.01	8.92 (0.57)	8.42 (0.67)	10.72	60	<0.01
	Rdo	-28.46 (2.68)	-30.05 (2.84)	6.93	65	<0.01	9.86 (0.98)	9.27 (0.78)	9.90	65	<0.01
	P	-23.65 (1.66)	NA	NA	NA	NA	5.64 (1.02)	NA	NA	NA	NA
	D	-24.17	-21.71	NA	NA	NA	10.19	10.71	NA	NA	NA
TS	Bck	-20.50 (1.21)	-21.11 (0.96)	7.04	47	<0.05	12.22 (2.54)	11.87 (2.70)	2.86	47	0.01
	Rup	-21.71	NA	NA	NA	NA	10.06	NA	NA	NA	NA
	Rdo	-22.44 (2.13)	-23.89 (2.15)	v-value = 66		<0.05	11.18 (1.51)	10.39 (1.12)	-0.29	10	0.77
	P	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	D	-21.59 (1.14)	-22.03 (1.79)	NA	NA	NA	10.67 (0.87)	10.59 (0.75)	NA	NA	NA

The $\delta^{13}C$ and $\delta^{15}N$ varied among the populations of *P. gorzugi*, from very narrow to relatively wide standard ellipses at the Rup and Rdo sites, respectively (Figure 9). $\delta^{13}C$–$\delta^{15}N$ centroids further revealed that isotopic niches were significantly different among *P. gorzugi* populations (F=75.85, P=0.001). The differences in niche size likely reflected the local availability of resources and their isotopic signature. However, the dispersion test showed a significant heterogeneity in the data (F=11.96, P=0.001; Table 3). Due to the heterogeneity in dispersions, the results of PERMANOVA might be affected (Anderson and Walsh 2013); therefore, we could not conclude that the differences in isotopic niches among poulations were due to differences in habitat.

Table 3. The results of Permutational Multivariate Analysis of Variance (PERMANOVA) analyses and heterogeneity in dispersions comparing the centroid locations of (1) *Pseudemys gorzugi* among study sites: Berrendo Creek (Bck), Black River (Rup and Rdo), and a pond (P), (2) *Trachemys scripta* between Rdo and Bck, and (3) *P. gorzugi* among sex classes (males, females, and juveniles) at Rup and Rdo. All study sites were located in New Mexico, USA.

Analyses	Factor	df	Sum of squares	R^2	F	p-value
PERMANOVA						
P. gorzugi among sites	site	3	0.074	0.59	75.85	0.001
T. scripta among sites	site	1	0.0039	0.083	5.70	0.01
P. gorzugi among age classes (Rdo)	sex	2	0.0085	0.22	10.82	0.001
P. gorzugi among age classes (Rup)	sex	2	0.00057	0.078	2.73	0.029
Betadisper						
P. gorzugi among sites	site	3			11.96	0.001
T. scripta among sites	site	1			0.052	0.83
P. gorzugi among age classes (Rdo)	sex	2			1.79	0.16
P. gorzugi among age classes (Rup)	sex	2			0.11	0.89

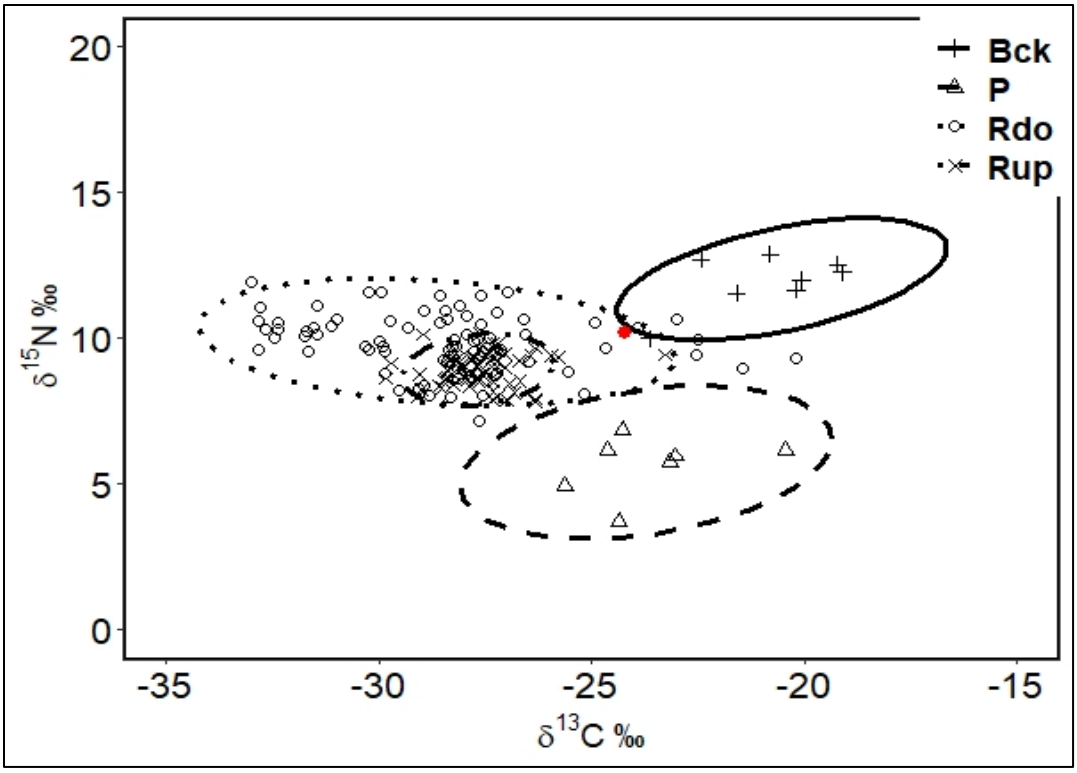

Figure 9. The standard ellipse areas (95%; SEA_B) of the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analyses of *Pseudemys gorzugi* claws collected in the summers of 2017 and 2019 across four surveyed sites throughout the Pecos River tributaries in New Mexico: the upstream stretch of the Black River (Rup; n=67), the downstream stretch of the Black River (Rdo; n=78), Berrendo Creek (Bck; n=8), and a pond located near the Black River headwaters (P; n=7). The solid red dot represents the only sample collected from Delaware River (D).

Among the populations of *P. gorzugi*, the population from site Rdo had the highest variation in isotopic signature (CR and NR), as well the largest niche area (Table 4; Figure 10). The lowest convex hull (TA) of *P. gorzugi* was observed at Site Bck, and the smallest SEA_C at the Rup site (Figure 10). A single *P. gorzugi* caught at Site D had $\delta^{13}C$ and $\delta^{15}N$ values of -24.2‰ and 10.2‰, respectively, which were within the range of values observed for the other river sites.

Table 4. Estimated Layman's metrics and stable isotope results for $\delta^{15}N$ and $\delta^{13}C$ of *Pseudemys gorzugi* (PG) and *Trachemys scripta* (TS) across five surveyed locations in New Mexico, USA: Berrendo Creek (Bck), Black River (Rup and Rdo), Delaware River (D), and a pond (Site P) located near the Black River headwaters. NR = $\delta^{15}N$ range, CR = $\delta^{13}C$ range, TA = convex hull area, CD = mean distance to centroid, MNND = mean nearest neighbor distance, SDNND = standard deviation of the nearest neighbor distance, SEA_B = Bayesian standard ellipse area, SEA_C = standard ellipse area for small samples sizes.

Site	Species	NR (‰)	CR (‰)	TA (‰^{2})	CD (‰)	MNND (‰)	SDNND (‰)	SEA_B (‰^{2})	SEA_C (‰^{2})
Bck	PG	2.89	4.52	5.76	1.50	0.98	0.82	26.11	4.36
	TS	10.54	7.27	46.39	2.22	0.50	0.69	58.46	9.76
Rup	PG	2.33	6.57	10.15	0.9	0.22	0.32	10.36	1.73
Rdo	PG	4.73	12.8	36.16	2.30	0.40	0.28	48.48	8.09
	TS	5.60	7.02	24.56	2.24	1.01	0.72	59.63	9.95
P	PG	3.12	5.18	8.03	1.57	1.13	0.88	36.18	6.04
D	TS	3.22	4.57	7.63	1.34	0.69	0.59	25.10	4.19

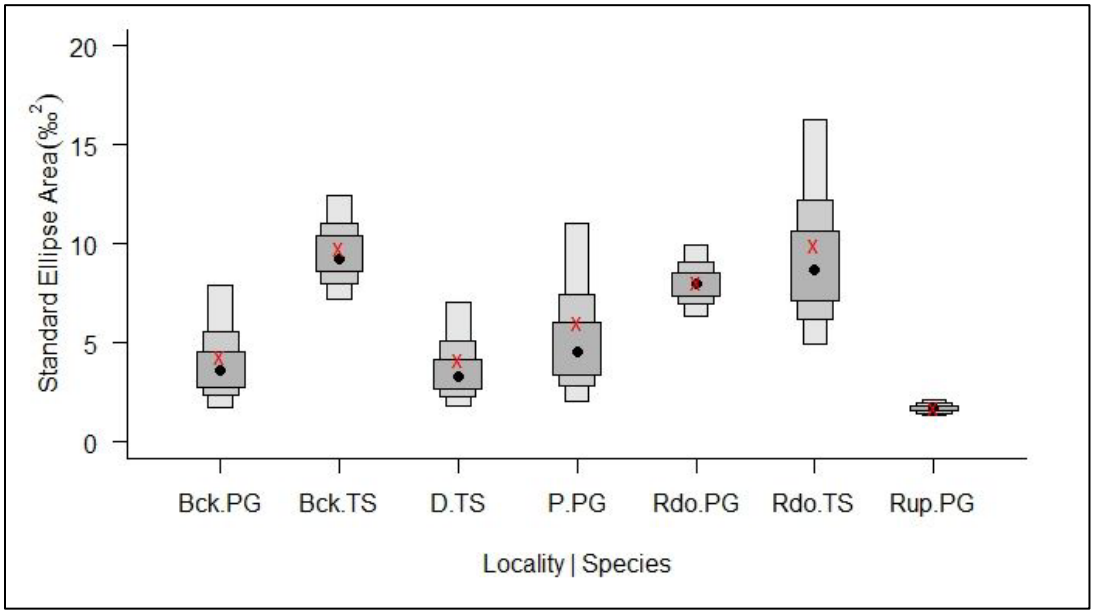

Figure 10. Density plots comparing the standard ellipse areas (95%; SEA_B) with the maximum likelihood estimates of the corrected standard ellipse areas (SEA_C; the red X marks) per species per survey location throughout the Pecos River tributaries in New Mexico, USA, including the upstream stretch of the Black River (Rup), the downstream stretch of the Black River (Rdo), Delaware River (D), Berrendo Creek (Bck), and a pond located near the Black River headwaters (P) using claw samples of *Pseudemys gorzugi* (PG) and *Trachemys scripta* (TS) collected during 2017 (only for site P) and 2019 (all other sites).

For the populations of *P. gorzugi* in the Black River, comparison of $\delta^{13}C$–$\delta^{15}N$ centroids revealed significant differences in mean niche positions among sex classes at Rdo and Rup ($F=10.83$, $P=0.001$ and $F=2.73$, $P=0.029$, respectively; Table 3). Based on the simple linear regression models, the relationship between the nitrogen isotopic signature and carapace length of *P. gorzugi* from Rdo was not significant ($P=0.5$, $r=-0.092$; Figure 11), while there was a significant negative relationship between the nitrogen isotopic signature and carapace length in the turtles at Rup ($P<0.05$, $r=-0.61$; Figure 11B). Our results generally corroborate the findings of Letter et al. (2019) regarding the various degrees of omnivory exhibited by *P. gorzugi* in the Black River. Letter et al. (2019) also found that turtle size positively correlated with algae consumption. Although we found differences in niche position among male, female, and juvenile *P. gorzugi* at Rup and Rdo, evidence of ontogenetic diet shift (change in $\delta^{15}N$) was observed only at Rup (Figure 5). While other *Pseudemys* species tend to be mainly herbivorous as adults (e.g., *P. texana*, *P. rubriventris*; Ernst and Lovich 2009), this study sheds new light on diet diversity of *P. gorzugi*.

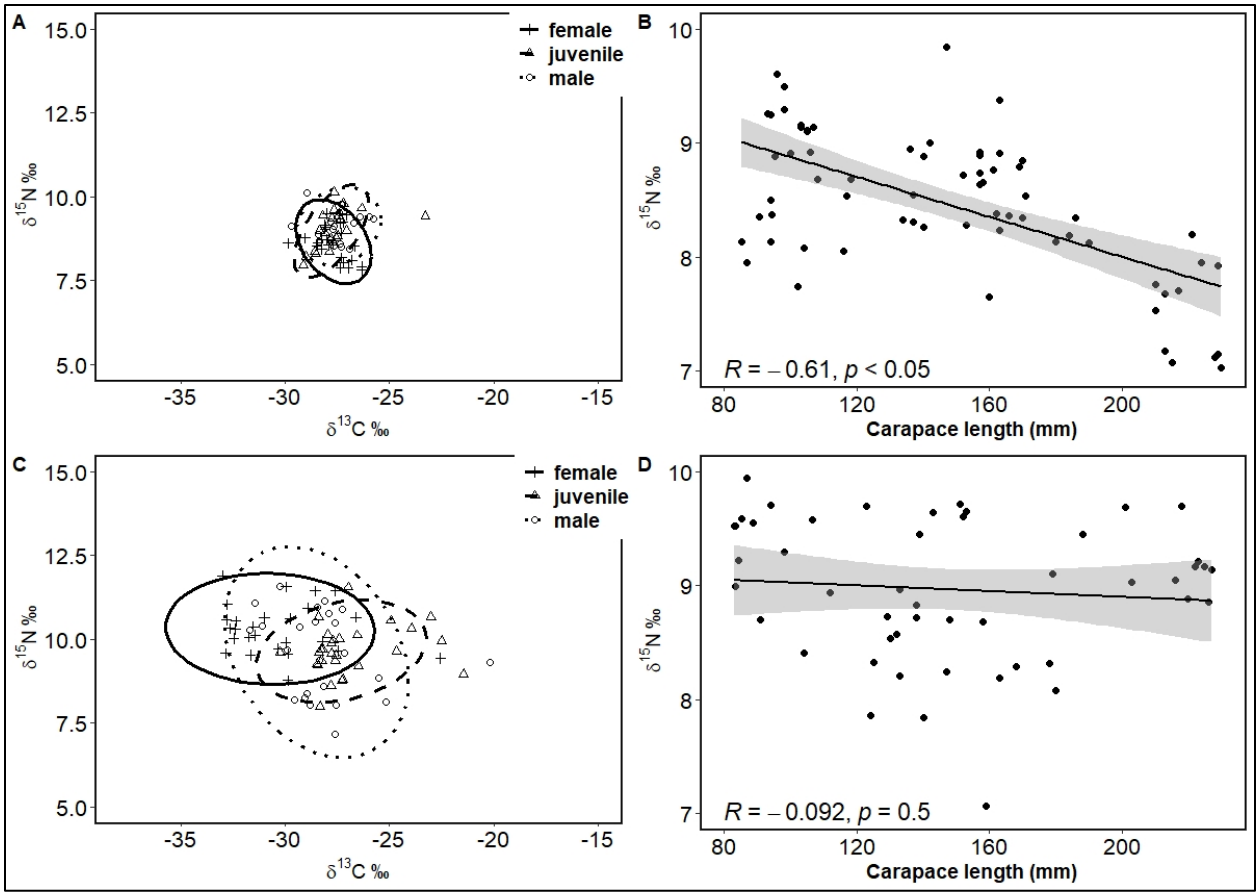

Figure 11. Standard ellipse areas (95%; SEA_B) of the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analyses of *Pseudemys gorzugi* claws collected in the summer of 2019 from (A) the upstream stretch of the Black River (Rup) and (C) the downstream stretch of the Black River (Rdo), New Mexico, USA, separated by sex classes: female, male, and juvenile. The relationship between turtle size and $\delta^{15}N$ was analyzed to assess the ontogenetic diet shift at each location, namely Rup (B) and Rdo (C) using blood collected in the summer of 2019 to represent the most recent diet for each size class at the time of survey.

For *T. scripta*, the mean values of claw samples ranged from -20.50‰ to -22.44‰ for $\delta^{13}C$, and from 10.06‰ to 12.22‰ for $\delta^{15}N$ (Table 2). Between two sites where it co-occurs with *P. gorzugi* (Bck and Rdo), *T. scripta* had similar CR but varied in NR, showing nearly two-fold higher values for NR and TA at the Bck site (Table 4; Figure 12). Standard ellipse area for small sample sizes were similar for both sites (Table 4). The comparison of $\delta^{13}C$–$\delta^{15}N$

centroids revealed significant differences in mean niche positions between the two populations of *T. scripta* (F=5.70, P=0.01; Table 3).

The area overlap between *P. gorzugi* and *T. scripta* based on SEA_C was 3.28 and 0 at Bck and Rdo, respectively; however, the area overlap based on SEA was ~20 (Table 5; Figure 13). These results should be interpreted with caution as the isotopic similarity does not neccesarily represent ecological or dietary similarity, and various species may be feeding on different food resources with identical isotopic compositions. However, there was no overlap of SEA_C between the two species at Rdo, and only a very small overlap at Bck (3.28; Table 5), supporting the differentiation of the mean core group isotopic niche. In addition, at both study sites, *T. scripta* demonstrated a wider standard ellipse area than their counterpart *P. gorzugi* (Figure 3), suggesting higher omnivory and plasticity of *T. scripta*.

Table 5. The area of overlap between standard ellipse area for small sample sizes (SEAc) and Bayesian standard ellipse area (SEAb) for *Trachemys scripta* and *Pseudemys gorzugi* using claw samples collected in 2019 at locations where the two species co-occur: Berrendo Creek (Bck) and the downstream stretch of the Black River (Rdo), New Mexico, USA.

	Bck SEAc (SEAb)	Rdo SEAc (SEAb)
Area overlap	3.28 (20.46)	0 (19.62)

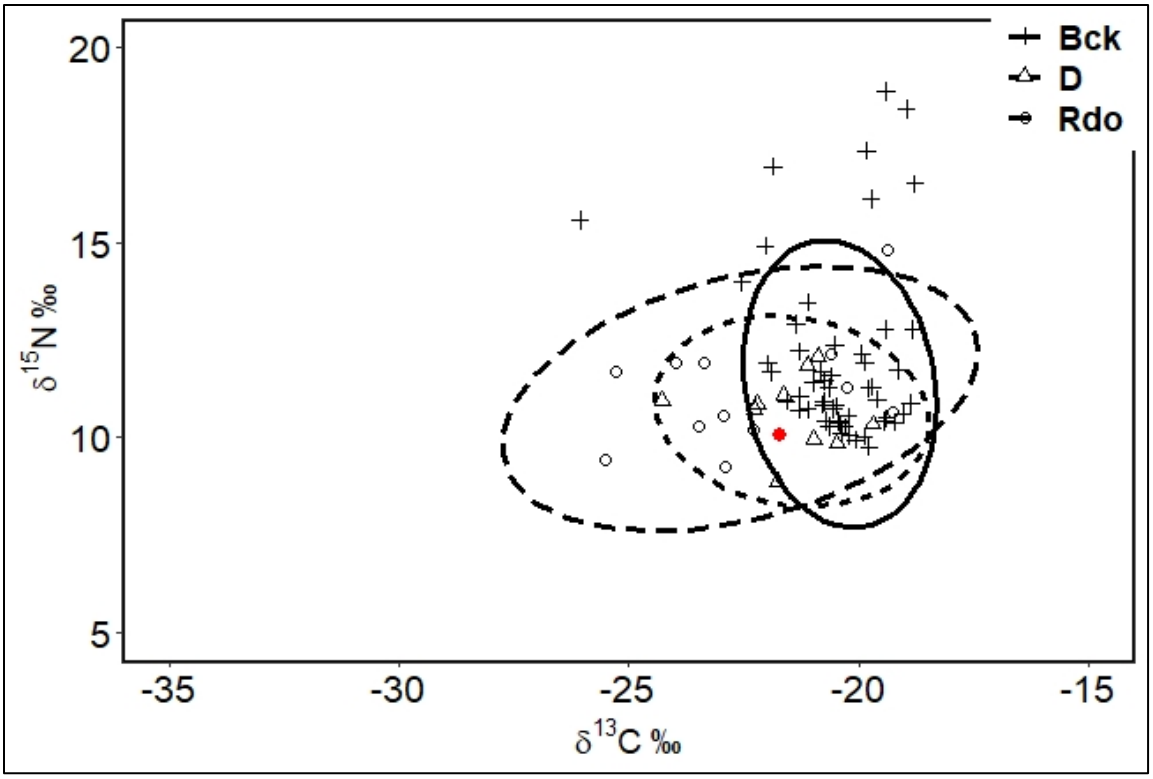

Figure 12. The standard ellipse areas (95%; SEA_B) of the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analyses of *Trachemys scripta* claws collected in 2019 across four surveyed sites throughout the Pecos River tributaries in New Mexico: Berrendo Creek (Bck; n=53), Delaware River (D; n=10), and the downstream stretch of the Black River (Rdo; n=12). The solid red dot represents the only sample collected from the upstream stretch of the Black River (Rup).

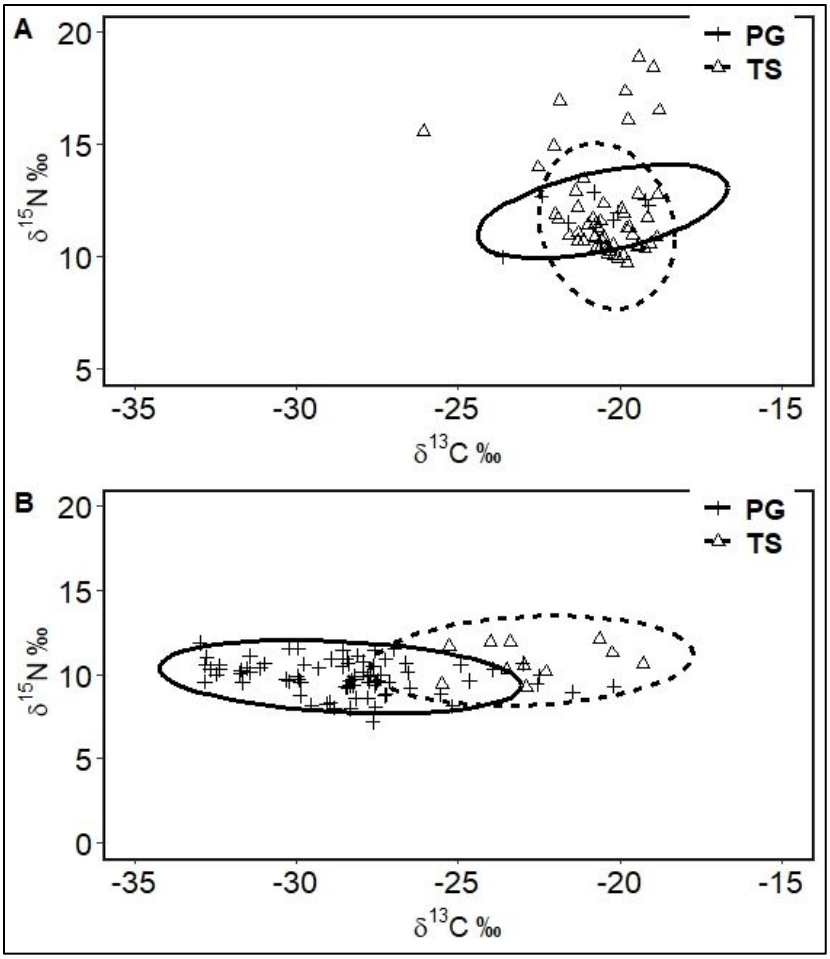

Figure 13. The standard ellipse areas (95%; SEA_B) comparing the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analyses of *Pseudemys gorzugi* (PG) claws and *Trachemys scripta* (TS) claws collected during 2019 surveys from the surveyed locations where the two species co-occurred: (A) the Berrendo Creek (Bck) and (B) the downstream stretch of the Black River (Rdo), New Mexico, USA.

For the *P. gorzugi* population at the Rup, net-leaf hackberry constituted the majority of the diet (24% for leaves and 10% for seeds). Other dicot vegetation constituted ~8% of the diet, while cottonwood seeds and willow leaves each constituted approximately 5% of the diet. Filamentous algae constituted ~8% of the diet. Animal sources, fish, and invertebrates, each constituted ~4% of the diet. Letter (2018) surveyed riparian vegetation along the Black River and noted that plants, in particular the net-leaf hackberry, occurred at a high frequency at Rup, which corresponds to the diet partitioning of *P. gorzugi*.

At Rdo, filamentous algae contributed most to the diet of *P. gorzugi* (20%), followed by cottonwood (19%). Animal food sources constituted ~12% of *P. gorzugi*'s diet (3% fish and 9% invertebrates). Net-leaf hackberry, willow, and other dicot vegetation constituted ~5% of diet each. Interestingly, salt cedar, which only occurs at Rdo, constituted ~8% of the diet. Based on the mixing model, the food items in the diet of *P. gorzugi* at Rdo were almost evenly distributed among animal sources, filamentous algae, and various dicots. These values indicate plasticity especially in carbon source use, or an attempt to compensate in the absence of the preferred prey.

For *T. scripta* at Rdo, the majority of the diet was composed of animal sources (~11% fish and ~11% invertebrates), followed by filamentous algae at ~13%, while other plant materials (i.e., cottonwood, net-leaf hackberry, willow, salt cedar, and other dicots) constituted between 3% and 9% of the diet. Based on the mixing models of prey distributions in diet analyses, it also appears that *T. scripta* and *P. gorzugi* consume different plant sources at Rdo, as characterized by differentiation in $\delta^{13}C$ (Figure 13B). The results of prey distributions in diet analyses further support the difference in resource selection between the two species. While the majority of the diet in *T. scripta* consisted of fish and invertebrates, the majority of the diet in *P. gorzugi* was filamentous algae and cottonwood leaves.

PRINCIPAL FINDINGS, CONCLUSIONS, AND RECOMMENDATIONS

This study is the first to compare isotopic niches of two naturally co-occuring freshwater turtle species of the Pecos River system. We were particularly aiming to understand niche width of *P. gorzugi* across different habitats, and the degree of its niche overlap with the wide spread *T. scripta*. *Pseudemys gorzugi* exhibited resource partitioning among populations, which reflected resource availability at each site. In addition, our results generally corroborate the findings of Letter et al. (2019) regarding the various degrees of omnivory exhibited by *P. gorzugi* in the Black River. Although evidence of ontogenic diet shift was not prominent in our study, we found differences in niche position among sex classes (i.e., male, female, juvenile). Previous studies demonstrated that introduced *T. scripta* has the potential for trophic competition and niche overlap with native turtles (Pearson et al. 2013; Balzani et al. 2016), but *T. scripta* niche overlap is seldom studied in its native range. Stable isotopes showed similar area overlap (SEA_B) of *T. scripta* and *P. gorzugi* between two study areas. However, we found the two species showed differences in food item preferences.

One caveat of our study design is that we opportunistically collected animal and plant matter only on the Black River and therefore were not able to reconstruct the diet and examine trophic positions among all four populations, since the isotopic signatures of different trophic levels can vary locally. Resource availability may also drive local turtle species abundances. The Black River is the only location in New Mexico known to support relatively large numbers of *P. gorzugi*, especially in comparison to *T. scripta* (Suriyamongkol et al. 2019; Mali et al. 2019). In contrast, Berrendo Creek (Bck) is dominated by *T. scripta* while *P. gorzugi* has only recently been discovered at this location, thus expanding the assumed species range ~80 km north (Mahan et al. 2020; Suriyamongkol et al. 2020). At this location, automatic feeders

dispense fish pellets daily, which can be oportunistically consumed by the freshwater turtles. The presence of automatic feeders could affect the overall isotopic signatures of samples from this site and may not represent the isotopic values related to the natural diet and resource availability in the area. They can also result in an increase in the overall nitrogen isotopic composition of the entire food chain (Filbrun et al. 2013; Mischke et al. 2019). Whereas lotic locations had relatively high water turbidity, site P showed high water clarity and dense occupation of filamentous algae on its bottom. Interestingly, *P. gorzugi* had the lowest mean nitrogen values at this site, alluding to more herbivorous local diet. However, caution should be taken when interpreting the results of isotopic values at site P due to the low sample size (n=7).

Another caveat is the temporal variability regarding the sampling events. Site P was sampled in 2017, two years prior to all other sampling efforts. The isotopic signature can vary not only spatially but also temporally (Haubrock et al. 2021). Although the dominant vegetation at site P did not observably change over time, resouce composition can differ between years, and therefore, affect the isotopic signatures of the turtles. In addition, it is important to emphasize that site P is a different habitat (i.e., lentic) in comparison with the other study sites (i.e., lotic). Niche differences observed in this study could therefore be the result of differences in habitat characteristics, as well as the absence of other turtle species at site P.

Overall, our study contributes to the growing literature on the application of stable isotope data to study freshwater turtle niche overlap. While traditional methods (i.e., fecal content analyses) can offer a high resolution of identified prey taxa, higher digestability of animal matter can provide biased results. Furthermore, stomach and fecal sample analyses may only represent a snapshot in time, while stable isotopes can provide insights to species diet over longer time periods. We strongly suggest that future studies on freshwater turtles, especially the

understudied *P. gorzugi*, on the Pecos River expand upon our initial investigation and focus on a thorough assessment of resource availability and collection, and isotopic analyses of all potential food sources across different habitats. Furthermore, future studies assessing biological aspects of *P. gorzugi* should focus on the nutritional status and body condition of turtles in different habitats in order to assess how dietary habits reflect the overall health of the population. We recommend conservation efforts focus on protecting and restoring riparian vegetation in habitats where *P. gorzugi* is found, along with ensuring that the quality of water and natural complexity of the flora and fauna meets their dietary needs.

SUMMARY

This work contributed to our understanding of the diet of one of the least studied freshwater turtle species in North America, *Pseudemys gorzugi*. The use of stable isotope analyses to study diets of *P. gorzugi* and sympatric turtle species allowed us to quantify isotopic signatures of *P. gorzugi* and determine the extent of niche overlap of *P. gorzugi* and *T. scripta* across different habitats in the Pecos River tributaries. While previous studies demonstrated that introduced *T. scripta* has the potential for trophic competition and niche overlap with native turtles, *T. scripta* niche overlap is seldom studied in its native range. This study also complements research conducted by Letter et al. (2019) who studied the *P. gorzugi* diet using fecal sample analyses. Here, we were able to further analyze levels of omnivory and highlight differences in niche width among four populations of *P. gorzugi*.

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