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Dietary Habits of Rio Grande Cooters (*Pseudemys gorzugi*) from Two Sites within the Black River, Eddy County, New Mexico, USA

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ABSTRACT.—Rio Grande Cooters (*Pseudemys gorzugi*) are one of the least studied species of freshwater turtles in North America. Rio Grande Cooters are listed as a state threatened species in New Mexico and near threatened by the International Union for Conservation of Nature. Given their conservation status, it is important to understand the natural history of this species. We conducted the first in-depth investigation of the dietary habits of *P. gorzugi* at two sites within the Black River in Eddy County, New Mexico, USA, by using fecal sample analyses. We captured and attempted to collect fecal samples from 277 turtles (205 nonrecapture, 72 recapture) and successfully collected 78 samples. Fecal content analysis revealed vegetation including netleaf hackberry (*Celtis reticulata*), cottonwood (*Populus* sp.), willow (*Salix* sp.), monocot grasses, and filamentous algae, along with animals including nine orders of insects, a crustacean, a feather, a fish vertebra, and monofilament fishing line. We found that turtle diets were omnivorous to varying degrees between sexes. Dicot vegetation, filamentous algae, and arthropods were found to be the most important diet components in male (index of relative importance [IRI] = 0.47), female (IRI = 0.53), and juvenile (IRI = 0.63) *P. gorzugi*, respectively. Juveniles were found to have a more specialized diet or lesser dietary breadth compared with diets of adult males and females. Future studies should investigate the nutritional value and digestibility of dietary resources, interspecific competition, and comparison of diet of *P. gorzugi* from different river systems.

Rio Grande Cooters (*Pseudemys gorzugi*) are relatively large freshwater turtles native to the southwestern United States and northern Mexico (Ernst and Lovich, 2009). In the USA, *P. gorzugi* occurs in New Mexico along the Pecos River and its tributaries from the Brantley Reservoir downstream into Texas, where populations can be found along the Rio Grande River and its tributaries from Big Bend National Park downstream to Starr County and, historically, to the city of Brownsville (Degenhardt et al., 1996; Ernst and Lovich, 2009; Dixon, 2013; Brush et al., 2017). In Mexico, populations of *P. gorzugi* occur along the Rio Grande River and tributaries extending to Coahuila, Nuevo León, and Tamaulipas (Ernst and Lovich, 2009). *Pseudemys gorzugi* appears to be locally abundant, but the overall densities are low throughout its range (Bailey et al., 2008).

On a state level, *P. gorzugi* is listed as threatened in New Mexico and a species of greatest conservation need in New Mexico and Texas (TPWD, 2012; NMDGF, 2016). In Mexico, *P. gorzugi* is listed as a threatened species (SEMARNAT, 2010). The species is not listed by the U.S. Fish and Wildlife Service, but the agency is currently reviewing its status for federal protection (USFWS, 2015). Threats to *P. gorzugi* populations include habitat loss and alteration, such as pollution and water diversion for industrial and agricultural use (reviewed in Pierce et al., 2016). The pet trade and human recreational activities may represent additional threats to *P. gorzugi* populations; several turtles have been found with bullet wounds and ingested fish hooks (Pierce et al., 2016; Waldon et al., 2017).

Generally, little is known about *P. gorzugi* natural history (Ernst and Lovich, 2009) including dietary habits. Our understanding of *P. gorzugi* diets is based on analyses of a few stomach and fecal samples as well as anecdotal foraging observations. Painter (1993) described the species as opportunistic and observed them eating the leaves of a fallen cottonwood branch. Legler (1958) found vegetable matter in the stomach of a single specimen, and Degenhardt et al. (1996) analyzed a few fecal samples and identified crayfish parts in

addition to vegetative matter. We hypothesized that *P. gorzugi* diet would reflect these single encounter observations and the diets of other freshwater emydid turtles, with the adult diet being more herbivorous and the juvenile omnivorous. Understanding the food requirements of species is a vital component in developing proper conservation strategies and understanding other natural history traits (i.e., reproduction and growth rate). The objective of this study was to determine the dietary habits of *P. gorzugi* along the Black River, New Mexico, through fecal sample analyses. We sought to identify most frequently consumed prey items and assess diet specialization, evaluate the relative importance of different prey items, and test for any evidence of ontogenetic diet shifts.

MATERIALS AND METHODS

Study Sites.— We surveyed *P. gorzugi* along the Black River in Eddy County, New Mexico, USA, from May to July 2017. The Black River is an ~87-km tributary of the Pecos River fed by the Capitan aquifer and contains bedrock composed of gypsum and limestone (BLM, 1993). The Black River's surface flow contains many deep pools separated by shallower stretches (BLM, 1993). We conducted research at two 1,500-m stretches of the Black River (Mali et al., 2018). These two sites were ~30 km apart, including 7 km of subterranean flow. Riparian vegetation consisted primarily of netleaf hackberry (*Celtis reticulata*), Fremont cottonwood (*Populus fremontii*), sawgrass (*Cladium mariscus jamaicense*), baccharis willow (*Baccharis salicina*), broadleaf cattail (*Typha latifolia*), Olney's three-square bulrush (*Schoenoplectus americanus*), and Canada goldenrod (*Solidago canadensis*).

Data Collection.— Turtles were captured using baited hoop net traps (Mali et al. 2014; Memphis Net & Twine Co., Memphis, Tennessee, USA). Traps were baited with shrimp and sardine-based lures, as they have proven successful in the trapping of *P. gorzugi* on the Black River (Degenhardt et al., 1996; Mirabal et al., 2018). For each capture, we took standard morphometric measurements such as straight line carapace length (CL) by using Haglof tree calipers (Haglof Inc., Madison, Mississippi,

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USA). Sex was determined via secondary sexual characteristics for turtles larger than 118 mm CL (Ernst and Lovich, 2009). Any turtle smaller than 118 mm CL was classified as a juvenile because we could not determine sex with certainty. We marked all captures by notching marginal scutes for adults using a Dremel tool (Cagle, 1939), a subcutaneous passive integrated transponder tag (Buhlmann and Tuberville, 1998) for juveniles and adults, or toe clipping in cohort for juveniles smaller than ~60 mm CL.

Fecal matter was collected by keeping turtles in clear plastic containers with a small amount of water. Turtles were released upon defecation or after a maximum of 12 h. Turtles were housed in shade or within an air-conditioned cabin and misted with water periodically to ensure hydration. Upon defecation, fecal material was collected by pouring the contents of the holding container through a sieve. We used tweezers to carefully place fecal components in sealed Falcon 50-mL conical centrifuge tubes (Fisher Scientific, Waltham, Massachusetts, USA) containing 95% ethanol for preservation and further analyses. Turtles were released at their capture locations. Holding containers were thoroughly rinsed with water before adding another turtle to ensure a clean environment.

Laboratory Analyses.— Fecal matter was rinsed with tap water in a 0.04-mm sieve and placed with water in a plastic tray. We separated components into four broad categories: animal, filamentous algae, vegetation, and unknown (i.e., some components were unidentifiable because of mechanical and digestive degradation). Vegetation and animal parts were then identified to the lowest possible taxonomic level by using a dissecting microscope (SM745, Amscope, Irvine, California, USA). For each fecal sample, we measured the volume of dicot leaves, monocot leaves, seeds, filamentous algae, arthropods, and unknown matter with the water displacement method.

Data Analyses.— We calculated percent occurrence (%F) of each food item recovered. We defined %F as the number of fecal samples in which a particular item occurred divided by the total number of fecal samples (Demuth and Buhlmann, 1997; Seminoff et al., 2002; Platt et al., 2016; Sung et al., 2016). Sums of occurrence values of different food items per sex class did not equal 100% because multiple food items can be found in a single sample. To determine diversity in *P. gorzugi* diets and determine whether any sex classes had dietary specializations (Platt et al., 2016) we used Shannon–Wiener diversity indices (H'), calculated using the formula

$$H' = - \sum pj \log pj$$

where pj is proportion of turtles consuming resource j . The index was standardized on a scale of 0 to 1 by using the measure of evenness, J'

$$J' = \frac{H'}{\log n}$$

where n is the total number of prey groups. A lower evenness value (i.e., closer to 0) indicates a more specialized and less diverse diet and a higher evenness value (i.e., closer to 1) indicates a broader and more diverse diet (Platt et al., 2016). To determine importance of dietary items relative to turtle sex class we calculated the index of relative importance (IRI) similar to methods used by Lindeman (2007)

$$IRI = \frac{V_i F_i}{\sum V_i F_i}$$

where V_i is the mean percent volume (%V) of food item i calculated across all samples and F_i is the %F of food item i . The sum of IRI values per sex class equals 1.

Finally, we assessed ontogenetic diet shift using overlapping group analysis (Platt et al., 2016). This method eliminates potential biases caused by differential digestibility in turtles of different sizes by using many overlapping groups to investigate ontogenetic trends. This permits identification of obscure trends with the assumption that digestion rates are the same within a dietary category (Platt et al., 2016). Similarly to Platt et al. (2016), we placed turtles into overlapping size classes based on CL. The first grouping was based on the 15 smallest turtles, the second grouping was formed by removing the 5 smallest of the first grouping and adding the next 5 larger measurements of sampled turtles. This was repeated until all turtles were included in a grouping. The final grouping had only 13 members because of sample size. We used mean %F of three dietary categories: vegetation, arthropods, and filamentous algae for each size grouping as a dependent variable. These data were transformed using square-root arcsine transformation (Platt et al., 2016). We used mean turtle size of each group as an independent variable and ran simple linear regressions. We inferred statistical significance at $\alpha = 0.05$. We conducted statistical analyses using program R 3.4.3 (R Core Team, 2017).

RESULTS

We captured 277 *P. gorzugi* over the course of 1280 trap days, including 72 recaptures. We obtained fecal samples from 24 males, 18 females, and 36 juveniles. Samples were dominated by dicot and monocot vegetation, arthropods, and filamentous algae. The main dicot taxa identified were *C. reticulata* and *Populus* sp., whereas monocots were not identified to any lower taxonomic levels. Among arthropods, class Insecta was the most dominant. Moreover, we identified nine orders of insects: Odonata, Orthoptera, Isoptera, Hemiptera, Coleoptera, Neuroptera, Hymenoptera, Lepidoptera, and Diptera. The uncommon prey items included three oligochaete worms (one in a male sample and two in different female samples), one bird feather in a male sample, one fish vertebra in a female sample, and one decapod crustacean part in a male sample.

Male and female samples had high %F for vegetation, filamentous algae, and arthropods (Table 1). Juveniles had a noticeably lower %F for filamentous algae (8.3%; Table 1). Females had the broadest dietary item range and least specialization ($J' = 0.805$), whereas juveniles had relatively specialized and less diverse diets ($J' = 0.515$) compared with diets of adults (Table 1). Relative to other diet components, dicot vegetation was most important in males (IRI = 0.47), filamentous algae were most important in females (IRI = 0.53), and arthropods were most important in juveniles (IRI = 0.63; Table 1). We found no significant relationship between turtle size and %F of vegetation or arthropods in fecal samples (Fig. 1A,B; $P > 0.05$). However, there was a significant positive relationship between %F of filamentous algae and turtle size (Fig. 1C; $P < 0.05$).

DISCUSSION

Our research is the first in-depth study on the diet of *P. gorzugi*. The results indicate that both adult and juvenile *P. gorzugi* are omnivorous, although to varying degrees. Primary diet components included netleaf hackberry, cottonwood, and grasses, as well as filamentous algae and insects. Species

TABLE 1. Fecal components of Rio Grande Cooters (*Pseudemys gorzugi*; male, $n = 24$; female, $n = 18$; juvenile, $n = 36$) along the Black River, Eddy County, New Mexico, USA. %F, %V, and IRI are given for each sex class. Percent occurrence for categories denoted with an asterisk (*) were used to calculate H' and J' .

| | Male | | | Female | | | Juvenile | | |
|--------------------|------|------|------|--------|------|------|----------|------|------|
| | %F | %V | IRI | %F | %V | IRI | %F | %V | IRI |
| Vegetation* | 95.8 | | | 66.6 | | | 86.1 | | |
| Dicot | 79.2 | 28.8 | 0.47 | 50 | 20.2 | 0.24 | 22.2 | 6.2 | 0.06 |
| Monocot | 66.6 | 8.4 | 0.12 | 38.8 | 4.6 | 0.04 | 80.5 | 7.4 | 0.25 |
| Seeds* | 75 | 4.2 | 0.07 | 83.3 | 4.1 | 0.08 | 19.4 | 7 | 0.06 |
| Filamentous algae* | 37.5 | 16 | 0.12 | 66.6 | 33.4 | 0.53 | 8.3 | 2.7 | 0 |
| Phylum Arthropoda* | 87.5 | 12.3 | 0.22 | 50 | 8.1 | 0.10 | 66.7 | 22.6 | 0.63 |
| Phylum Annelida* | 4.1 | 0.3 | 0 | 11.1 | 1.9 | 0.01 | | | |
| Phylum Chordata* | 4.1 | 0.2 | 0 | 5.5 | 0.5 | 0 | | | |
| Diversity (H') | 0.42 | | | 0.63 | | | 0.40 | | |
| Evenness (J') | 0.54 | | | 0.81 | | | 0.52 | | |

identification of different grasses in fecal samples was challenging because of their lack of distinguishing flowering parts. Insects were found in >50% of male, female, and juvenile samples. Interestingly, of the nine orders of insects found, most were terrestrial or aerial insects including orders Hymenoptera, Lepidoptera, Hemiptera, Orthoptera, Odonata, Isoptera, Neuroptera, and Coleoptera. Larval Odonata, larval Coleoptera, and larval Diptera were aquatic insect components of the diet. These findings suggest that at least some foraging occurred at, or near,

the water's surface. Indeed, we observed a juvenile *P. gorzugi* consuming a winged insect, from what appeared to be order Isoptera, at the water's surface during our summer surveys.

Our analyses found that juvenile *P. gorzugi* are more specialized and have a less diverse diet relative to the adults as they consumed primarily monocot vegetation and arthropods. In the only other study to record juvenile *Pseudemys* diet, Lindeman (2007) found that juvenile *P. texana* consumed primarily algae and arthropods. In comparison, we found very low %F and negligible IRI of algae in juvenile *P. gorzugi* samples. However, arthropods had a particularly high IRI in juvenile diet. This supports previous hypotheses that a carnivorous diet in juvenile turtles is essential because it may induce rapid growth and increase survivability (Bouchard and Bjorndal, 2006). We describe adult *P. gorzugi* as opportunistic. We found high IRI for dicot vegetation in males and filamentous algae in females. However, there was a high frequency of occurrence of both vegetation and arthropods among all three sex classes (i.e., males, females, and juveniles; Table 1). One clear trend in our data is an increase of filamentous algae as turtles grew larger. Other emydid turtles such as Red-Eared Sliders (*Trachemys scripta*) and Ouachita Map Turtles (*Graptemys ouachitensis*) are known to undergo an ontogenetic diet shift that is thought to allow for increased growth in juveniles with a protein-rich, animal-based diet and as they grow to adult size, they shift to a more opportunistic and vegetation-based diet (Clark and Gibbons, 1969; Moll, 1976; Bouchard and Bjorndal, 2006).

When using %V as a direct metric of prey consumption in fecal samples or indirectly through calculation of IRI like in our study, one must understand that differential digestibility of prey categories is a possibility. The %V of stomach contents is more reliable than %V of fecal samples because of stomach contents not undergoing the full digestive process that could reduce volume for more digestible dietary components. Diets of *Pseudemys* turtles have been studied in the past using both fecal sample analyses and Legler stomach flushing techniques, with a combination of both techniques providing the most comprehensive diet analyses (Bjorndal and Bolten, 1993; Lagueux et al., 1995; Fields et al., 2003; Lindeman, 2007; Caputo and Vogt, 2008). Because of the threatened status of *P. gorzugi* in New Mexico, we passively collected fecal samples to ensure quick data collection and an immediate return of the turtles to the capture locations. Our study assessed diet in the summer and may lack the full scope of *P. gorzugi* diet components throughout the turtles' active period. We hope to use stable isotope analyses of different *P. gorzugi* tissues (i.e., claw and

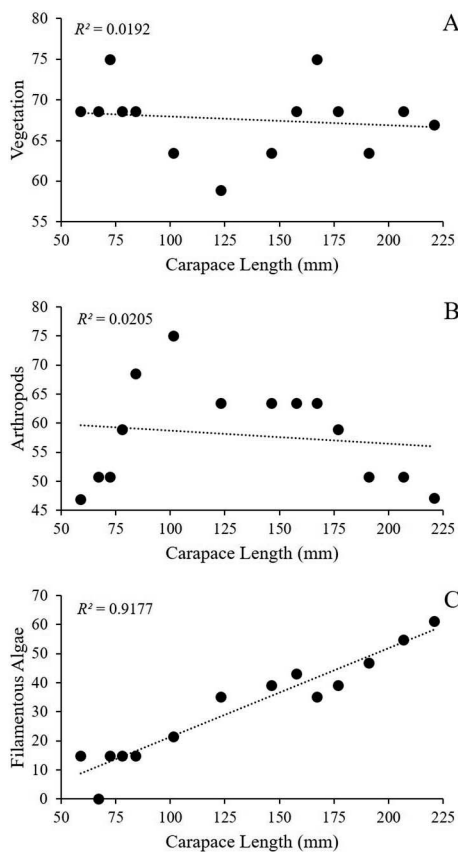


FIG. 1. Test of ontogenetic diet shift of Rio Grande Cooters (*Pseudemys gorzugi*) on the Black River, New Mexico, USA. (A) Carapace length vs. %F of vegetation in diet. (B) Carapace length vs. %F of arthropods in diet. (C) Carapace length vs. %F of filamentous algae in diet. The occurrence of vegetation and arthropods in the diet was not affected by turtle size; however, the occurrence of filamentous algae in the diet was greater with increased size of turtle.

shell) to further investigate assimilation of plant vs. animal matter, especially relative to age and sex classes.

We found monofilament fishing lines in two fecal samples. Possibilities for monofilament line ingestion include an incidental consumption of lost or broken line stuck in vegetation or active foraging for baited hooks. Moreover, we captured one *P. gorzugi* via snorkeling that contained a fishing hook lodged in its throat (Waldon et al., 2017). The presence of monofilament line in fecal samples and evidence of fishing hook ingestion indicated that turtles may be negatively affected by recreational fishing (Steen et al., 2014; Steen and Robinson, 2017). It also suggests more opportunistic feeding habits of *P. gorzugi* than previously thought.

Notably, our analysis does not necessarily represent resource selection. Because of the sensitivity of the Black River system and the overlap of Rio Grande cooters' range with federally listed Texas Hornshell Mussels (*Popenaias popeii*), we did not conduct an in-depth assessment of different prey items' availability in the habitat. Also not assessed was microhabitat use by *P. gorzugi* of different sizes (i.e., juveniles vs. adults), which could have consequences for their diets. Therefore, resource availability and microhabitat use should be of interest for future studies. A better understanding of the habitat requirements and natural history traits of *P. gorzugi* is critical for effective planning and enacting future management policies on the Black River ecosystem. Future research should focus on expanding the surveys to other localities in New Mexico such as Pecos and Delaware rivers, evaluating habitat suitability, comparing diets and body conditions of *P. gorzugi* across localities, and determining diet during spring and fall active periods. Future research should also investigate interspecific food resource competition with other turtles in the Black River including Red-Eared Sliders (*T. scripta*), Western Painted Turtles (*Chrysemys picta*), Common Snapping Turtles (*Chelydra serpentina*), Yellow Mud Turtles (*Kinosternon flavescens*), and Texas Spiny Softshells (*Apalone spinifera*).

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