Final Report

DEVELOPMENT AND APPLICATION OF A NOVEL SUITE OF FIELD SURVEY METHODS TO INFORM CONSERVATION OF THE RIO GRANDE COOTER, *PSEUDEMYS GORZUGI*

Prepared for the Texas Comptroller of Public Accounts
#18-6725CS
(Principal Investigators: Abdullah Rahman, Richard Kline, Md Rahman)

by

DREW R. DAVIS, AMY P. BOGOLIN, MD SAYDUR RAHMAN, RICHARD J. KLINE, AND ABDULLAH F. RAHMAN

School of Earth, Environmental, and Marine Sciences,
University of Texas Rio Grande Valley,
South Padre Island, Texas 78597

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

We thank the Texas Parks and Wildlife Department (TPWD), National Park Service (NPS), The Nature Conservancy (TNC), and the International Boundary and Water Commission (IBWC) for issuing permits to allow for sampling on their property, as well as the Federal Aviation Administration for issuing a waiver to conduct drone flights in a no-fly zone. We also wish to thank the following private landowners and public organizations who granted us permission to sample and access sites on their property: J. Chandler and R. Jasso (Chandler Ranch), J. Lugo, Fort Clark Springs Association, City of Del Rio, Eagle Pass Golf Course, San Felipe Springs Golf Course, The National Butterfly Center. Further, we thank C. Guadiana for allowing us to collect water samples for preliminary eDNA analysis from the Gladys Porter Zoo and D. Lucio for providing information on zoo records. All research was conducted under a TPWD Scientific Research Permit (SPR-1018-294), TPWD State Park Scientific Study Permit (2019_R2_RGV_02), TPWD Aerial Wildlife and Exotic Animal Management Permit (M-1603), NPS Scientific Research and Collecting Permit (AMIS-2018-SCI-0007), TNC (Texas Chapter) Scientific Investigation and Collection Permit, IBWC U.S. Section Permit (USIBWC-19-2-0011), Certificate of Waiver or Authorization (2019-P107-CSA-10089), and a University of Texas Rio Grande Valley IACUC protocol (AUP-18-28). Our special thanks go to UTRGV graduate students P. Robinson and K. Ruppert for their immeasurable help with both field and lab work and I. Hinson for her assistance with early drone test flights.

EXECUTIVE SUMMARY AND RECOMMENDATIONS

There is a paucity of information on the Rio Grande Cooter (*Pseudemys gorzugi*) given its limited range, recent species designation, and elusive behavior. This overall lack of information on *P. gorzugi*, combined with numerous threats due to climate change and anthropogenic pressures, highlights an urgent need for data and research on this species. In this project, we developed a novel suite of methods for detecting the presence of *P. gorzugi* in southwestern Texas using high-resolution color photography from a drone-mounted camera and developing and validating an assay to detect *P. gorzugi* environmental DNA (eDNA) through the collection of water samples. Additionally, at each data collection site we recorded habitat variables, water quality parameters, and collected images from a drone-mounted multispectral spectrometer to better understand habitats used by *P. gorzugi* throughout this region. Listed below are the two main objectives of this project, which include a summary and recommendations.

Documenting the presence of *Pseudemys gorzugi* and identifying habitat associations along the Rio Grande and Pecos River.—We sampled 61 sites for turtles in southwestern Texas ranging from Pecos County to Cameron County. Sites were chosen based on the historical distribution of *P. gorzugi*, current recognized populations from literature, and from early scouting events. At these sites we collected data on turtle species and abundance using four different survey techniques: visual, trapping, drone, and environmental DNA (eDNA) surveys. Additionally, we collected and recorded water quality data, habitat characteristics, and multispectral images to better characterize habitats associated with *P. gorzugi* presence.

During 84 visual surveys, we had 91 observations of *P. gorzugi* at 15 (of 44) sites and were able to identify an average of 51% of the turtles observed. Dense shoreline vegetation, inaccessible habitat, and obscured turtles were among the major challenges with visual surveys that reduced our ability to confidently identify turtles to species and likely reduced overall abundance counts. Our trapping efforts resulted in a total of 86 adult *P. gorzugi* captured from 18 (of 39) sites. Trapping efforts were hindered by variable water depths due to releases from upstream dams and limited water access but provided us the opportunity to assess individual health and size as well as allowed us to collect tissue samples. In total, 73 drone surveys produced 84,441 photographs. From these photographs we detected 307 *P. gorzugi* from 18 (of 42) sites. Compared to visual surveys, drone surveys resulted in a higher percentage of turtles (82%) that were able to be identified to species due to a unique aerial view that facilitated the observation of diagnostic characteristics. We detected *P. gorzugi* eDNA at 22 of the 42 sites sampled, including the northernmost and southernmost detection of *P. gorzugi* from these four survey methods. The results from these survey methods, combined with opportunistic detections during the survey period, resulted in *P. gorzugi* being detected at 43 (of 61) sites (Figure 1).

Water quality data showed that sites where *P. gorzugi* was detected had a significantly lower minimum pH (mean = 8.07) compared to sites where we did not detect *P. gorzugi* (mean pH = 8.22). We also found significantly lower minimum conductivity (mean = 1961.9 µS/cm) at sites where *P. gorzugi* was detected compared to sites where they were not (mean = 3906.8 µS/cm). Additional analyses of water quality are on-going, and we are attempting to understand how factors may influence the sites used by *P. gorzugi*. We are continuing to explore whether other habitat parameters, such as vegetation associations and abundance, also affect the presence of *P. gorzugi* in an area.

Evaluating the effectiveness and efficiency of drone-based photography and eDNA analyses as alternate survey methods for *Pseudemys gorzugi*.—We compared data collected from drone surveys to the traditional survey methods of trapping and visual surveys to determine if a particular method outperformed the others in its ability to detect turtles. eDNA surveys were not able to be included in this comparison as analyses only result in presence/absence data, not abundance data. We did not detect a significant difference in the number of total turtles detected among these three survey methods or the total number *P. gorzugi* detected. Trapping resulted in 100% identification and species identification from drone surveys (82%) was significantly greater than that for visual surveys (51%). This significant difference highlights the potential use of drones for turtle surveys and may also be superior than visual surveys for other wildlife (Table 1).

Although the drone surveys faced numerous logistical challenges regarding permitting and licenses, developing optimal camera and flight parameters, and issues and limitations of the equipment, once the protocol was established, drone surveys resulted in high-quality images of turtles that allowed us to count and identify individuals, with seemingly little to no disturbance on turtles themselves or other wildlife (Table 1). Drones were also able to capture additional aspects of *P. gorzugi* behavior, which may be informative to understand foraging and reproductive behaviors of this species.

Results from eDNA assays at most of the sites included in this validation matched our expectations: at sites where *P. gorzugi* was observed, we had positive eDNA detection and at sites where *P. gorzugi* was never observed we had no eDNA detection. Occasionally, we did not detect *P. gorzugi* eDNA at sites where they were consistently detected. Usually, these sites where eDNA assays results did not match other survey results were spring-fed or associated with urban development. We continue to analyze eDNA samples from repeat visits to these sites to determine if these patterns are consistent. eDNA surveys resulted in the northernmost and southernmost detection of *P. gorzugi* across the geographic extent of our surveys, an important consideration that helps to inform surveys for additional populations of *P. gorzugi* in these regions.

Despite unique challenges and advantages associated with each survey methodology (Table 1), the development of drone and eDNA surveys appear to be useful tools in the detection of *P. gorzugi*. These survey techniques can be used to monitor the status of known populations as well used to help identify previously unreported populations of *P. gorzugi*. If assessing the health of individuals, particularly if there is a need to collect tissue samples, then trapping must remain a component of *P. gorzugi* surveys (Table 1). Trapping can be labor intensive and the use of drone and/or eDNA surveys can help to refine locations where trapping may be more successful. From our results, visual surveys appear to be outperformed by drone surveys, and limitations to visual surveys make them less efficient than drone surveys. Future survey efforts should attempt to locate additional populations of *P. gorzugi* within the upper Pecos River, from the confluence of Independence Creek, Terrell County upriver to the border with New Mexico, as well as the lower reaches of the Rio Grande within Hidalgo County, and by incorporating drone and eDNA methods into survey protocols, detections of additional populations may be possible.

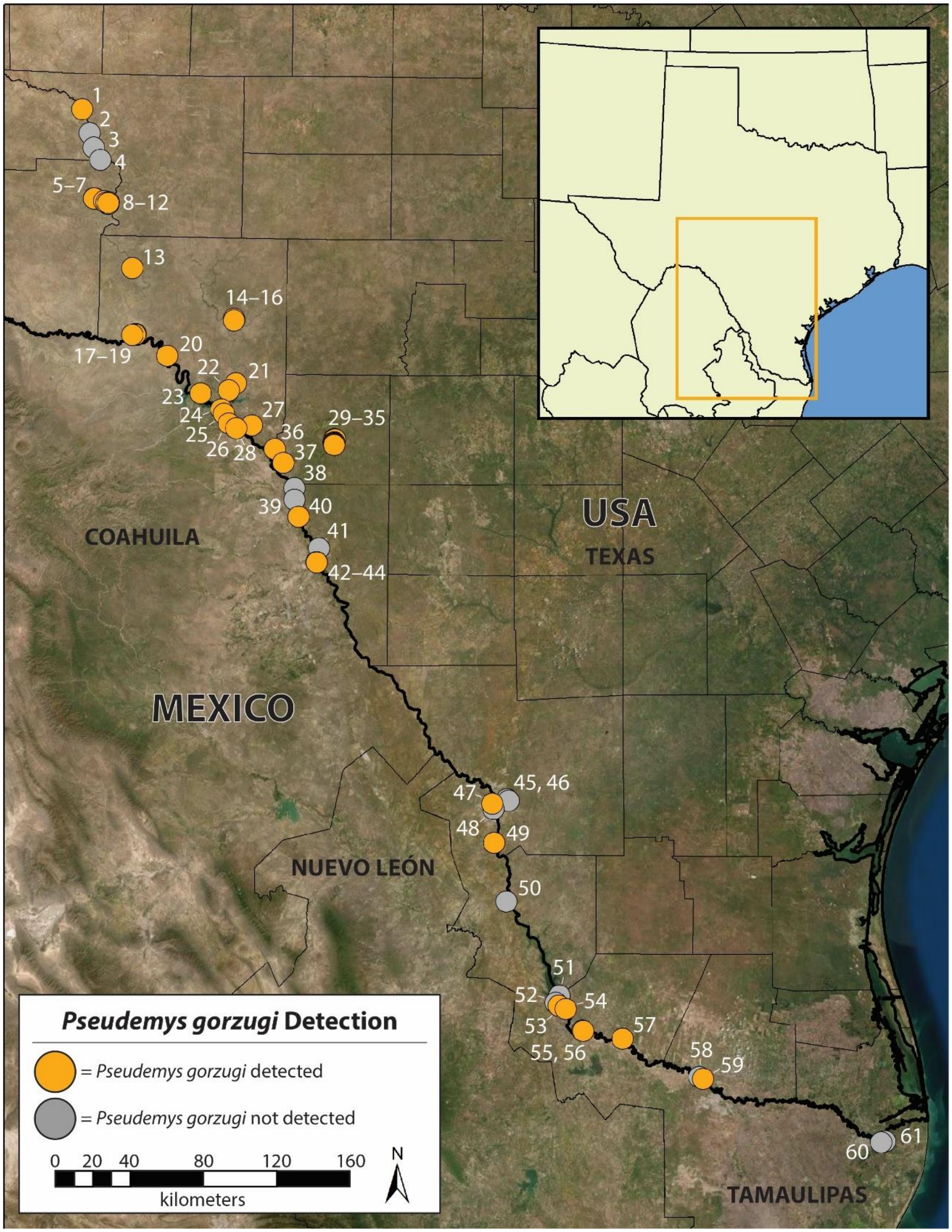

Figure 1. Map of sites where *Pseudemys gorzugi* was detected in southwestern Texas, USA. Detections were from visual, trapping, drone, and eDNA surveys as well as opportunistic captures and observations. Sites where *P. gorzugi* was detected are indicated in orange. Sites where *P. gorzugi* was not detected are indicated in gray. Site numbers correspond to those used in Table 2.1 and throughout the report.

Table 1. Summarized comparison of survey methods used during this study. Survey type, invasiveness, effort, identification (ID) percentage, cost, required conditions, challenges, and advantages are mentioned.

Survey Method	Invasiveness	Effort	ID Percentage	Cost	Required Conditions	Challenges	Advantages
VISUAL	medium	low	medium	low	shore access; sunny conditions	observer bias; low ID %	quick assessment
TRAPPING	high	high	100%	medium	water access; penetrable substrate	trap theft; sampling bias	provides size and health data
DRONE	low	low/medium	high	high initial, then low/medium	low wind, no rain; launch area	technological issues; short flight time	aerial viewpoint; improved identification
eDNA	low	low/medium	high	high initial, then low/medium	water access	not quantifiable; delayed results	detection without observing turtles

CHAPTER I

INTRODUCTION

Rio Grande Cooter

The Rio Grande Cooter (*Pseudemys gorzugi*), is a large, aquatic, freshwater turtle species found in the southwestern United States, including southwestern Texas and southeastern New Mexico, as well as northeastern Mexico, including the states of Tamaulipas, Nuevo León, and Coahuila (Figure 1.1; Iverson 1992a; Degenhardt et al. 1996). Within Texas, *P. gorzugi* is restricted to the Rio Grande, Pecos, and Devils rivers, as well as their associated drainages. The population of *P. gorzugi* in New Mexico is disjunct from populations in Texas and Mexico and this separation is attributed to anthropogenic degradation of the Pecos River from water extraction, modification of flow rates, and reductions in water quality (Ward 1984). However, despite this separation of ca. 160 km, both populations remain genetically similar (Bailey et al. 2008). *Pseudemys gorzugi* only recently received designation as a full species due to it being allopatric from other *Pseudemys*, the absence of any evidence of gene flow, and morphological differences (Ernst 1990; Collins 1991; Ernst et al. 1994; Degenhardt et al. 1996). *Pseudemys gorzugi* has an elongate oval carapace (ca. 23.5 cm carapace length) covered in black, yellow-orange, and green concentric circles (Figure 1.2; Ernst 1990; Degenhardt et al. 1996; Hibbitts and Hibbitts 2016). Older, mature males often become melanistic with their carapace developing a dark, reticulated pattern, obscuring the concentric circle pattern (Figure 1.2C; Bailey et al. 2005). Sexual dimorphism is pronounced in *P. gorzugi*, with females reaching larger adult sizes and males having a broader tail and longer foreclaws (Figure 1.2; Degenhardt et al. 1996; Hibbitts and Hibbitts 2016). Little is known about the diet of *P. gorzugi*. It was long assumed that juveniles were omnivorous and became increasingly herbivorous as adults, but recent research suggests that adults are opportunistic, consuming algae, plant, and animal material (Lindeman 2007; Letter et al. 2019). They are active year-round and can be found in both clear and turbid habitats, as well as lentic and lotic water bodies (Degenhardt et al. 1996; Pierce et al. 2016), suggesting this species may be a habitat generalist.

There is a paucity of information on *P. gorzugi*, especially compared to other turtles given its limited range, recent species designation, and elusive behavior. In an analysis of available literature on turtles, Lovich and Ennen (2013) found that *P. gorzugi* ranked 57th out of 58 turtle species. These results may partially be skewed because Lovich and Ennen (2013) failed to include literature from Mexico, where *P. gorzugi* also occur, but these results still highlight the notable absence of information concerning this species.

Considered locally abundant in a few locations, *P. gorzugi* is recognized as having an overall low population density, though it remains uncertain if this is widespread characteristic of the species (Bailey et al. 2008; Dixon 2013). Recent studies by Bailey et al. (2008) and Forstner et al. (2004) have suggested that populations are patchy and restricted to few stretches of waterways in the United States and noted the lack of juveniles in Texas. In recent years, *P. gorzugi* populations have been subjected to numerous threats, such as habitat degradation and collection for the pet trade (Mali et al. 2017). Modifications to river flow rates, flood control practices including construction of dams and channels, and water pollution from untreated sewage inflows, runoff from agriculture and mining, and atmospheric deposits, all place *P. gorzugi* populations at risk, and have led to the designation of the Rio Grande as one of the top ten most endangered rivers in America (American Rivers 2003; Bailey et al. 2014). Fishing bycatch and wanton killing of *P. gorzugi* by commercial and recreational river users have further threatened populations (MacLaren et al. 2017). These concerns have led to *P. gorzugi* being designated as Threatened in New Mexico, Near Threatened by the IUCN, and a Species of Greatest Conservation Need in Texas (Pierce et al. 2016). Currently, its status is under review by the United States Fish and Wildlife Service regarding potential federal listing (USFWS 2015).

The numerous threats facing *P. gorzugi*, in combination with an overall lack of knowledge of this species, highlights the need for data and a call to research. It is essential that a thorough survey effort be undertaken throughout the Rio Grande and its tributaries to determine the population health and current distribution of *P. gorzugi*. Additionally, it is imperative that the ecological characteristics of *P. gorzugi* habitat are identified to assist in the discovery of new populations. The combination of this data can then be used to inform conservation efforts to ensure the continued presence of *P. gorzugi* on the landscape.

Traditional Sampling Methods

Aquatic freshwater turtle species are often surveyed using traditional sampling methodologies, including baited hoop-net or basking traps, seining, and visual, snorkeling, and SCUBA surveys. The use of baited hoop-net traps appears to be the most prominent survey method for turtles (Beauvais and Buskirk 1999; Buckland et al. 2000; Lanica et al. 2005). *Pseudemys gorzugi* populations have been surveyed using many of these methodologies with mixed success (Christman and Kamees 2007; Bailey et al. 2008; Mali et al. 2014, 2018). Traditional survey methodologies are often time consuming, labor intensive, and expensive, making it difficult to adequately assess turtle populations (Beauvais and Buskirk 1999; Gu and Swihart 2004; Lancia et al. 2005). Furthermore, biases exist amongst trapping methodologies with differential escape probabilities, varying bait preferences, influences from trapping intensity and duration, and individual responses to traps, all potentially affecting results (Frazer et al. 1990; Thomas et al. 2008; Mali et al. 2012). Less invasive sampling methodologies such as visual surveys are often less effective than trapping, especially for elusive species such as *P. gorzugi,* and limited to areas where water access is available (Mali et al. 2017). Lack of dietary knowledge, or perhaps trap avoidance behaviors, have further hindered trapping efforts of *P. gorzugi* with little trapping success occurring thus far in Texas (Degenhardt et al. 1996).

Drone Surveys

Numerous limitations exist to traditional sampling methodologies, and as a result, new technologies are being employed by wildlife biologists to survey for a variety of different species. With increasing prevalence and affordability of small, unmanned aerial vehicles (drones), wildlife biologists have begun incorporating their use into surveys (Jones et al. 2006; Hodgson et al. 2013). To date, surveys for numerous different species have been successful, including orangutans, elephants, rhinoceros, whales, and sea turtles (Koski et al. 2009; Hodgson et al. 2013; Vermeulen et al. 2013; Mulero-Pázmány et al. 2014). Recently, freshwater aquatic turtle species have been added to the list with a single published drone survey of freshwater turtles in Bulgaria (Biserkov and Lukanov 2017). Drones can conduct programmed flights over a survey area and camera attachments take photographs or obtain video to be analyzed to measure abundance, threats, tracks, nesting sites, as well as many other types of data (Van Germart et al. 2015). Drone surveys are relatively inexpensive when compared to traditional sampling methodologies, are less labor intensive, and drones can often survey areas where access is limited (Van Gemert et al. 2015). Drones also have the benefit of being less invasive and documented wildlife response to drone flights has been minimal (Bevan et al. 2018). With technology and efficiency being continually improved, drones are expected to become widely incorporated into wildlife surveys (Rees et al. 2018).

Multispectral cameras can also be attached to drones and record light reflectance from various bands of the electromagnetic spectrum. Images are created from these reflectance values, and bands such as near-infrared go beyond the visible light portion of the spectrum and allow valuable data to be obtained that is not visible to human eyes. With this information, the presence of various types of vegetation, as well as their abundance, biomass, distribution, and structural attributes may be mapped (Goncalves et al. 2015). Farmers have recently taken advantage of this technology surveying crop fields, analyzing crop distribution, reactions to pesticides, establishing vegetation indices and much more, and forestry management and geosciences have followed with their own applications (Grenzdörffer et al. 2008; Westoby et al. 2012; Candiago et al. 2014; Ouédraogo et al. 2014). Imagery from multispectral cameras can facilitate habitat and vegetation assessments, that will provide information on habitat characteristics preferred by *P. gorzugi*.

Environmental DNA

Environmental DNA (eDNA) is another novel survey methodology that has shown promise in the detection of wildlife, particularly aquatic species (Goldberg et al. 2015). Organisms continually shed DNA into their surrounding environments from skin cells, urine, and feces, and these minute amounts of DNA can be collected and analyzed (Hofreiter et al. 2003; Ficetola et al. 2008). For aquatic organisms, water can be collected and filtered through a small pore filter to trap the eDNA (Goldberg et al. 2011; Jerde et al. 2011; Takahara et al. 2012; Thomsen et al. 2012; Turner et al. 2014; Renshaw et al. 2015; Takara et al. 2013). Environmental DNA can then be extracted from the filter, amplified through polymerase chain reaction, purified through a gel, and sequenced to confirm that the DNA is from the species of interest (Goldberg et al. 2016). Primers are developed to ensure species-specificity, which is often confirmed through Sanger sequencing the amplified DNA product. Frequently, primers are selected from the cytochrome oxidase I mitochondrial gene, including previous work with turtles (Reid et al. 2011). The use of eDNA surveys to detect species can be less time consuming, less labor intensive, and less invasive than traditional methods, and has the unique characteristic of being able to confirm a species presence despite lack of visual or auditory detection (Ficetola et al. 2008; Hoffman et al. 2016); however, eDNA assays are currently unable to provide reliable abundance estimates for species.

Water Quality

Turtles are often considered biological indicators as they are often susceptible to contaminants and choose habitats in minimally impacted areas (Gibbons 1990). *Pseudemys gorzugi* is presumed to select habitats with higher water quality and thus presence or absence at a location may be due to water quality parameters (Ward 1984). The 160 km gap between *P. gorzugi* populations has been attributed to water quality degradation from oil and natural gas well runoff (Ward 1984). The Rio Grande, Pecos River, and their tributaries are subject to contaminants from sewage inflow as well as agriculture and mining activities (Levings et al. 1998). With the Rio Grande already considered an endangered river system with significantly degraded water quality (USDOI 1998), collecting basic water quality information such as dissolved oxygen, pH, conductivity, oxidation-reduction potential, and nutrient concentrations, will better inform us of the health of the river and how this may affect *P. gorzugi*. Little research has studied the effects of specific water quality parameters on *P. gorzugi*, but individuals have been detected at sites with conductivity values ranging from 2264–2593 µS/cm along the Delaware River in Texas (Bonner and Littrell 2016).

Project Scope

In this project, we developed a novel suite of methods for detecting the presence of *P. gorzugi* in southwestern Texas. The novel methodologies included in this study involved using high-resolution color photography from a drone-mounted camera to locate and identify *P. gorzugi* in difficult-to-reach areas and developing and validating an assay to detect *P. gorzugi* environmental DNA through the collection of water samples. These two novel survey methods were compared against two more-traditional methods of surveying for turtles: visual surveys and trapping. We sampled numerous habitats in the Rio Grande, Pecos River, and their tributaries in Texas from near Iraan, Pecos County down to near Brownsville, Cameron County, including sites where *P. gorzugi* were historically known as well newly reported localities. Additionally, at each site we recorded habitat variables, water quality parameters, and collected images from a drone-mounted multispectral sensor to better understand habitats used by *P. gorzugi* throughout this region. Our project involved two specific objectives, described in further detail below:

1. Documenting the presence of *P. gorzugi* and identifying habitat associations along the Rio Grande and Pecos River

2. Evaluating the effectiveness and efficiency of drone-based photography and eDNA analyses as alternate survey methods for *P. gorzugi*

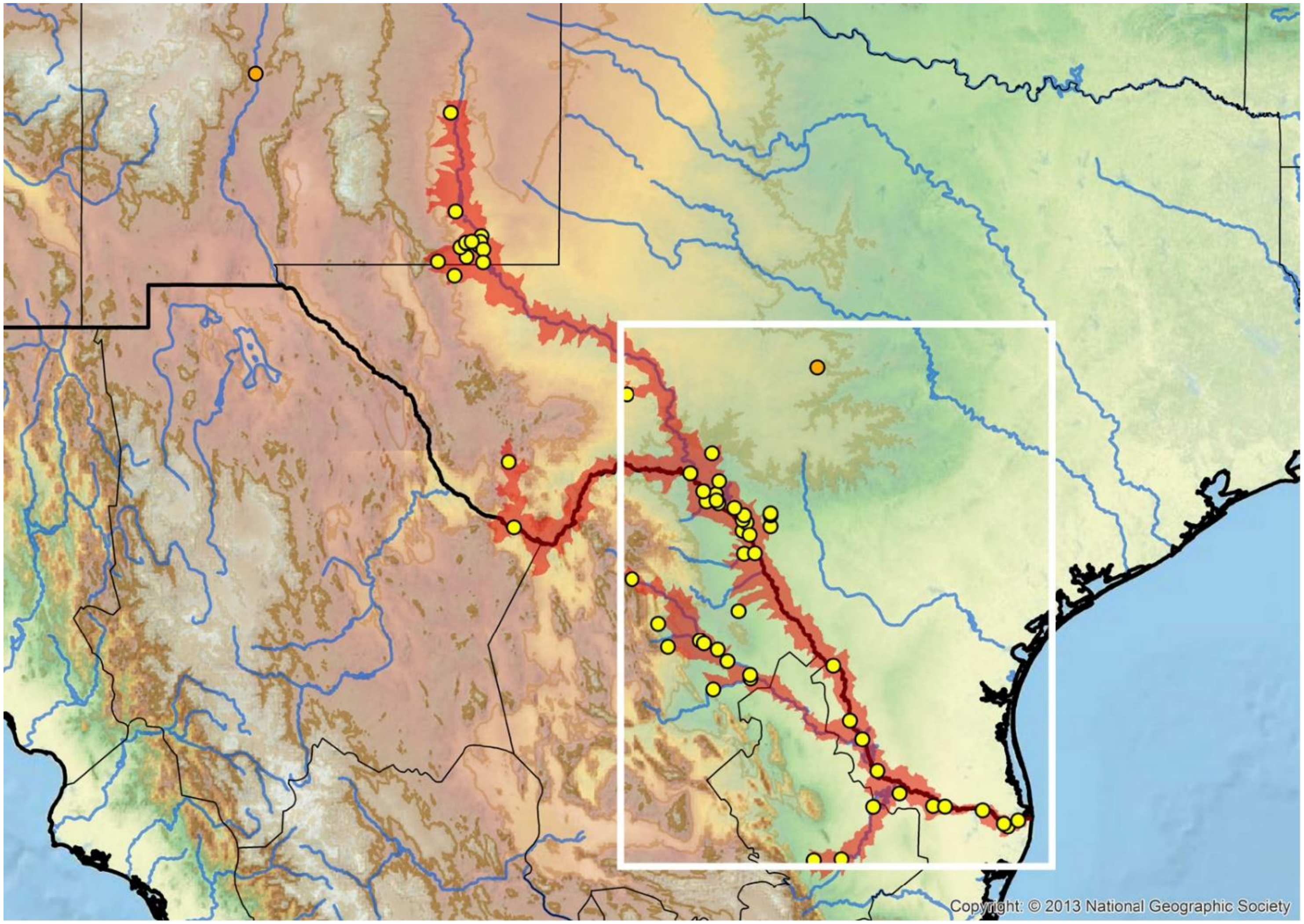

Figure 1.1. Historic distribution map of *Pseudemys gorzugi* in southwestern USA and northeastern Mexico. Map adapted from Pierce et al. (2016). Yellow dots indicate museum and literature occurrence records of native populations and orange dots indicate introduced or misidentified specimens. Red shading is the projected historic distribution of *P. gorzugi*. The white polygon represents the approximate extent of our sampling within Texas, USA that is included in this report (see Figure 2.1).

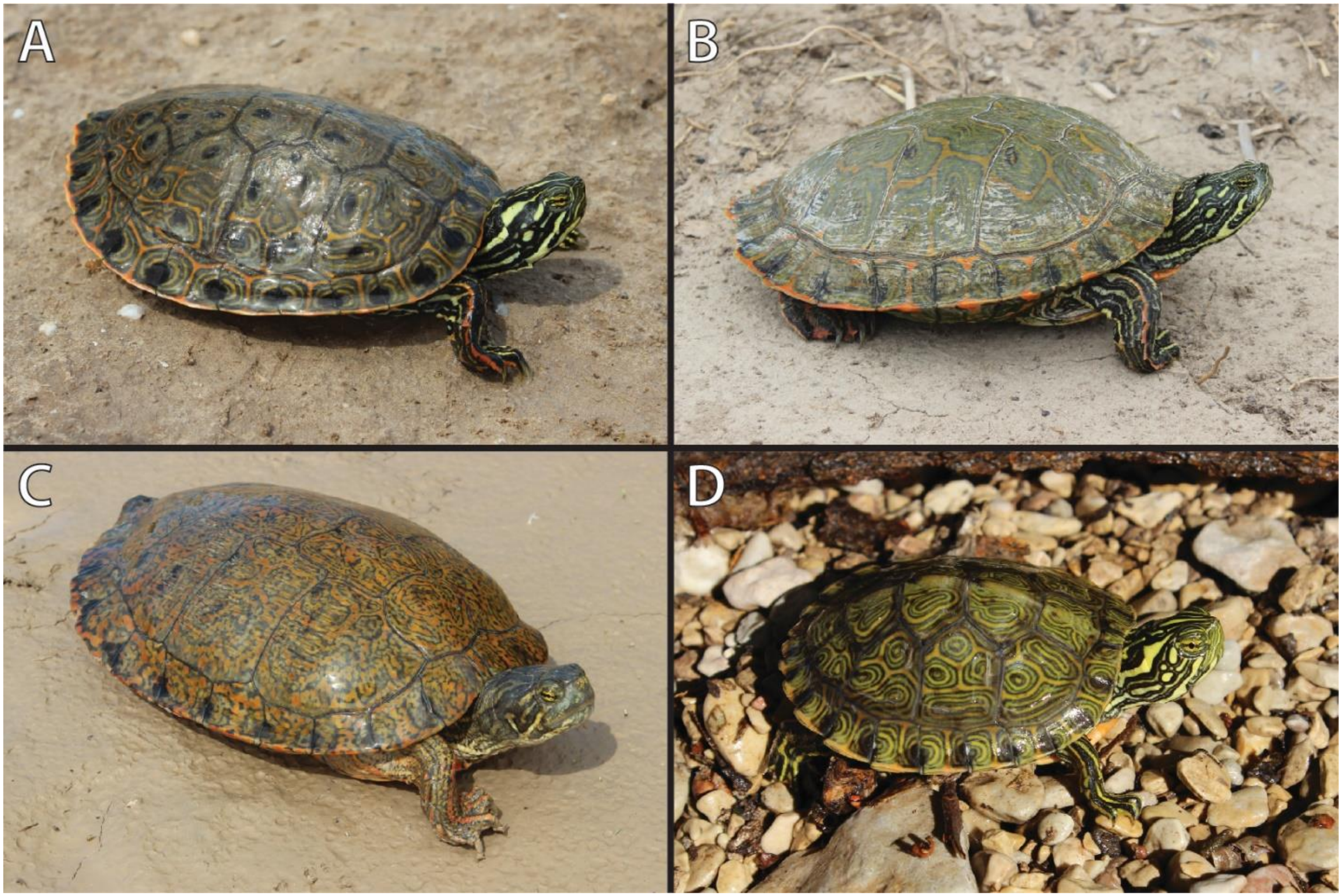

Figure 1.2. Representative photos of *Pseudemys gorzugi*: A) adult male (DRD 5673); B) adult female (DRD 6101); C) adult male showing reticulate melanism (DRD 6080); D) juvenile (iNaturalist 35863373). All individuals were captured during the survey period. All photos by DRD.

CHAPTER II

TASK 1. Conduct *P. gorzugi* surveys at representative sites (including historic sites) in the Rio Grande and Pecos River in Texas

A. Collect and record water quality data, habitat characteristics, species presence, and species physical characteristics
B. Analyze survey results to estimate the species' presence, persistence at historic sites, health and size of populations, habitat status and habitat associations
C. Record GPS coordinates for all survey data collected

1. OVERVIEW

Chapter II discusses the sites sampled over the entire survey period, water quality and habitat data collected during each sampling visit, and the methods, results, and discussion from traditional survey methodologies. The traditional survey methodologies included in Chapter II are visual surveys and trapping. Subtasks A–C are addressed throughout this chapter in relevant sections. Distribution maps, figures, and tables containing information related to Chapter II are included at the end of the chapter, and appendices are included at the end of the report.

2. STUDY LOCATION

Historic records of *Pseudemys gorzugi* are located throughout southwestern Texas, southeastern New Mexico, and northeastern Mexico (Figure 1.1). From November 2018–October 2019 we surveyed 61 unique localities in southwestern Texas for the presence of *P. gorzugi* (Figure 2.1; Table 2.1). These sites were primarily located in the Rio Grande and Pecos River watersheds, including both mainstem rivers and their tributaries (Figure 2.2). Some of these locations were opportunistic sites that were added upon discovery of *P. gorzugi* in the area. Due to logistical constraints, not every survey method (e.g., visual survey, trapping) was conducted at each site. We attempted to sample each site twice throughout the study period, and the final number of visits for each site ranged from 1–4.

Sites ranged from Pecos County, Texas, our northernmost sampled county, south down to Cameron County, Texas (Figure 2.1). Locations were chosen based on the historical distribution of *P. gorzugi* (natural history collection records and literature; Figure 1.1) as well as from early scouting events. Unfortunately, a large gap exists in our sampling along the Rio Grande from Laredo, Webb County to Eagle Pass, Maverick County due to limited access to the river in this region. Across this large geographic range of sampled sites in Texas there is substantial habitat variation, with noticeable differences in waterbody size, depth, flow, vegetation, hydrology, and level of anthropogenic disturbance (Figure 2.2). Additionally, differences in the surrounding habitat were observed, including both urban and rural sites, riverbank height, topography, and ecoregion.

3. WATER QUALITY AND HABITAT CHARACTERISTICS

3.1 Materials and Methods

Water Quality and Habitat Characteristics.—Water quality information was gathered during each sampling event. A Hach HQ40D Portable Multi Meter water quality sonde was placed just off the shore in the water, ca. 1 m from the shoreline. We measured water temperature (°C), pH, dissolved oxygen (mg/L), conductivity (µS/cm), and oxidation-reduction potential (mV). Water quality strips were also used to measure nitrate (ppm), nitrite (ppm), ammonia (ppm), alkalinity (ppm), and hardness (ppm). During each visit we also collected data on turbidity, depth, flow, connectivity, as well as the presence of dredging, surface films, algal mats, trees, and woody debris. We also estimated the percentage cover of open substrate, submerged vegetation, emergent vegetation, and floating vegetation.

Multispectral Imaging.—The MAIA, an eight spectral band multispectrometer, was used for multispectral imaging, and attached to a DJI Matrice 600 Pro unmanned aerial vehicle. Additional information on drone flights is included below in Chapter III. Bands ranged from blue to near-infrared regions of the light spectrum (390–950 nm) mimicking the Worldview-2 satellite sensors (Global Scan Technologies LLC 2019). Camera triggering was set at one image per second. The multispectral images were stitched together using Metashape photogrammetry software. Imagery was used in habitat assessment, mapping vegetation species, presence, abundance, biomass, distribution, and structure, which may be useful when determining potential *P. gorzugi* habitat.

3.2 Results

We measured water quality parameters and recorded site characteristics at 52 sites during each sampling event (Appendix 1, 2). We observed a significant difference in minimum pH measured between sites with and without *Pseudemys gorzugi* (Mann Whitney U-test: $H = 5.4$, $p = 0.02$). Sites where *P. gorzugi* was detected had a significantly lower minimum pH (mean = 8.07) compared to sites where we did not detect *P. gorzugi* (mean = 8.22; Figure 2.3). We also observed a significant difference in minimum conductivity measured between sites with and without *P. gorzugi* ($H = 5.8$, $p = 0.02$). Sites where *P. gorzugi* was detected had a significantly lower minimum conductivity (mean = 1961.9 µS/cm) compared to sites where we did not detect *P. gorzugi* (mean = 3906.8 µS/cm; Figure 2.4). There was not a significant difference between sites with and without *P. gorzugi* for maximum pH and maximum conductivity measures (Figure 2.3, 2.4).

Given the large geographic extent of sampling sites, we observed numerous habitat differences. A total of 18 of our sites were associated with springs, 28 sites were located on the main stem of the Rio Grande or Pecos River, and 8 sites were located within large reservoirs or lentic systems. We are continuing to investigate whether specific habitat parameters are associated with *P. gorzugi* presence and are analyzing MAIA data to better understand preferred habitats (Figure 2.5).

4. VISUAL SURVEYS

4.1 Materials and Methods

Visual surveys were conducted from the shore at sampled sites using 10× magnification binoculars. All turtles visible from the shoreline were counted and identified to species, if possible.

During the survey period, the observer moved up and down the shoreline to gain additional vantage points when possible but remained at least 3-m away from the shoreline to minimize disturbance on turtle behavior or detection. The visual survey duration was 15 min to coincide with the average drone flight duration. Additionally, we attempted to match the visual survey location with the area that the drone survey covered. Preliminary data using these methods suggest that the 15 min duration was adequate to view an entire area, with additional time failing to produce additional turtle detections or increase identification percentage.

To minimize biases in turtle detection, we randomly chose whether the drone flight or the visual survey would be conducted first when visiting a site. Additionally, we waited 15 min between the first method chosen and the second survey method in order to allow for potentially startled turtles to return to basking locations. A single observer (Amy P. Bogolin) conducted all visual surveys to minimize observer bias in detections and identifications.

4.2 Results

In total, 84 visual surveys were conducted at 44 sites during the survey period (Figure 2.6; Table 2.2). Visual surveys resulted in 315 turtles observed. Species identified in visual surveys included *Pseudemys gorzugi* (n = 91), *Trachemys scripta* (n = 20), and *Apalone spinifera* (n = 25), as well as unidentified turtles (n = 171). *Pseudemys gorzugi* was identified at 15 (34.1%) of the 44 sites through visual surveys (Figure 2.6; Table 2.2). Overall (n = 44 sites), the mean number of individual turtles (± 1 SD) detected during visual surveys were 1.1 ± 2.3 *P. gorzugi*, 0.3 ± 0.7 *T. scripta*, 0.3 ± 1.0 *A. spinifera*, and 2.0 ± 3.7 unidentifiable turtles. Site-specific detections for each species are located in Table 2.2. The highest mean number of *P. gorzugi* detected (± 1 SD) was 7.7 ± 4.2 individuals at TNC Dolan Falls Preserve, Devils River, Dolan Falls (Site 16; Figure 2.6; Table 2.2). Including only sites where *P. gorzugi* was detected (n = 15 sites), we observed a mean (± 1 SD) of 2.8 ± 2.2 individuals. Identification percentage varied among sites, with an overall mean identification percentage (± 1 SD) of 50.8 ± 35.1% (Table 2.2).

4.3 Discussion

The three turtle species identified in visual surveys (*Apalone spinifera*, *Trachemys scripta*, *Pseudemys gorzugi*) were all expected to occur in the survey area. Differences in shoreline habitat drastically affected the quality of visual surveys. Areas with tall shoreline vegetation, consisting mostly of *Phragmites* sp., greatly reduced the amount of survey area that we could observe from the shoreline. This was primarily an issue at sites along the Rio Grande. The sites with the highest detections of *P. gorzugi* were TNC Dolan Falls Preserve, Devils River, Dolan Falls (Site 16), Fort Clark Springs, Headwater Pond (Site 27), and Del Rio, San Felipe Springs Golf Course, San Felipe Creek (Site 29). These sites had higher detections of *P. gorzugi* than other sites, which is likely due to a combination of large *P. gorzugi* populations and a clear, easily accessible shoreline, both facilitating detections. Additionally, identification of turtles proved to be more difficult than expected, as the majority of turtles observed during visual surveys were swimming in the water and not basking on woody debris above the water's surface. Characteristics used to differentiate species of turtles were difficult to detect in swimming turtles and were obscured by aquatic vegetation, glare from the sun, and shadows. This resulted in increased numbers of unidentified turtles during visual surveys. Sites where high numbers of *P. gorzugi* were observed could indicate large population sizes or a tendency for multiple individuals to subaerially bask, resulting in increased visibility.

5. TRAPPING

5.1 Materials and Methods

Three standard hoop-net traps, measuring 121.9 × 182.9 cm with 4.45 cm mesh openings, were deployed at each locality where trapping surveys occurred. Traps were set 1–5 m from shore, at a distance where the water level covered the trap mouth, but the top of the trap remained above the water level to prevent trapped turtles from drowning. A combination of stakes and string was used ensure traps remained open and secured to the shoreline, and occasionally additional PVC pipe was secured along the length of the trap to help keep the trap open (primarily in sites with rock substrates). Traps were baited with canned sardines in oil, and the trap mouths were set facing downstream. Some localities were not suitable for trapping due to characteristics of the shoreline that prevented us from securing traps to the shore, lack of shoreline access, or variable water depth due to fluctuating water releases from upstream dams. To remedy this, floating traps were designed and implemented in the latter portion of our survey period. Floating traps consisted of hoop-net traps held open with two pieces of PVC pipe, with multiple foam pool noodles secured lengthwise along the outside of the trap, which kept the trap afloat. These traps were placed further away from shore, in deeper water, closer to known basking areas, and were kept anchored with 5.9-kg kayak anchors. All traps were checked ca. 24 h after deployment, and all captured turtles were removed, processed, and released (described below). During the 24-h check, traps were visually inspected for any tears in the mesh and we ensured that bait remained. If needed, traps were repaired with zip ties and new bait was added. Upon ca. 48 h in the water, traps were removed, and trapped turtles were processed and released.

We collected basic measurement and life history information on all turtles that were trapped and a few individuals that were opportunistically captured by hand. All shell measurements were collected with Haglöf Mantax calipers (in mm) and mass was collected on a digital scale (in g). From each individual turtle, we measured straightline carapace length (SCL), carapace width (CW) at the widest point, plastron length (PL) down the midline of the plastron, plastron width (PW) measured between the junction of the marginal, pectoral, and abdominal scutes, and maximum shell height (SH). Turtles were then sexed, notched with a unique identification number on their marginals following a modified version of the system presented by Ernst et al. (1974; Figure 2.7), and photographed. Before being released, a small tissue clip was collected from the hind foot. Occasionally, trapped turtles had notches on their marginal scutes corresponding with marks from previous researchers. We used these existing notches as part of our numbering scheme when possible and assigned a new number (usually by adding notches on the marginals corresponding to the thousands values) if the existing number was already used (Figure 2.7). Due to their morphology, we did not record PW or notch *Apalone spinifera*. Instead, we detected previously captured turtles by comparing the individual to existing photographs and examining the hind foot for the tissue clip that would have been collected when the individual was first captured.

5.2 Results

Trapping surveys occurred at 39 sites (Figure 2.8; Table 2.3), corresponding to 66 separate trapping events and a total of 8,096 trap hours. The mean (± 1 SD) number of hours each individual trap was deployed was 43.8 ± 6.2 h. On occasion, we found traps ripped open from turtles or collapsed due to increased water flow which resulted in our trap hours being less than our goal of a 48-h deployment. We caught all three expected species of turtles in our traps (*Pseudemys gorzugi*, *Trachemys scripta*, *Apalone spinifera*), though we did not catch all three species at every location or during each trapping event. *Pseudemys gorzugi* was trapped at 18 (46.2%) of the 39 sites (Figure 2.8; Table 2.3). Overall (n = 39 sites), the mean number of individual turtles trapped (± 1 SD) was 1.03 ± 1.82 *P. gorzugi*, 1.65 ± 3.31 *T. scripta*, and 0.80 ± 1.29 *A. spinifera*. Site-specific detections for each species are located in Table 2.3. The highest mean number of *P. gorzugi* trapped (± 1 SD) was 7.0 (± N/A) individuals at the Pecos River, 0.3 river km upstream of confluence with Independence Creek (Site 11; Figure 2.8; Table 2.3), and the highest number of *P. gorzugi* per trap hour (± 1 SD) was 0.0302 ± 0.0312 at Fort Clark Springs, Las Moras Creek, upstream of the golf pro shop (Site 32; Figure 2.8; Table 2.3). Including only sites where *P. gorzugi* was detected (n = 18 sites), the mean (± 1 SD) number of *P. gorzugi* trapped was 2.96 ± 1.97.

Overall, 242 unique turtles were processed, including 86 *P. gorzugi*, 101 *T. scripta*, and 55 *A. spinifera.* All trapped turtles (n = 219) were adults. Seven turtles were recaptured throughout the course of the study. An additional 23 turtles (19 *P. gorzugi*, two *T. scripta*, two *A. spinifera*) were opportunistically hand-captured during our field work. Included in these 19 hand-captured *P. gorzugi* were two juveniles, which represent our only detections of juveniles during the survey period. No juvenile *T. scripta* or *A. spinifera* were captured during the survey period. The notable absence of juvenile turtles from our trapping effort is not surprising, as the trap mesh size allows for juveniles to escape and the microhabitat where our traps were placed favor habitats used by adult turtles. Sex-specific measurements of all trapped and hand-captured turtles can be found in Table 2.4. The average SCL (± 1 SD) for *P. gorzugi* was 193.8 ± 43.3 mm for males and 233.3 ± 55.1 mm for females, and the average mass (± 1 SD) was 1026.1 ± 573.6 g for males and 1886.3 ± 1066.4 g for females (Table 2.4). All turtles appeared outwardly healthy and robust. Occasionally, at a few sites, leeches were present on a small number of individuals. One hand captured *P. gorzugi* from TNC Independence Creek Preserve, raceway below Upper Lake, Terrell County (Site 7; Figure 2.1) had severe damage to the limbs, likely from a recent encounter with a predator, and as a result, the individual was collected as a voucher specimen (DRD 5884; Biodiversity Collections, University of Texas at Austin [TNHC] 114465). Predation of a juvenile *P. gorzugi* was also observed while scouting sampling sites in Del Rio, Texas on 16 May 2019. A juvenile *P. gorzugi* (ca. 5 cm) was caught and killed by a Yellow-crowned Night Heron (*Nyctanassa violacea*), but we were unable to observe it being consumed before it flew off (Bogolin et al. 2019a). At the completion of fieldwork, photographs of all captured turtles were uploaded to the Herps of Texas project on iNaturalist (https://www.inaturalist.org/projects/herps-of-texas) and given a tag ("TX Comptroller – UTRGV – Pseudemys gorzugi") to allow these records to be aggregated more efficiently (Figure 2.9).

5.3 Discussion

On occasion, trapping efforts were subjected to issues such as trap collapse, trap theft, variable water levels, and inadvertent removal of traps. Unfortunately, much of the access to sites, such as those along the Rio Grande, were at public access points. Human disturbance of traps may have occurred more frequently than realized, and preventing it completely was challenging. Traps were stolen on one occasion from a site in Laredo. TPWD Game Wardens removed traps that we set in the Rio Grande, near Salineño, thinking they were set illegally. Fluctuating water levels due to variable water releases from upstream dams resulted in some traps being fully out of water during periods of low-flow. To address this issue, floating traps were used during subsequent trips. Floating traps allowed the trap to move with rising and falling water levels and allowed traps to remain submerged for the full 48-h trap period.

The main advantage to trapping was that it resulted in turtles in hand, which allowed us to collect measurement data and tissue samples from individuals. Trapping success appeared to vary seasonally, as we observed lower capture numbers during the summer months, which matches studies on other turtle species (Plummer 1977). Surprisingly, trapping *P. gorzugi* was largely unsuccessful at some high-abundance sites, suggesting that bait type or trap placement could be preventing their capture and future studies should examine this further.

Turtle measurements fell within the expected ranges previously reported in the literature (Pierce et al. 2016). Females were larger than males, a trend typically seen in many turtles, as a larger body size allows for greater reproductive output (Iverson 1992b). We were unable to trap juveniles in traps, likely because the trap openings were too large and traps were placed in microhabitats not used by juveniles, and as a result, juveniles are underrepresented in our data. Measurements from juveniles were the result of opportunistic hand captures. A small number of turtles were recaptured during our study (n = 7), and the duration between sampling events was not long enough to note differences in size or health of recaptured individuals.

6. OPPORTUNISTIC OBSERVATIONS

Some of these locations included in Figure 2.1 and Table 2.1 were opportunistic sites that were added upon discovery of *Pseudemys gorzugi* in the area, but more rigorous sampling efforts (visual surveys, trapping, drone surveys, eDNA) were not conducted there. We include these observations in the report as they provide important additional occurrence data on *P. gorzugi* within our sampling area. At a few of these locations we were able to hand-capture *P. gorzugi* on land (Site 8), crossing roads (Site 28), or in water while we snorkeled (Site 6). Conversations with the public during sampling trips yielded additional information on turtle occurrence. One individual shared a video recording of an adult *P. gorzugi* from the Rio Grande, Roma Island, north end, Starr County (Site 55), which we uploaded as a photographic observation (iNaturalist 35924758). At the time, it was the furthest downriver site in the Rio Grande that *P. gorzugi* had been observed. This prompted an additional scouting trip to Roma and Rio Grande City to look for and record *P. gorzugi*. We were able to observe a young adult *P. gorzugi* in the Rio Grande, Roma Island, south end (Site 56; iNaturalist 35886829). Additionally, we observed two adult *P. gorzugi* basking in the Rio Grande, near Rio Grande City (Site 57; iNaturalist 35887108, 35887109; Figure 2.10). These two individuals observed near Rio Grande City represent the furthest downriver that *P. gorzugi* has been observed in recent decades.

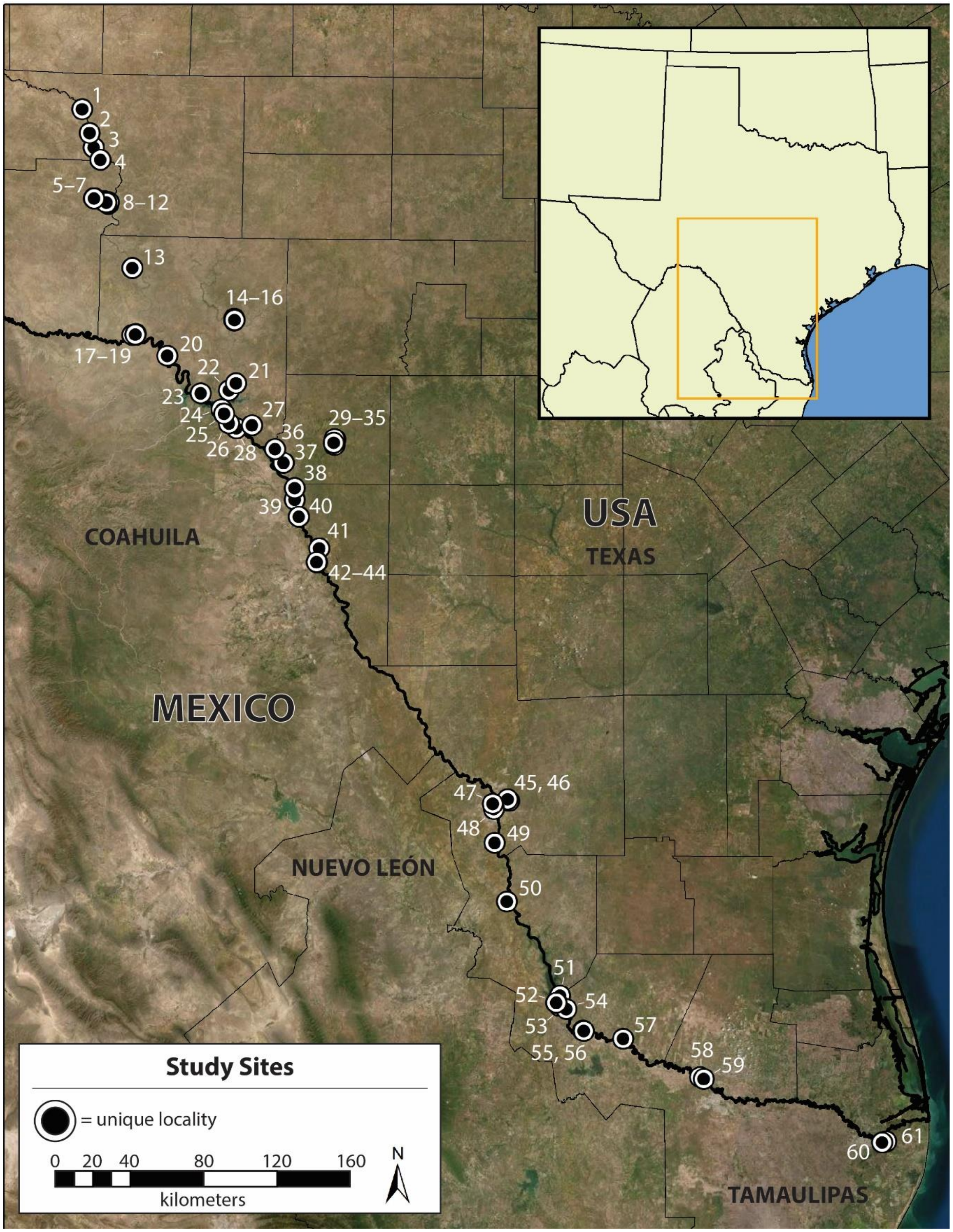

Figure 2.1. Map of 61 study sites visited from November 2018–October 2019 as part of surveys for *Pseudemys gorzugi* in southwestern Texas, USA. Sites represent both areas where multiple survey methodologies were used to detect *P. gorzugi* as well as opportunistic collection of individuals. Site numbers correspond to those used in Table 2.1.

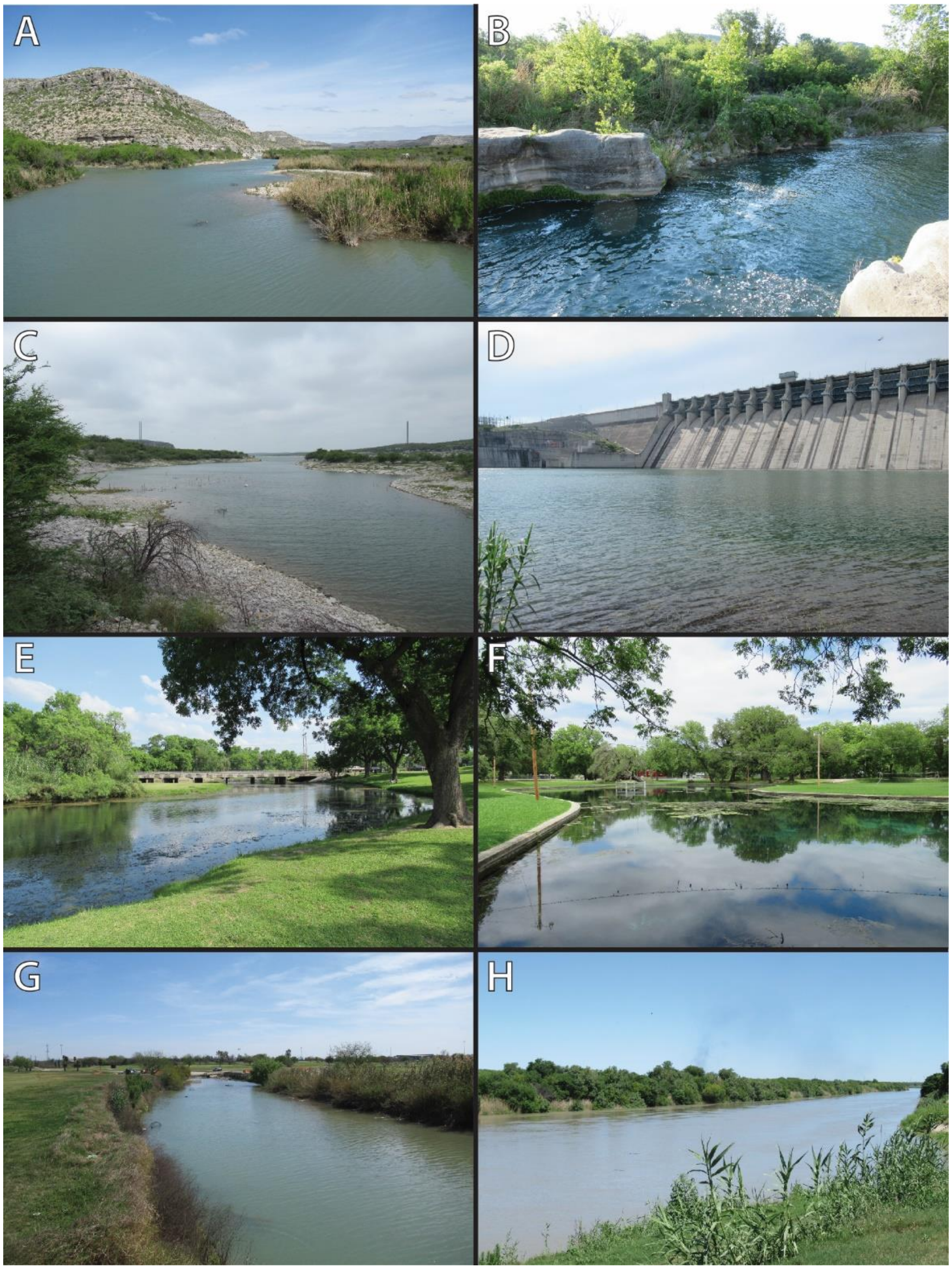

Figure 2.2. Representative sites sampled during the survey period in Texas, USA. A) Pecos River, at Pandale crossing, Val Verde County (Site 13); B) TNC Dolan Falls Preserve, Devils River, Dolan Falls, Val Verde County (Site 16); C) Lake Amistad, Rough Canyon, Val Verde County (Site 21); D) Rio Grande, spillway below Amistad Dam, Val Verde County (Site 24); E) Del Rio, San Felipe Springs Golf Course, San Felipe Creek, Val Verde County (Site 27); F) Fort Clark Springs, Headwater Pond, Kinney County (Site 29); G) Eagle Pass Golf Course, spillway into Rio Grande, Maverick County (Site 42); H) Rio Grande, Laredo, near water treatment center, Webb County (Site 47). All photos by APB.

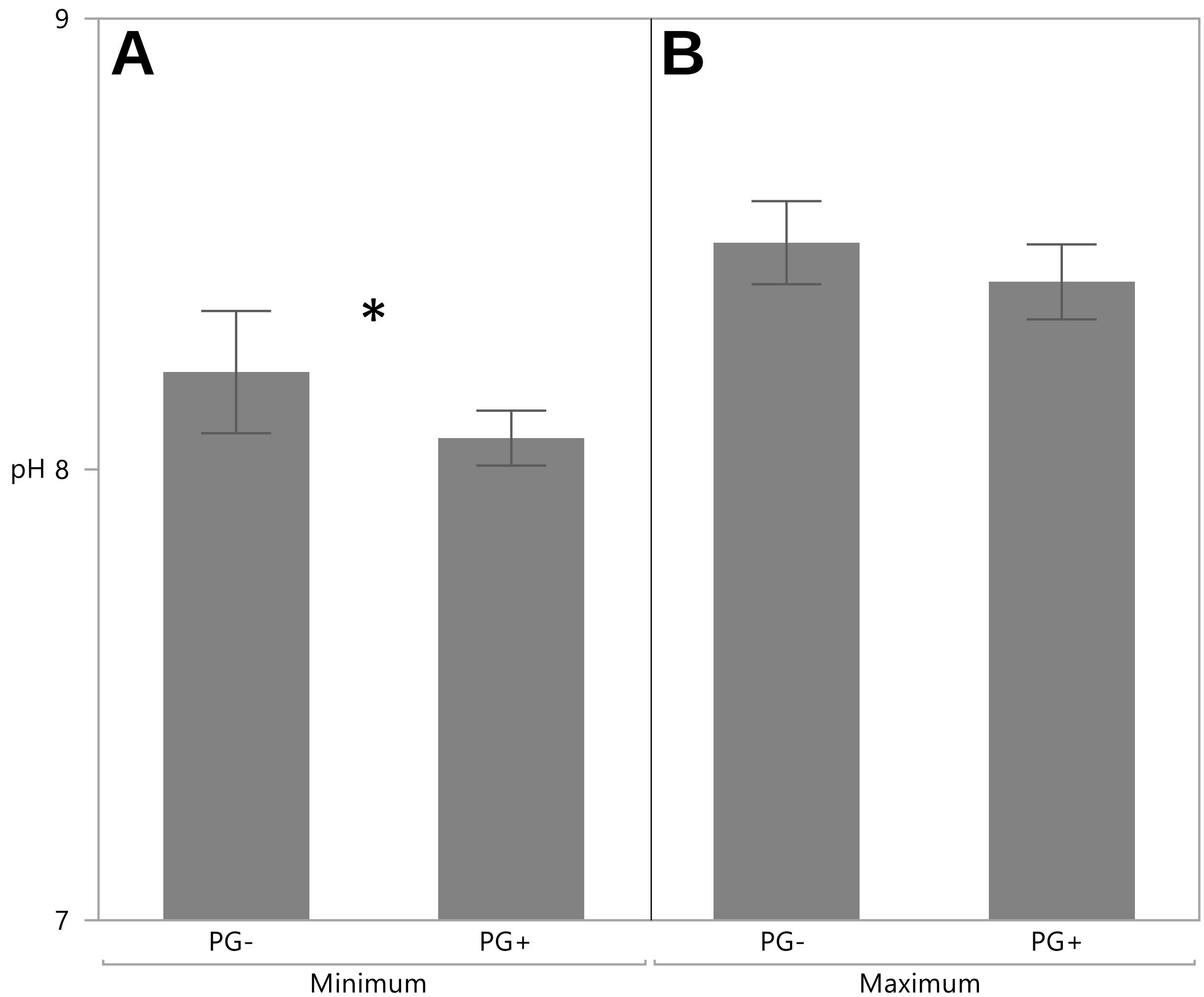

Figure 2.3. Mean (± 1 SE) minimum (A) and maximum (B) pH at sites sampled over the survey period where *Pseudemys gorzugi* (PG) was positively detected (PG+) and never detected (PG-). Asterisk indicates a significant difference between the two groups ($\alpha = 0.05$).

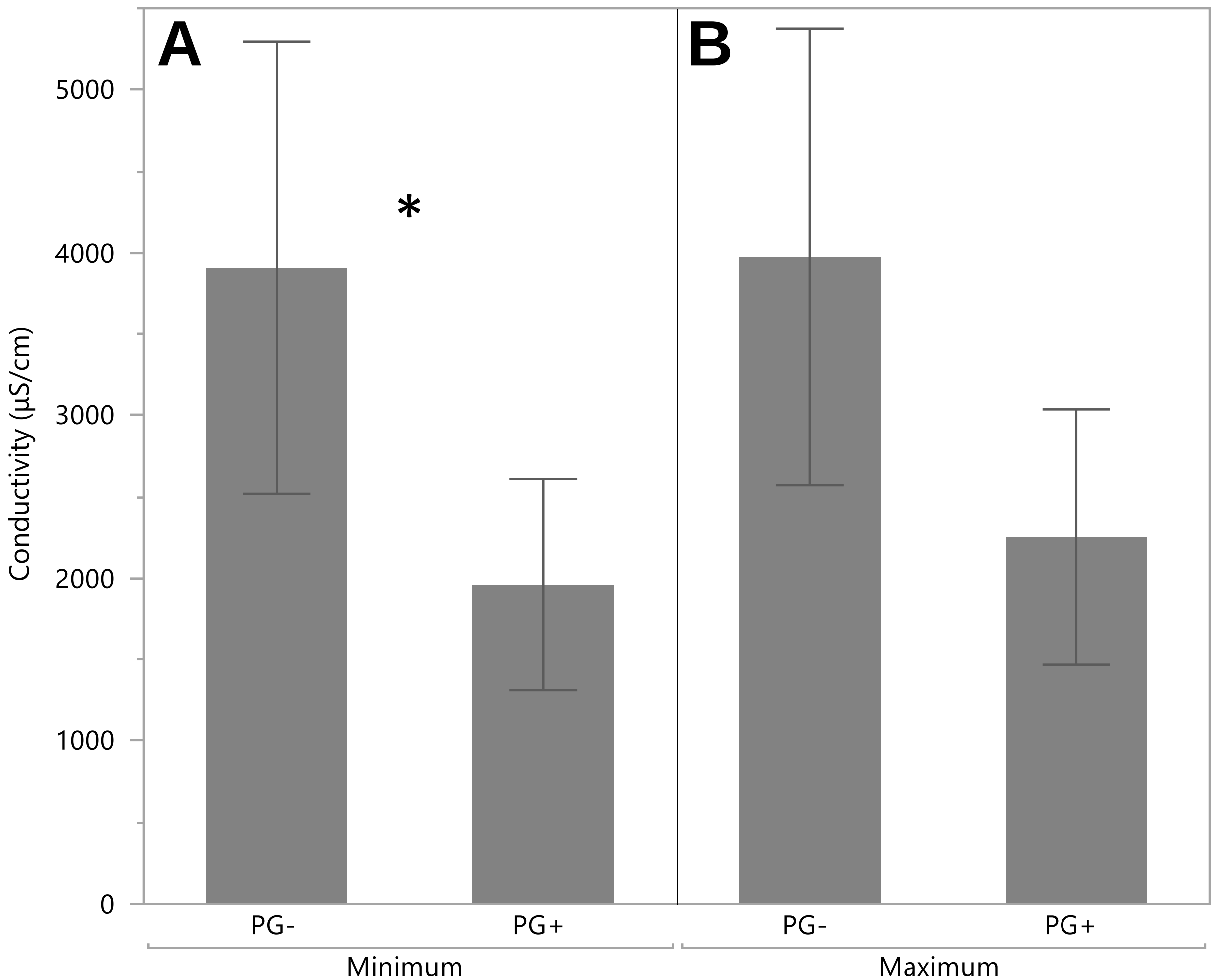

Figure 2.4. Mean (± 1 SE) minimum (A) and maximum (B) conductivity measures at sites sampled over the survey period where *Pseudemys gorzugi* (PG) was positively detected (PG+) and never detected (PG-). Asterisk indicates a significant difference between the two groups (α = 0.05).

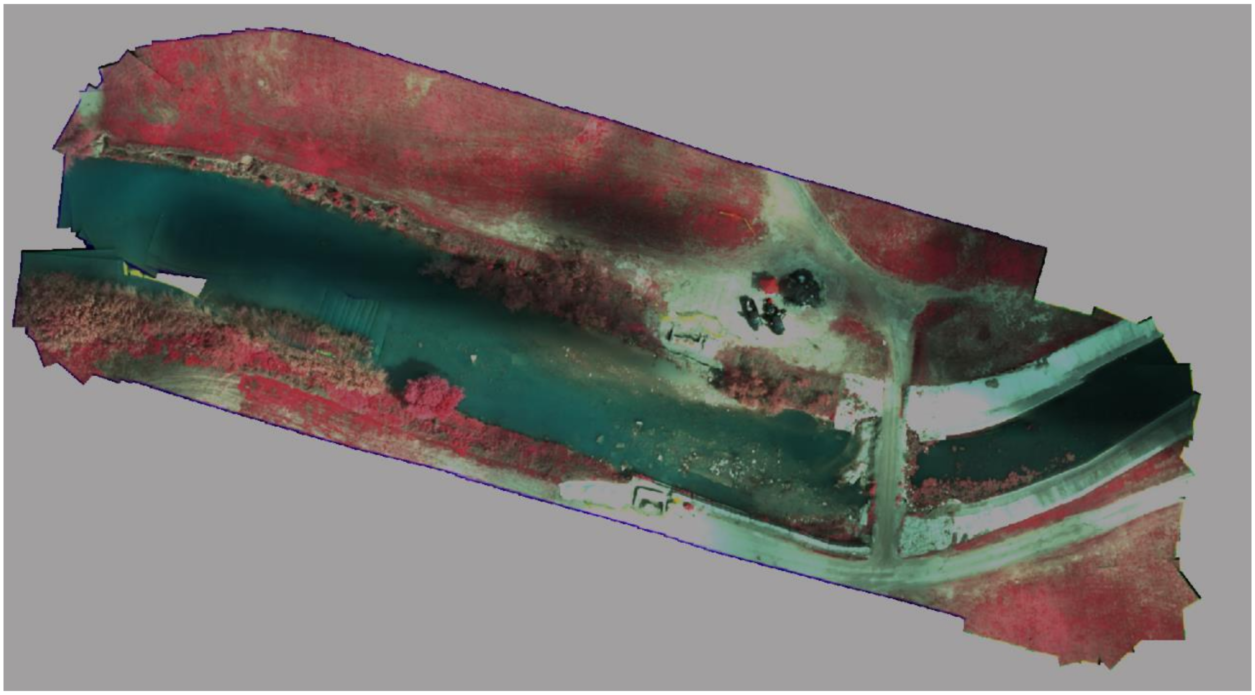

Figure 2.5. Example of multispectral imagery from the Eagle Pass Golf Course, spillway into Rio Grande, Maverick County. We are continuing to analyze data from multispectral images to describe habitat characteristics of *Pseudemys gorzugi*.

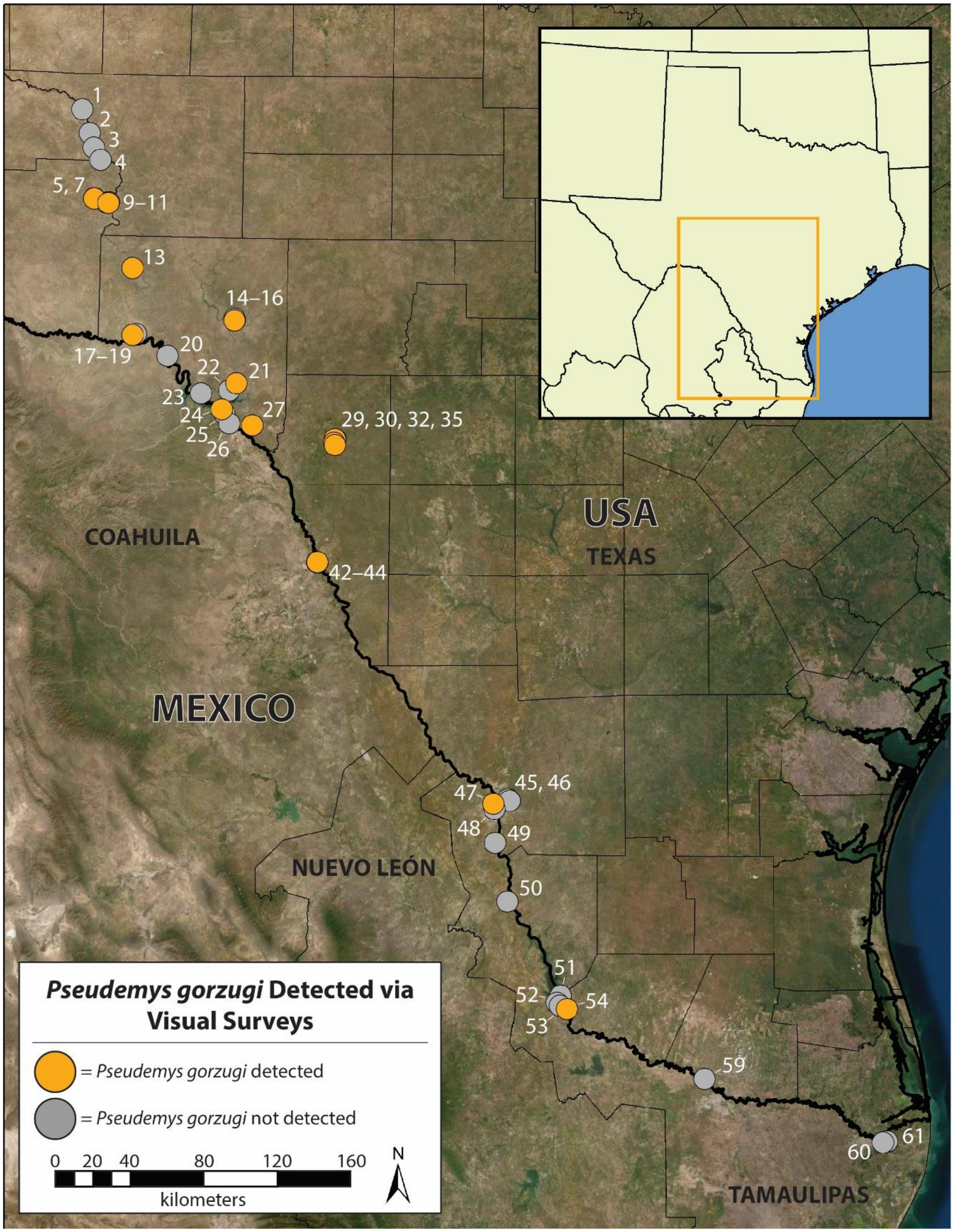

Figure 2.6. Map of 44 sites where visual surveys were conducted for *Pseudemys gorzugi* in southwestern Texas, USA. Sites where *P. gorzugi* was positively detected are indicated in orange. Sites where *P. gorzugi* was not detected are indicated in gray. Site numbers correspond to those used in Table 2.2.

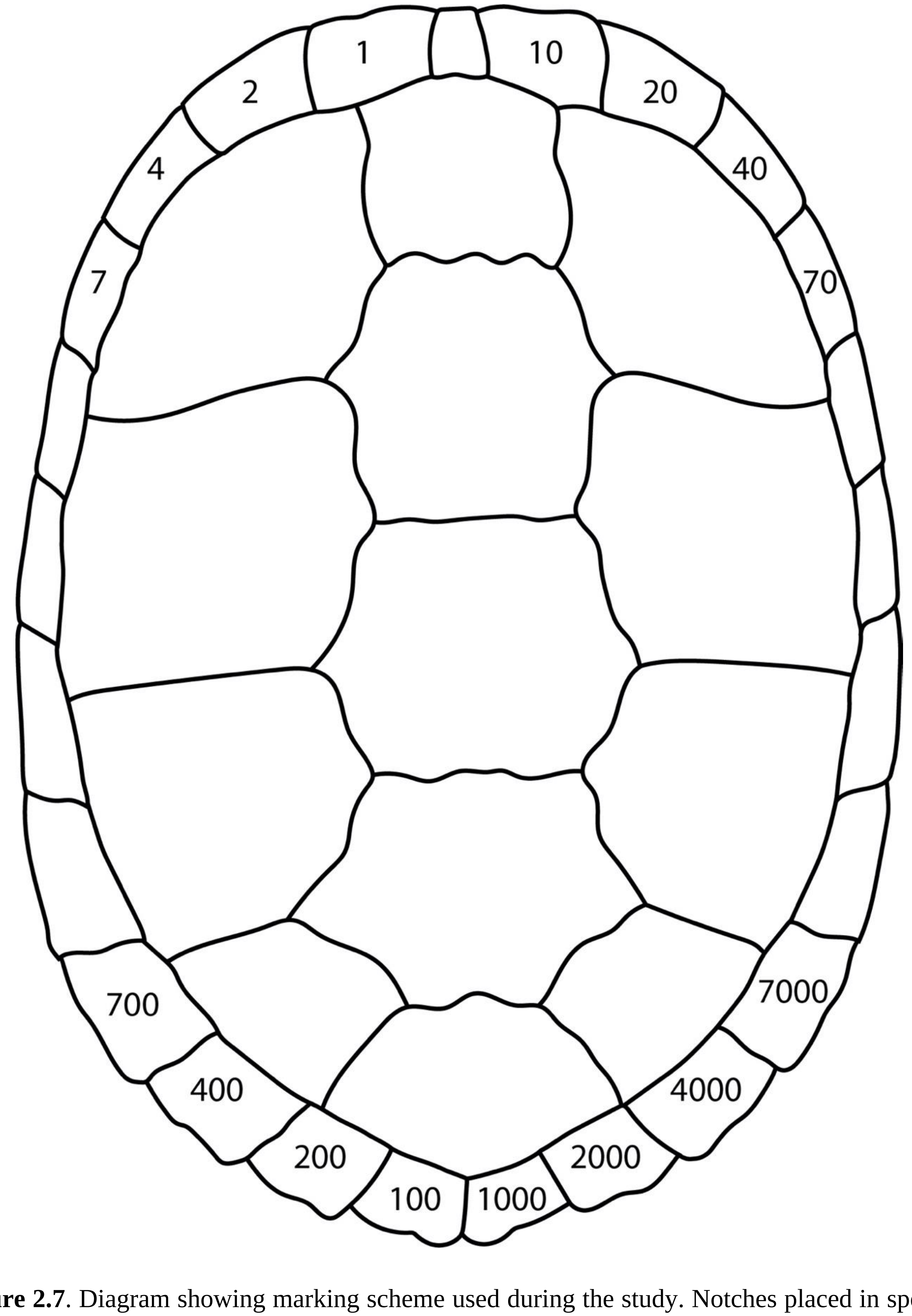

Figure 2.7. Diagram showing marking scheme used during the study. Notches placed in specific marginals are summed to result in the turtle ID number and is derived from the marking scheme in Ernst et al. (1974). Figure drawn from a preserved specimen (TNHC 114463 [DRD 5628]).

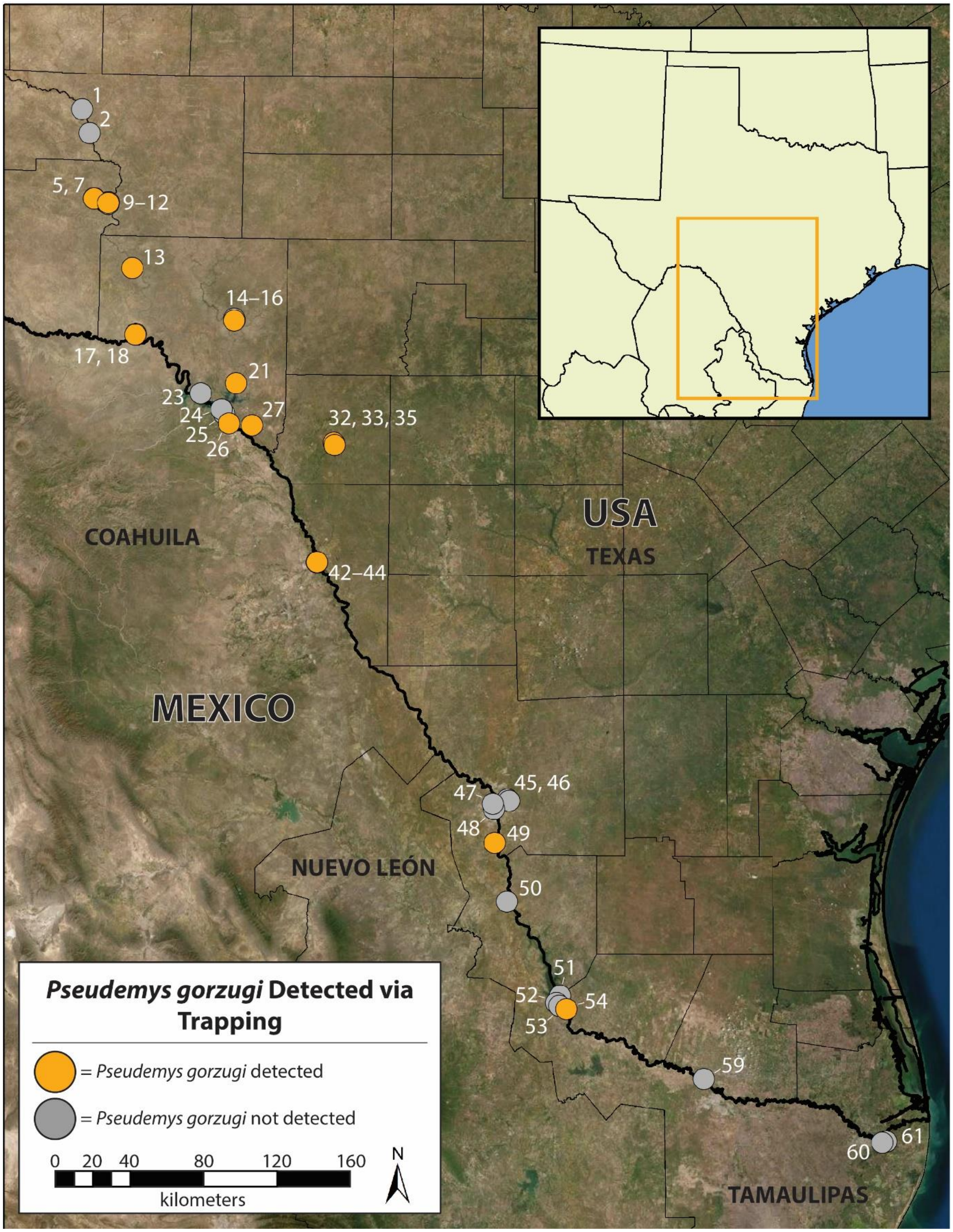

Figure 2.8. Map of 39 sites where trapping for *Pseudemys gorzugi* occurred in southwestern Texas, USA. Sites where *P. gorzugi* was trapped are indicated in orange. Sites where *P. gorzugi* were not trapped are indicated in gray. Site numbers correspond to those used in Table 2.3.

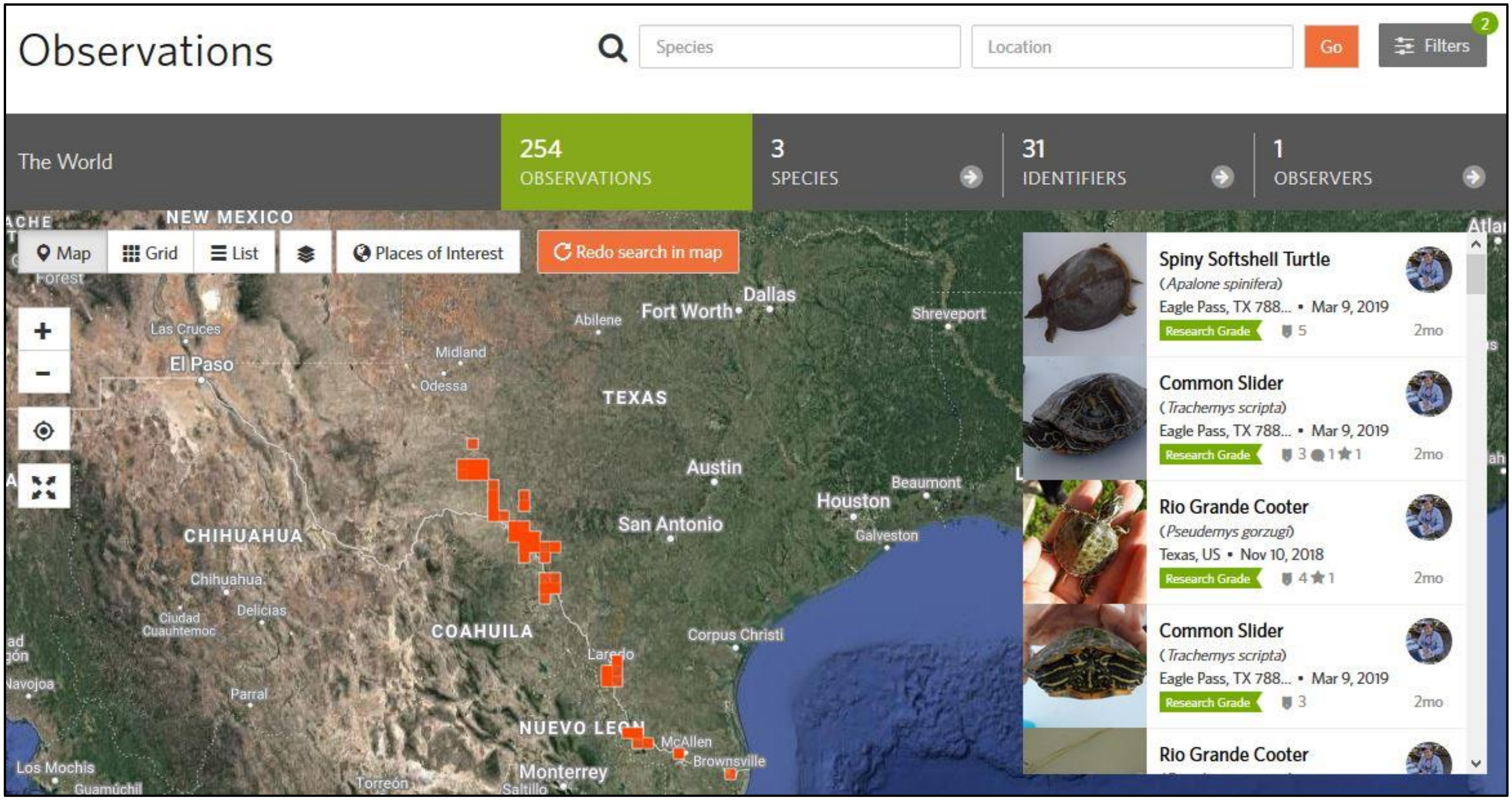

Figure 2.9. Website image of the 254 observations of three species of turtles (*Pseudemys gorzugi*, *Trachemys scripta*, *Apalone spinifera*) uploaded to the Herps of Texas project on iNaturalist (*www.inaturalist.org*) with a coarse map showing the geographic extent of these records. All observations were submitted in November 2019 and were given the tag: "TX Comptroller – UTRGV – Pseudemys gorzugi" to allow these records to be aggregated more efficiently.

Figure 2.10. *Pseudemys gorzugi* observed in the Rio Grande, near Rio Grande City, Starr County (Site 57). These two adults represent the furthest downriver that *P. gorzugi* has been observed in the Rio Grande in recent decades. A) iNaturalist 35887108; B) iNaturalist 35887109.

Table 2.1. List of 61 study sites visited from November 2018–October 2019 as part of surveys for *Pseudemys gorzugi* in Texas. Sites represent both areas where multiple survey methodologies were used to detect *P. gorzugi* as well as opportunistic collection of individuals. Site numbers correspond to those used in Figure 2.1.

Site #	County	Site	Latitude	Longitude	# of Visits
1	Pecos	Pecos River, at US Hwy 190 crossing	30.90516	-101.88083	3
2	Pecos	Pecos River, at Texas Rock Rd (Crockett Co Rd 306)	30.78851	-101.83502	2
3	Pecos	Pecos River, at I-10 crossing	30.71808	-101.80954	1
4	Pecos	Pecos River, at TX Hwy 290 crossing	30.65960	-101.77022	1
5	Terrell	TNC Independence Creek Preserve, Lower Lake	30.46955	-101.80131	2
6	Terrell	TNC Independence Creek Preserve, Upper Lake	30.46893	-101.80204	1
7	Terrell	TNC Independence Creek Preserve, raceway below Upper Lake	30.46736	-101.80181	2
8	Terrell	Chandler Ranch, Cement Pond	30.45747	-101.74300	1
9	Crockett	Pecos River, 0.8 river km upstream of confluence with Independence Creek	30.45259	-101.71940	2
10	Terrell	Independence Creek, at County Road crossing	30.45026	-101.73124	2
11	Crockett	Pecos River, 0.3 river km upstream of confluence with Independence Creek	30.44767	-101.72119	2
12	Terrell	Pecos River, ca. 0.4 river km below confluence with Independence Creek	30.44183	-101.72089	1
13	Val Verde	Pecos River, at Pandale crossing	30.13120	-101.57450	2
14	Val Verde	TNC Dolan Falls Preserve, Devils River, upstream of confluence with Dolan Creek	29.89387	-100.99561	3
15	Val Verde	TNC Dolan Falls Preserve, Dolan Creek, near confluence with Devils River	29.88591	-100.99292	2
16	Val Verde	TNC Dolan Falls Preserve, Devils River, Dolan Falls	29.88385	-100.99397	3
17	Val Verde	Rio Grande, at Eagle Nest Canyon	29.80829	-101.54893	1
18	Val Verde	Rio Grande, near Langtry	29.80564	-101.55088	2
19	Val Verde	Pump Canyon, Langtry	29.80343	-101.56750	1
20	Val Verde	Pecos River, near confluence with Rio Grande	29.70431	-101.36667	2

21	Val Verde	Lake Amistad, Rough Canyon	29.57490	-100.97809	3
22	Val Verde	Lake Amistad, along Spur 406	29.54023	-101.01623	1
23	Val Verde	Lake Amistad, Box Canyon	29.52420	-101.17585	3
24	Val Verde	Rio Grande, spillway below Amistad Dam	29.44737	-101.05667	2
25	Val Verde	Rio Grande, weir below Amistad Dam	29.42455	-101.04118	2
26	Val Verde	Rio Grande, near Lugo property	29.37719	-101.01348	3
27	Val Verde	Del Rio, San Felipe Springs Golf Course, San Felipe Creek	29.37029	-100.88526	3
28	Val Verde	Del Rio, Vega Verde Rd	29.35488	-100.97136	1
29	Kinney	Fort Clark Springs, Headwater Pond	29.30944	-100.42125	4
30	Kinney	Fort Clark Springs, Las Moras Creek, near guard station	29.30740	-100.41745	1
31	Kinney	Fort Clark Springs, Las Moras Creek, near Scales Rd	29.29273	-100.42075	1
32	Kinney	Fort Clark Springs, Las Moras Creek, upstream of golf pro shop	29.29043	-100.42386	3
33	Kinney	Fort Clark Springs, Las Moras Creek	29.28638	-100.42263	1
34	Kinney	Fort Clark Springs, Las Moras Creek, NW end of Buzzard Roost	29.28238	-100.42325	1
35	Kinney	Fort Clark Springs, Las Moras Creek, Buzzard Roost	29.28034	-100.42076	3
36	Val Verde	Sycamore Creek, at US Hwy 277 crossing	29.25473	-100.75216	1
37	Kinney	Pinto Creek, at US Hwy 277 crossing	29.18898	-100.70340	1
38	Maverick	Tequesquite Creek, at US Hwy 277 crossing	29.06453	-100.63899	1
39	Maverick	irrigation canal along US Hwy 277, near Las Moras Creek	29.00785	-100.63817	1
40	Maverick	Quemado Creek, along US Hwy 277	28.92578	-100.61490	1
41	Maverick	Elm Creek, near US Hwy 277	28.77016	-100.49828	1
42	Maverick	Eagle Pass Golf Course, spillway into Rio Grande	28.70416	-100.51046	2
43	Maverick	Rio Grande, along Eagle Pass Golf Course	28.70294	-100.51089	2
44	Maverick	Eagle Pass Golf Course, settling pond along Rio Grande	28.70146	-100.50979	2
45	Webb	Lake Casa Blanca International State Park, Casa Blanca Lake, near El Ranchito pavilion	27.54447	-99.44098	2

46	Webb	Lake Casa Blanca International State Park, Casa Blanca Lake, fishing pier	27.53861	-99.43475	2
47	Webb	Rio Grande, Laredo, near water treatment center	27.52372	-99.52431	3
48	Webb	Rio Grande, Laredo, near international railroad bridge crossing	27.49835	-99.51674	2
49	Webb	Rio Grande, near El Cenizo	27.33117	-99.51195	2
50	Zapata	Rio Grande, near San Ygancio	27.04330	-99.44496	1
51	Starr	Falcon State Park, Falcon Lake	26.58179	-99.15259	3
52	Starr	Rio Grande, spillway below Falcon Dam	26.54608	-99.17093	3
53	Starr	Rio Grande, near Chapeno	26.53233	-99.15546	1
54	Starr	Rio Grande, near Salineño	26.51429	-99.11662	4
55	Starr	Rio Grande, Roma Island, north end	26.40985	-99.02465	1
56	Starr	Rio Grande, Roma Island, south end	26.40657	-99.02073	1
57	Starr	Rio Grande, near Rio Grande City	26.36799	-98.80555	1
58	Hidalgo	Bentsen-Rio Grande Valley State Park, La Parida Banco	26.17906	-98.38716	1
59	Hidalgo	Rio Grande, near National Butterfly Center	26.16934	-98.36742	2
60	Cameron	Rio Grande, downstream of TNC Southmost Preserve	25.85462	-97.37676	1
61	Cameron	Rio Grande, near TNC Southmost Preserve Office	25.85008	-97.39865	2

Table 2.2. Mean (± 1 SD) number of three species of turtles (*Pseudemys gorzugi*, *Trachemys scripta*, *Apalone spinifera*), as well as unidentified turtles, observed during 15-min visual surveys at sampled sites. Site locality information, number of visits, *Pseudemys gorzugi* (PG) detection, and mean (± 1 SD) percent identification of observed turtles is also provided (ID %). Site numbers correspond to those used in Table 2.1.

Site #	County	Site	Latitude	Longitude	# of Visits	PG Detected	*Pseudemys gorzugi*	*Trachemys scripta*	*Apalone spinifera*	Unidentified	ID %
1	Pecos	Pecos River, at US Hwy 190 crossing	30.90516	-101.88080	3	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	–
2	Pecos	Pecos River, at Texas Rock Rd (Crockett Co Rd 306)	30.78851	-101.83502	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0.5 (± 0.7)	0 (± N/A)
3	Pecos	Pecos River, at I-10 crossing	30.71808	-101.80954	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)	–
4	Pecos	Pecos River, at TX Hwy 290 crossing	30.65960	-101.77020	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)	–
5	Terrell	TNC Independence Creek Preserve, Lower Lake	30.46955	-101.80131	2	yes	2.0 (± 1.4)	0 (± 0)	0.5 (± 0.7)	5.0 (± 0)	30.6 (± 19.6)
7	Terrell	TNC Independence Creek Preserve, raceway below Upper Lake	30.46736	-101.80181	1	yes	4.0 (± N/A)	0 (± N/A)	0 (± N/A)	2.0 (± N/A)	66.7 (± N/A)
9	Crockett	Pecos River, 0.8 river km upstream of confluence with Independence Creek	30.45259	-101.71940	1	no	0 (± N/A)	0 (± N/A)	3.0 (± N/A)	0 (± N/A)	100.0 (± N/A)
10	Terrell	Independence Creek, at County Road crossing	30.45026	-101.73124	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	–
11	Crockett	Pecos River, 0.3 river km upstream of confluence with Independence Creek	30.44767	-101.72119	1	yes	2.0 (± N/A)	0 (± N/A)	0 (± N/A)	1.0 (± N/A)	66.7 (± N/A)
13	Val Verde	Pecos River, at Pandale crossing	30.13120	-101.57450	1	yes	2.0 (± N/A)	0 (± N/A)	0 (± N/A)	6.0 (± N/A)	25.0 (± N/A)
14	Val Verde	TNC Dolan Falls Preserve, Devils River, upstream of confluence with Dolan Creek	29.89387	-100.99561	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	–
15	Val Verde	TNC Dolan Falls Preserve, Dolan Creek, near confluence with Devils River	29.88591	-100.99292	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	–
16	Val Verde	TNC Dolan Falls Preserve, Devils River, Dolan Falls	29.88385	-100.99397	3	yes	7.7 (± 4.2)	0 (± 0)	0 (± 0)	5.7 (± 3.5)	56.0 (± 26.2)
17	Val Verde	Rio Grande, at Eagle Nest Canyon	29.80829	-101.54893	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)	–
18	Val Verde	Rio Grande, near Langtry	29.80564	-101.55088	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	–
19	Val Verde	Pump Canyon, Langtry	29.80343	-101.56750	1	yes	4.0 (± N/A)	0 (± N/A)	4.0 (± N/A)	6.0 (± N/A)	57.1 (± N/A)
20	Val Verde	Pecos River, near confluence with Rio Grande	29.70431	-101.36667	2	no	0 (± 0)	1.0 (± 1.4)	0 (± 0)	1.0 (± 1.4)	50.0 (± N/A)
21	Val Verde	Lake Amistad, Rough Canyon	29.57490	-100.97809	3	yes	0.3 (± 0.6)	0 (± 0)	0 (± 0)	1.3 (± 2.3)	20.0 (± N/A)
22	Val Verde	Lake Amistad, along Spur 406	29.54023	-101.01623	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)	–
23	Val Verde	Lake Amistad, Box Canyon	29.52420	-101.17585	3	no	0 (± 0)	0 (± 0)	0 (± 0)	1.0 (± 1.7)	0 (± N/A)

24	Val Verde	Rio Grande, spillway below Amistad Dam	29.44737	-101.05667	2	yes	0.5 (± 0.7)	0 (± 0)	0 (± 0)	9.5 (± 0.7)	5.0 (± 7.1)
25	Val Verde	Rio Grande, weir below Amistad Dam	29.42455	-101.04118	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0.5 (± 0.7)	0 (± N/A)
26	Val Verde	Rio Grande, near Lugo property	29.37719	-101.01348	3	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	–
27	Val Verde	Del Rio, San Felipe Springs Golf Course, San Felipe Creek	29.37029	-100.88526	3	yes	4.0 (± 1.0)	1.3 (± 1.5)	2.0 (± 2.6)	11.0 (± 7.0)	43.5 (± 17.5)
29	Kinney	Fort Clark Springs, Headwater Pond	29.30944	-100.42125	3	yes	6.7 (± 3.5)	0.7 (± 0.6)	0 (± 0)	6.7 (± 6.4)	57.4 (± 31.6)
30	Kinney	Fort Clark Springs, Las Moras Creek, near guard station	29.30740	-100.4175	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)	–
32	Kinney	Fort Clark Springs, Las Moras Creek, upstream of golf pro shop	29.29043	-100.42386	3	yes	3.0 (± 1.7)	0.7 (± 0.6)	0 (± 0)	1.7 (± 1.5)	73.8 (± 25.1)
35	Kinney	Fort Clark Springs, Las Moras Creek, Buzzard Roost	29.28034	-100.42076	2	yes	0.5 (± 0.7)	0 (± 0)	0 (± 0)	0 (± 0)	100.0 (± N/A)
42	Maverick	Eagle Pass Golf Course, spillway into Rio Grande	28.70416	-100.51046	1	yes	3.0 (± N/A)	1.0 (± N/A)	0 (± N/A)	2.0 (± N/A)	66.7 (± N/A)
43	Maverick	Rio Grande, along Eagle Pass Golf Course	28.70294	-100.51089	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)	–
44	Maverick	Eagle Pass Golf Course, settling pond along Rio Grande	28.70146	-100.50979	1	no	0 (± N/A)	4.0 (± N/A)	0 (± N/A)	15.0 (± N/A)	21.1 (± N/A)
45	Webb	Lake Casa Blanca International SP, Casa Blanca Lake, near El Ranchito pavilion	27.54447	-99.44098	2	no	0 (± 0)	1.0 (± 0)	1.5 (± 0.7)	1.5 (± 0.7)	63.3 (± 4.8)
46	Webb	Lake Casa Blanca International SP, Casa Blanca Lake, fishing pier	27.53861	-99.43475	2	no	0 (± 0)	1.0 (± 0)	0.5 (± 0.7)	0.5 (± 0.7)	75.0 (± 35.4)
47	Webb	Rio Grande, Laredo, near water treatment center	27.52372	-99.52431	3	yes	0.3 (± 0.6)	0 (± 0)	0.7 (± 1.2)	0.3 (± 0.6)	75.0 (± N/A)
48	Webb	Rio Grande, Laredo, near international railroad bridge crossing	27.49835	-99.51674	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	–
49	Webb	Rio Grande, near El Cenizo	27.33117	-99.51195	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	–
50	Zapata	Rio Grande, near San Ygancio	27.04330	-99.44496	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	1.0 (± N/A)	0 (± N/A)
51	Starr	Falcon State Park, Falcon Lake	26.58179	-99.15259	3	no	0 (± 0)	1.3 (± 1.5)	0 (± 0)	0.3 (± 0.6)	87.5 (± 17.7)
52	Starr	Rio Grande, spillway below Falcon Dam	26.54608	-99.17093	3	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	–
53	Starr	Rio Grande, near Chapeno	26.53233	-99.15546	1	no	0 (± N/A)	1.0 (± N/A)	0 (± N/A)	0 (± N/A)	100.0 (± N/A)
54	Starr	Rio Grande, near Salineño	26.51429	-99.11662	3	yes	1.3 (± 2.3)	0.7 (± 1.2)	1.7 (± 2.9)	3.3 (± 3.1)	32.4 (± 45.7)
59	Hidalgo	Rio Grande, near National Butterfly Center	26.16934	-98.36742	2	no	0 (± 0)	0 (± 0)	0 (± 0)	3.5 (± 0.7)	0 (± 0)
60	Cameron	Rio Grande, downstream of TNC Southmost Preserve	25.85462	-97.37676	1	no	0 (± N/A)	1.0 (± N/A)	0 (± N/A)	0 (± N/A)	100.0 (± N/A)
61	Cameron	Rio Grande, near TNC Southmost Preserve Office	25.85008	-97.39865	2	no	0 (± 0)	0.5 (± 0.7)	0 (± 0)	0 (± 0)	100.0 (± N/A)

Table 2.3. Mean (± 1 SD) number of three species of turtles (*Pseudemys gorzugi*, *Trachemys scripta*, *Apalone spinifera*) trapped over 48-h trapping periods at sampled sites. Site locality information, number of visits, *Pseudemys gorzugi* (PG) detection, and mean (± 1 SD) number of *P. gorzugi* per trap hour is also provided. Site numbers correspond to those used in Table 2.1.

Site #	County	Site	Latitude	Longitude	# of Visits	PG Detected	*Pseudemys gorzugi*	*Trachemys scripta*	*Apalone spinifera*	# PG/trap hour
1	Pecos	Pecos River, at US Hwy 190 crossing	30.90516	-101.88083	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
2	Pecos	Pecos River, at Texas Rock Rd (Crockett Co Rd 306)	30.78851	-101.83502	2	no	0 (± 0)	0.5 (± 0.7)	0 (± 0)	0 (± 0)
5	Terrell	TNC Independence Creek Preserve, Lower Lake	30.46955	-101.80131	2	yes	1.5 (± 0.7)	7.0 (± 0)	3.0 (± 1.4)	0.0099 (± 0.0036)
7	Terrell	TNC Independence Creek Preserve, raceway below Upper Lake	30.46736	-101.80181	1	yes	4.0 (± N/A)	1.0 (± N/A)	3.0 (± N/A)	0.0284 (± N/A)
9	Crockett	Pecos River, 0.8 river km upstream of confluence with Independence Creek	30.45259	-101.71940	2	yes	1.0 (± 1.4)	0 (± 0)	0 (± 0)	0.0077 (± 0.0108)
10	Terrell	Independence Creek, at County Road crossing	30.45026	-101.73124	2	no	0 (± 0)	0 (± 0)	1.0 (± 1.4)	0 (± 0)
11	Crockett	Pecos River, 0.3 river km upstream of confluence with Independence Creek	30.44767	-101.72119	1	yes	7.0 (± N/A)	0 (± N/A)	2.0 (± N/A)	0.0262 (± N/A)
12	Terrell	Pecos River, ca. 0.4 river km below confluence with Independence Creek	30.44183	-101.72089	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)
13	Val Verde	Pecos River, at Pandale crossing	30.13120	-101.57450	2	yes	2.5 (± 3.5)	0 (± 0)	0 (± 0)	0.0157 (± 0.0222)
14	Val Verde	TNC Dolan Falls Preserve, Devils River, upstream of confluence with Dolan Creek	29.89387	-100.99561	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
15	Val Verde	TNC Dolan Falls Preserve, Dolan Creek, near confluence with Devils River	29.88591	-100.99292	2	no	0 (± 0)	0.5 (± 0.7)	0 (± 0)	0 (± 0)
16	Val Verde	TNC Dolan Falls Preserve, Devils River, Dolan Falls	29.88385	-100.99397	2	yes	0.5 (± 0.7)	1.5 (± 0.7)	1.0 (± 1.4)	0.0037 (± 0.0053)
17	Val Verde	Rio Grande, at Eagle Nest Canyon	29.80829	-101.54893	1	no	0 (± N/A)	0 (± N/A)	1.0 (± N/A)	0 (± N/A)
18	Val Verde	Rio Grande, near Langtry	29.80564	-101.55088	2	yes	0.5 (± 0.7)	0.5 (± 0.7)	2.0 (± 1.4)	0.0043 (± 0.0061)
21	Val Verde	Lake Amistad, Rough Canyon	29.57490	-100.97809	3	yes	1.3 (± 2.3)	0 (± 0)	0.3 (± 0.6)	0.0085 (± 0.0147)
23	Val Verde	Lake Amistad, Box Canyon	29.52420	-101.17585	3	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
24	Val Verde	Rio Grande, spillway below Amistad Dam	29.44737	-101.05667	2	no	0 (± 0)	0.5 (± 0.7)	2.5 (± 3.5)	0 (± 0)
25	Val Verde	Rio Grande, weir below Amistad Dam	29.42455	-101.04118	2	no	0 (± 0)	1.0 (± 1.4)	0 (± 0)	0 (± 0)
26	Val Verde	Rio Grande, near Lugo property	29.37719	-101.01348	1	yes	1.0 (± N/A)	2.0 (± N/A)	0 (± N/A)	0.0093 (± N/A)
27	Val Verde	Del Rio, San Felipe Springs Golf Course, San Felipe Creek	29.37029	-100.88526	2	yes	3.5 (± 0.7)	5.5 (± 5.0)	0 (± 0)	0.0268 (± 0.0048)

32	Kinney	Fort Clark Springs, Las Moras Creek, upstream of golf pro shop	29.29043	-100.42386	2	yes	4.5 (± 5.0)	5.5 (± 3.5)	0.5 (± 0.7)	0.0302 (± 0.0312)
33	Kinney	Fort Clark Springs, Las Moras Creek	29.28638	-100.42263	1	yes	4.0 (± N/A)	1.0 (± N/A)	1.0 (± N/A)	0.0079 (± N/A)
35	Kinney	Fort Clark Springs, Las Moras Creek, Buzzard Roost	29.28034	-100.42076	2	yes	1.0 (± 0)	1.0 (± 1.4)	1.0 (± 1.4)	0.0071 (± 0.0010)
42	Maverick	Eagle Pass Golf Course, spillway into Rio Grande	28.70416	-100.51046	2	yes	3.5 (± 2.1)	2.0 (± 0)	3.0 (± 2.8)	0.0293 (± 0.0093)
43	Maverick	Rio Grande, along Eagle Pass Golf Course	28.70294	-100.51089	1	yes	3.0 (± N/A)	0 (± N/A)	3.0 (± N/A)	0.0227 (± N/A)
44	Maverick	Eagle Pass Golf Course, settling pond along Rio Grande	28.70146	-100.50979	1	yes	2.0 (± N/A)	16.0 (± N/A)	3.0 (± N/A)	0.0139 (± N/A)
45	Webb	Lake Casa Blanca International SP, Casa Blanca Lake, near El Ranchito pavilion	27.54447	-99.44098	2	no	0 (± 0)	0 (± 0)	2.0 (± 0)	0 (± 0)
46	Webb	Lake Casa Blanca International SP, Casa Blanca Lake, fishing pier	27.53861	-99.43475	2	no	0 (± 0)	0.5 (± 0.7)	0 (± 0)	0 (± 0)
47	Webb	Rio Grande, Laredo, near water treatment center	27.52372	-99.52431	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
48	Webb	Rio Grande, Laredo, near international railroad bridge crossing	27.49835	-99.51674	1	no	0 (± N/A)	0 (± N/A)	3.0 (± N/A)	0 (± N/A)
49	Webb	Rio Grande, near El Cenizo	27.33117	-99.51195	1	yes	3.0 (± N/A)	0 (± N/A)	0 (± N/A)	0.0200 (± N/A)
50	Zapata	Rio Grande, near San Ygancio	27.04330	-99.44496	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)
51	Starr	Falcon State Park, Falcon Lake	26.58179	-99.15259	2	no	0 (± 0)	8.5 (± 10.6)	0.5 (± 0.7)	0 (± 0)
52	Starr	Rio Grande, spillway below Falcon Dam	26.54608	-99.17093	2	no	0 (± 0)	4.0 (± 4.2)	1.0 (± 1.4)	0 (± 0)
53	Starr	Rio Grande, near Chapeno	26.53233	-99.15546	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)
54	Starr	Rio Grande, near Salineño	26.51429	-99.11662	2	yes	1.5 (± 2.1)	3.0 (± 2.8)	0 (± 0)	0.0105 (± 0.0148)
59	Hidalgo	Rio Grande, near National Butterfly Center	26.16934	-98.36742	2	no	0 (± 0)	1.5 (± 2.1)	0.5 (± 0.7)	0 (± 0)
60	Cameron	Rio Grande, downstream of TNC Southmost Preserve	25.85462	-97.37676	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)
61	Cameron	Rio Grande, near TNC Southmost Preserve Office	25.85008	-97.39865	1	no	0 (± N/A)	1.0 (± N/A)	0 (± N/A)	0 (± N/A)

Table 2.4. Number (N), mean (± 1 SD) shell measurements (mm), and mass (g) of male, female, and juvenile *Pseudemys gorzugi*, *Trachemys scripta*, and *Apalone spinifera* captured during trapping events and opportunistically by hand during the survey period. No juvenile *T. scripta* or *A. spinifera* were captured during the survey period. SCL = straightline carapace length, CW = carapace width at the widest point, PL = plastron length down the midline of the plastron, PW = plastron width measured between the junction of the marginal, pectoral, and abdominal scutes, SH = maximum shell height.

Species	Sex	N	SCL	CW	PL	PW	SH	Mass
Pseudemys gorzugi	male	54	193.8 (± 43.3)	170.5 (± 193.4)	167.9 (± 33.4)	109.0 (± 18.7)	71.3 (± 22.8)	1026.1 (± 573.6)
Pseudemys gorzugi	female	30	233.3 (± 55.1)	176.2 (± 38.2)	209.3 (± 48.6)	134.9 (± 30.7)	92.7 (± 21.9)	1886.3 (± 1066.4)
Pseudemys gorzugi	juvenile	2	45.5 (± 16.3)	43.0 (± 12.7)	41.5 (± 12.0)	31.5 (± 9.2)	21.0 (± 4.2)	20.6 (± 17.8)
Trachemys scripta	male	52	168.4 (± 26.9)	128.2 (± 16.4)	151.0 (± 24.3)	98.1 (± 13.4)	65.5 (± 11.9)	718.3 (± 317.9)
Trachemys scripta	female	59	208.2 (± 38.8)	157.9 (± 25.5)	190.0 (± 44.0)	121.2 (± 21.3)	84.8 (± 17.7)	1419.4 (± 626.8)
Apalone spinifera	male	28	168.8 (± 20.2)	136.9 (± 14.3)	122.3 (± 13.7)	N/A	45.2 (± 8.2)	2438.4 (± 1450.7)
Apalone spinifera	female	26	294.5 (± 76.5)	225.1 (± 54.9)	209.8 (± 55.9)	N/A	75.1 (± 23.3)	516.0 (± 187.6)

CHAPTER III

TASK 2. Conduct a pilot study to evaluate the effectiveness and efficiency of drone-based imaging and eDNA survey methods for *P. gorzugi*

A. Conduct Task 2 concurrently with Task 1 and at the same sites identified under Task 1
B. Utilize drones to take high-resolution photographs and multispectral images of each site to assess *P. gorzugi* presence and habitat characteristics
C. Collect and analyze water samples from each site to assess the presence of *P. gorzugi* eDNA
D. Analyze the results of Task 2 to evaluate the effectiveness and efficiency of drone-based imaging and eDNA survey methods for *P. gorzugi*
E. Record GPS coordinates for all survey data collected

1. OVERVIEW

Chapter III expands the survey methodologies conducted at the study sites initially described in Chapter II and focuses on two novel survey methodologies: drone surveys and environmental DNA (eDNA) surveys. The explanation and description of novel survey methodologies used includes methods, results, and brief discussion sections. Subtasks A–E are addressed throughout this chapter in relevant sections. Additionally, Chapter III emphasizes drone surveys, and we highlight examples of benefits to the use of drones in the text and with figures. Distribution maps, figures, and tables containing information related to Chapter III are included at the end of the chapter.

2. STUDY LOCATION

Study locations in Chapter III of this report match those described in the Chapter II. Not every survey method was conducted at each site during each visit. Chapter III (below) describes sites where drone surveys and eDNA sampling occurred. Sites where novel survey methods were tested covered the same geographic extent as did the sampling using traditional methodologies, with many novel and traditional survey methods being used at many of the same sites during the sample sampling visit.

3. DRONE SURVEYS

3.1 Materials and Methods

Drone surveys.—A DJI Matrice 600 Pro unmanned aerial vehicle was used to conduct drone surveys (Figure 3.1A). A Gremsy T-3 gimbal was attached and slightly modified to accommodate the digital and multispectral cameras that were used (Figure 3.1B). Flights were programmed using the MapsMadeEasy app with flight parameters set at a height of 30 m AGL, 82% overlap between transects, and at a maximum speed of 2.2 m/s (Figure 3.2). These parameters were chosen after a series of test flights to determine the optimal photograph resolution with minimal disturbance on turtle behavior. Flights were conducted in linear transects along a site to assist in photo-stitching. The entire study area was surveyed when possible, amounting to ca. 1.2 ha with 10 m of shoreline surveyed as well to detect basking turtles. This survey area was the maximum area that could be surveyed with one set of batteries. Permitting constraints prohibited the surveying of the Mexican side of the Rio Grande, thus limiting the survey area to the Texas shoreline of the river. On occasion the DJI GSPro app was also utilized to conduct flights. These flights had a front overlap of 55% and a side overlap of 50% with a maximum speed of 2.5 m/s which assisted in photo-stitching efforts. Due to battery limitations, drone surveys with this app consisted of two flights, as the drone would have to return to its launching point for a change of batteries. To minimize biases in turtle detection, we randomly chose whether the drone flight or the visual survey would be conducted first when visiting a site. Additionally, we waited 15 min between the first method chosen and the second survey method in order to allow for potentially startled turtles to return to basking locations. All drone flights were conducted by Amy P. Bogolin and under a Federal Aviation Administration remote pilot license (#4189203).

High Resolution Digital Camera.—A SONY ILCE α6000 E-mount camera with APS-C sensor was attached to the drone via the gimbal (Figure 3.1B). A SONY FE 85 mm F1.8 prime lens and a Platinum 67-mm UV lens filter were attached to the camera to enhance imagery, providing additional zoom and reducing glare from the sun. A GeoSnap Express was attached to the digital camera to control camera triggering, and to provide GPS locations for photographs to use in post-flight processing and analysis. Photographs were taken on a one second interval over the flight duration in both JPEG and ARW format. Prior to each flight, the camera was manually focused to the camera prompt distance of 29 m, the ISO set to 320, the F-stop at 6.3, and the shutter speed at 1/1000. Each photograph covered an area of 46 m, with a pixel size of 1.4 mm/pixel. Post-flight, images were individually analyzed, and all turtles present were identified. In order to differentiate between species, each detected turtle was examined to see if it had any of the unique characteristics of each species. *Pseudemys gorzugi* has distinctive yellow bands on top of its head and webbing between the toes that is a vibrant red-orange color (Figure 3.3, 3.4). At times images were clear enough to depict the concentric circles on the carapace of *P. gorzugi*. *Trachemys scripta* has bright red bands on the head by their tympana and often have bright yellow bands extending down the sides of the carapace (Figure 3.3). However, the red markings are often not very visible in melanistic males, which likely led to some of these individuals being categorized as unknown turtles. *Apalone spinifera* is a solid light gray or tan color, with the vertebrae of the backbone visible through their leathery carapace (Figure 3.4). The head of *A. spinifera* is narrower and they have an elongated, protruding snout, which was also visible in photos (Figure 3.4). A combination of these characteristics was used to determine the identification of each species. Turtles that were unable to confidently be assigned to a species from the photograph were counted but were classified as unknown. With a size of 1.4 mm/pixel, the photograph resolution was sufficient to detect species-specific characteristics. Unidentified turtles were likely due to individuals being obscured by water or vegetation, dapple shade, and wind (due to movement of the camera during flights). Photographs containing turtles were then uploaded onto GoogleMaps utilizing the GPS stamps to determine their proximity to one another. Adjacent photographs were analyzed to determine whether any of the turtles were duplicates from other photographs. This was likely due to the large overlap between transects and high photograph interval rate. Once accounting for duplicate turtles, final counts were determined for each species.

3.2 Results

We conducted 73 drone surveys at 42 unique localities throughout the sampling period (Figure 3.5; Table 3.1). Drone flights conducted with the MapsMadeEasy app were on average 14 min 23 sec in duration and both apps covered on average a survey area of 1.18 ha. A total of 84,441 photographs were collected during drone surveys and 640 turtles were counted from these photographs. *Pseudemys gorzugi* (n = 307), *Trachemys scripta* (n = 93), and *Apalone spinifera* (n = 89), as well as unidentifiable turtles (n = 151), were all detected in drone surveys and the overall identification percentage of turtles from drone surveys was (n = 34) was 82.3% (± 27.8). *Pseudemys gorzugi* was detected at 18 (42.8%) of these unique localities (Figure 3.5; Table 3.1). Overall (n = 42 sites), the mean number of individual turtles (± 1 SD) detected during drone surveys were 4.21 (± 10.18) *P. gorzugi*, 1.29 (± 2.74) *T. scripta*, 1.22 (± 2.66) *A. spinifera*, and 2.07 (± 4.22) unidentifiable turtles (Table 3.1). Site-specific detections for each species are located in Table 2.2. The highest mean number of *P. gorzugi* detected (± 1 SD) was 56 (± N/A) individuals at Rio Grande, spillway below Amistad Dam (Site 24; Figure 3.5; Table 3.1). Including only sites where *P. gorzugi* were detected (n = 18 sites), we observed a mean (± 1 SD) of 9.59 (± 13.68) individuals.

3.3 Discussion

Drone surveys faced numerous logistical challenges regarding permitting and licenses (e.g., issues with flying a drone along an international border), developing optimal camera and flight parameters, and issues and limitations of the equipment (e.g., equipment overheating). However, with time and experience, we were able to address issues that surrounded the use of the drone. Technological advances and increasing familiarity of drone use in scientific studies will likely help prevent many of these issues in the future. Once the protocol was established, the use of drone surveys resulted in high-quality images of turtles that allowed us to count and identify individuals, with seemingly little disturbance on turtles themselves or other wildlife. The benefits from drone-based surveys, such as the ability to document turtles that were not visible from the shoreline, appear to largely outweigh the challenges.

A major benefit of drone surveys was that they resulted in higher identification percentages than from visual surveys. Aerial (dorsal) photographs of turtles resulted in better identification of species-specific characters, such as carapace patterns and coloration on the feet. Though many turtles observed in drone surveys were swimming, these characteristics remained visible with an aerial photograph as opposed to a view from the shoreline in a visual survey. It is likely that identification of turtles will continue to improve with technological advances. Drone surveys were able to document the largest number of *Pseudemys gorzugi* during an individual survey compared to other methodologies. The detection of large *P. gorzugi* populations supports existing literature that this species is considered locally abundant at some sites (Bailey et al. 2014).

4. DRONE HIGHLIGHTS

Once the settings on the drone and camera were optimized, photos from drone surveys produced an abundance of additional data to supplement our baseline data of species identifications and abundance. Photographs from drone surveys documented numerous identifiable behaviors of *P. gorzugi*, such as basking, courtship, and foraging. On 9 March 2019 at the Eagle Pass Golf Course, spillway into Rio Grande, Maverick County, drone surveys captured mass basking of 26 *Pseudemys gorzugi* sharing a single basking rock (Figure 3.6). On repeat visits this behavior was not observed, suggesting that this could be due to seasonality, as basking is more prominent in the cooler spring months. Subaerial basking was also observed on several occasions at numerous sites, which is a common behavior of *P. gorzugi* where individuals bask on top of submerged aquatic vegetation (Figure 3.7). Courting behaviors were captured multiple times during drone surveys and were more prevalent at sites with large *P. gorzugi* populations, such as at the Eagle Pass Golf Course, spillway into Rio Grande, Maverick County and Rio Grande, spillway below Amistad Dam, Val Verde County (Figure 3.8). Courting was observed throughout the sampling period (March–October), suggesting that reproduction could occur over a large portion of the year. Little is known about the specific diet of *P. gorzugi*, and we were able to capture foraging behaviors of *P. gorzugi* with drone imagery (Figure 3.9). In a series of photographs, the drone documented an adult male *P. gorzugi* approaching and consuming a piece of aquatic vegetation that was floating at the surface of the water at TNC Dolan Falls Preserve, Devils River, Dolan Falls, Val Verde County on 27 April 2019 (Figure 3.9).

There were also advantages to drone surveys over other sampling methodologies in its ability to detect turtles where other methods failed. Visibility from the shoreline was often poor due to vegetation and habitat characteristics, however with an aerial viewpoint the drone was able to document several turtles than were not visible from shore (Figure 3.10). The drone was also able to document *P. gorzugi* that were underwater and undetectable through other survey methods (Figure 3.11). The first detection of *P. gorzugi* in Crockett County, a county where *P. gorzugi* has been unreported, was from a drone survey image (Figure 3.12). This observation from the drone survey occurred on 5 June 2019 and guided more extensive sampling at this location on subsequent sampling trips, resulting in the first vouchered specimens from Crockett County (Bogolin et al. 2019b).

The use of drones to survey for other wildlife was also supported by our drone surveys. Throughout the study, numerous species of non-target wildlife were documented in drone imagery, including several species of birds (Figure 3.13), fish (Figure 3.14), and invertebrates (Figure 3.15). Animals seemed to not be disturbed by the presence of the drone flying overhead, with little impact on their behavior. On one occasion we observed an Osprey (*Pandion haliaetus*) catch and consume a fish while the drone was flying overhead. The fine resolution of the imagery allows for even butterflies to be identified by species (Figure 3.15). Additionally, drone-based surveys gathered valuable habitat data. We were able to document several instances where *P. gorzugi* were observed basking near trash, showing that pollution or degraded habitats may not necessarily prevent their occurrence (Figure 3.16). Drone imagery was also able to document tracks in the mud created from turtles moving through shallow water (Figure 3.17). Observation of tracks and other signs of wildlife highlights the potential of drone-based surveys to target suitable habitats used by species, even when they are not directly observed.

5. ENVIRONMENTAL DNA (eDNA)

5.1 Materials and Methods

eDNA Sample Collection.—Water was collected from sites using a 2-l plastic pitcher attached to the end of a telescoping pole. When possible, water was collected at least 2 m from shore and 1 m below the water's surface. We filtered water samples using a 47-mm filter cup that was attached to a PVC arm and inserted into a hand-powered automotive fluid evacuator (Figure 3.17). Water samples were filtered using 47-mm Whatman Grade 4 cellulose filters that have a pore size of ca. 25 microns. To begin, a field blank sample was collected by filtering 1 l of DI water through a filter. Afterwards, three more field samples were collected by filtering at least 2 l of site water through a filter. Occasionally, filters began to clog prior to having filtered 2 l of water and we recorded the total volume of water that was filtered. After water had been filtered through each filter, the filter was folded and placed in sterile 2 ml microcentrifuge tubes containing 700 µL of DNAzol (Molecular Research Center, Inc.), a DNA buffer/extraction solution. Before initial sampling and between sites, all water collection and filtering equipment was cleaned with a 50% bleach solution, rinsed with a sodium thiosulfate wash to neutralize the bleach, and rinsed with DI water; nitrile gloves were worn throughout eDNA sample collection and changed between sites.

Filter Extraction.—Filters were stored in DNAzol for a minimum of 3 d at room temperature before being extracted following a modified protocol from the DNAzol manual. The sample was heated at 55°C for 30 min, vortexed, and centrifuged 1 min at 5000 *g*. The filter was then removed from the tube and squeezed using forceps (cleaned with a 50% bleach solution) to retain all fluid (DNAzol) from the filter in the microcentrifuge tube. Afterwards, we added 500 µl of chloroform and vortexed the samples, then let the samples rest for 1 min before centrifuging at 12000 *g* for 2 min. We extracted the supernatant into a clean 1.5 ml microcentrifuge tube and added 500 µl of absolute ethanol, inverted until mixed, and centrifuged for 10 min at 16000 *g* to pellet out the DNA. The supernatant was then discarded. The DNA pellet was washed with 500 µl 95% ethanol, vortexed, and centrifuged for 1 min at 5000 *g*, before we discarded the supernatant. This step was repeated with 500 µl 75% ethanol. The pellet was then air dried for at least 30 min before being dissolved in 22 µl of 30% TE buffer at 55°C. A subset of extracted samples was quantified for total DNA concentration using a Qubit Flourometer (Invitrogen) following the procedure outlined in the manual, then stored at -20°C until analyzed. All eDNA extractions took place in a separate clean lab away from where PCRs occurred to help prevent contamination of samples (Goldberg et al. 2016). The benchtop and micropipettors were cleaned before extractions, and only sterile filter pipette tips were used. Nitrile gloves were worn throughout the extraction procedure.

Primer Design.—Forward and reverse oligomer primers were designed in Geneious v11.0.1 using publicly-available nucleotide sequences (GenBank: HQ329656.1). Primer design was completed in Primer3, with the goal of developing primer sets that are specific to *P. gorzugi* with similar melting temperatures and minimal dimer formation. Polymerase chain reaction (PCR) methods were used to amplify both tissue DNA and eDNA and follow the methods outlined below. Primer specificity was confirmed by screening designed primers against tissue sample extracts from *P. gorzugi* and two abundant sympatric species, the Pond Slider (*Trachemys scripta*; DRD 6170 [Fort

Clark Springs, Las Moras Creek]) and Spiny Softshell (*Apalone spinifera*; DRD 6289 [Casa Blanca Lake, Lake Casa Blanca International State Park, Webb County, Texas, USA]). Afterwards, primers were tested against a positive-control eDNA sample to ensure that selected primers could detect eDNA in more dilute field conditions. This positive-control eDNA sample was generated by placing a juvenile *P. gorzugi* (Chandler Ranch, cement pond, Terrell County, Texas, USA) in ca. 8 l of water collected from TNC Independence Creek Preserve, Lower Lake, Terrell County, Texas, USA for 48 h. Results from PCR assays were visualized using gel electrophoresis in a 1% agarose gel in TBE buffer at 100 V for 45 min. All samples were run against a 100 base-pair ladder and a no-template control (NTC) to ensure no contamination had occurred.

eDNA Assays.—eDNA samples were run through an initial and nested round of PCR, both of which were optimized specifically for use with *P. gorzugi* eDNA. For both rounds of PCR, the total volume of the reactions was 25 µl and consisted of 12.5 µl GoTaq G2 HotStart MasterMix, 5.5 µl water, 1 µl 10 µM forward primer, 1 µl 10 µM reverse primer, and 5 µl of sample (or 5 µl water for the NTC). For the initial PCR, the sample consisted of the 5 µl of the filter extract. For the nested PCR, the sample consisted of 5 µl of the purified product from the initial PCR. To purify the product from the initial PCR, we used Monarch PCR & DNA Cleanup Kits following a modified protocol. The optimized protocol for the initial PCR was as follows: hot start (94°C), initial denaturing (94°C for 3 min), followed by 35 cycles of denaturing (94°C for 30 sec), annealing (57°C for 30 sec), and extension for (72°C for 30 sec), and afterwards, a cooling period of 4°C for 10 min. The nested PCR protocol was similar to the initial PCR protocol except that it was run for 38 cycles with an annealing temperature of 55°C and the cooling period was 4°C for 5 min. After the nested PCR, we purified the products again using Monarch PCR & DNA Cleanup Kits and quantified them for total DNA concentration using a Qubit Fluorometer following the procedure outlined in the manual. Samples that had total DNA concentrations that were unmeasurable by the Qubit Fluorometer were considered eDNA-negative and samples that had measurable total DNA concentrations were mailed off for sequencing. Samples were sequenced using Sanger sequencing methods at Eurofins Genomics LLC (Louisville, Kentucky, USA). Sequence data was then compared to sequence data available on GenBank using the BLAST query and aligned with a known *P. gorzugi* sequence (GenBank: KC687314) to designate samples as eDNA-positive.

5.2 Results

The initial PCR primer set amplified a 155-bp sequence, and the nested PCR primer set amplified a 118-bp sequence, both in the CO1 region (Table 3.2). A total of 42 unique sites were chosen to validate the *Pseudemys gorzugi* eDNA assay (Table 3.3). These sites included those where *P. gorzugi* was abundant and detected using several different survey methodologies and sites where no individuals were observed. Additionally, these sites ranged across the full geographic extent of where surveys were conducted during the survey period. The mean volume of water (± 1 SD) filtered through eDNA filters was 1650 ± 690 ml. We detected *P. gorzugi* eDNA at 22 sites and failed to detect eDNA at 20 sites (Figure 3.19; Table 3.2). Included in sites where we detected *P. gorzugi* eDNA was the Pecos River at US Hwy 190 crossing, Pecos County (Site 1) the northernmost detection of *P. gorzugi* resulting from this study, and the Rio Grande, near the National Butterfly Center, Hidalgo County (Site 59; Figure 3.19; Table 3.2) the southernmost detection of *P. gorzugi* resulting from this study.

5.3 Discussion

Environmental DNA (eDNA) surveys faced few implementation challenges in the field and we filtered water at almost every site we sampled. Only one site prevented the collection of eDNA samples: Pump Canyon, Langtry, Val Verde County. At this site we could not access the water without boat access on the Rio Grande and steep canyon walls prevent us from accessing it from shore. Total volume of water able to be filtered varied among sites due to some sites being highly turbid (e.g., most Rio Grande sites) or with high amounts of algae (e.g., Eagle Pass Golf Course, settling pond along Rio Grande). At other sites, the target volume (2 l) was easily filtered due to clear, often spring-fed, water and low turbidity. The major disadvantage to eDNA surveys is that only presence/absence information can be generated and not abundance data.

We validated our eDNA survey protocol by screening 42 sites for *P. gorzugi* eDNA. The samples were collected from March–October and covered the full geographic extent of our sampled sites. Results from eDNA assays at most of the sites included in this validation matched our expectations. At sites where *P. gorzugi* was observed, we had positive eDNA detection and at sites where *P. gorzugi* was never observed we had no eDNA detection. Occasionally, we did not detect *P. gorzugi* eDNA at sites where they were consistently detected. Usually, these sites where eDNA assays results did not match other survey results were spring-fed or associated with urban development. It may be possible that dilution due to spring outflow may dilute eDNA and that contaminants present from urban discharge may degrade eDNA at a faster rate, making detections more difficult. Future work with *P. gorzugi* eDNA should investigate these issues further.

Assays indicated the presence of *P. gorzugi* eDNA at two sites where turtles were never observed through any of the other survey methods. We detected *P. gorzugi* eDNA at our northwestern-most site (Pecos River, at US Hwy 190 Crossing, Pecos County) and one of our southeastern sites (Rio Grande, near National Butterfly Center, Hidalgo County). These two positive detections of *P. gorzugi* eDNA represent the furthest northwest and furthest southeast detection of this species within our sampled sites and in recent decades. *Pseudemys gorzugi* is known to occur in the Delaware River in Texas along the New Mexico border (Bonner and Littrell 2016), further upstream of the Pecos River drainage from our northwestern-most eDNA detection. There is a ca. 160-km gap between these two localities and the presence of *P. gorzugi* eDNA may suggest the presence of unreported populations between these two sites. The positive eDNA detection at the Rio Grande, near National Butterfly Center is ca. 92 river-km downriver from observations of *P. gorzugi* individuals in the Rio Grande, near Rio Grande City, and similarly, the detection of *P. gorzugi* eDNA may suggest additional unrecognized populations are located nearby. The positive eDNA detection at these sites may be attributed to eDNA drifting downstream from populations that exist upstream nearby. Many factors can affect eDNA degradation such as sunlight, temperature, and microbes, and it is currently unknown how far eDNA can travel in these systems before it becomes too degraded for detection. Future studies should attempt to better understand the longevity of eDNA in these systems in order to better understand how geographically proximate sites need to be for water flow to result in detections further downstream.

No *P. gorzugi* individuals were observed at either of these sites and it may be that these are unsuitable habitat for this species. The physical appearance of the Rio Grande, near National Butterfly Center site is similar to the Rio Grande further upriver (e.g., Rio Grande, near Salineño) and it appears as though it should be suitable habitat for *P. gorzugi*. Additional samples from additional sites not previously screened and from samples from repeat sampling visits will help to better understand these issues with eDNA detection, and this work is on-going.

6. SURVEY METHODS COMPARISON

6.1 Materials and Methods

One of the goals of this project was to determine which methodology was the most effective at surveying for *Pseudemys gorzugi*. This was determined by comparing number of total turtles detected, identification percentage, and number of *P. gorzugi* detected among visual, trap, and drone surveys. Environmental DNA analyses could not be included in this comparison due to the absence of abundance data with this method. Sampling effort and units varied among methodologies, which made comparisons between methods difficult. In order to make these comparisons, we assumed that our target survey effort for each of these three survey methods were standard and generally acceptable in the field. Our target effort for each survey method was an area of 1.25 ha (ca. 15 min of flight time) for drone surveys, 15 min duration for visual surveys, and three traps, each deployed for 48 h, for trapping efforts. Any surveys not meeting these conditions were not included in the comparison. Additionally, sites where turtles were never detected in any survey methodology were removed from the comparison to avoid our data becoming zero heavy. Turtle abundance counts and identification percentages were averaged by site to avoid pseudoreplication in our final dataset. The data was non-normally distributed and groups (survey methods) had unequal variance. As a result, non-parametric analyses ($\alpha = 0.05$), primarily Kruskal-Wallis test and Wilcoxon multiple comparisons, were conducted on the final dataset. All analyses were conducted in JMP v14.

6.2 Results

We did not detect a significant difference in the total number of turtles detected among survey types ($H = 2.55$, $df = 2$, $p = 0.28$; Figure 3.20). We did detect a significant difference in identification percent among survey methods ($H = 42.94$, $df = 2$, $p < 0.0001$; Figure 3.21). The identification percent for trapping was significantly higher than both the drone ($p = 0.0002$) and visual surveys ($p < 0.0001$) and the identification percent was higher for drone surveys compared to visual surveys ($p < 0.0001$; Figure 3.21). Finally, we did not detect a significant difference in the number of *Pseudemys gorzugi* detected among survey methods ($H = 1.93$, $df = 2$, $p = 0.38$; Figure 3.22).

6.3 Discussion

Even though we did not detect significant differences in total number of turtles detected and the number of *Pseudemys gorzugi* detected across survey methodologies, the mean values for drone surveys were much higher than those from trapping and visual surveys. Non-parametric comparisons are rank-based and as a result, the magnitude of difference between values is obscured. Further, high variance in count data from drone surveys likely helps obscure true differences among sampling methods. At sites where the number of turtles detected were large, drone surveys resulted in much higher numbers of detections. For example, on 2 October 2019 at Rio Grande, spillway below Amistad Dam, Val Verde County, we detected a total of 80 unique turtles (n = 56 *P. gorzugi*) during the drone survey compared to 10 turtles (n = 0 *P. gorzugi*) in the visual survey and 6 turtles (n = 0 *P. gorzugi*) trapped. This pattern is similar from a trip to TNC Dolan Falls Preserve, Devils River, Dolan Falls, Val Verde County on 19 September 2019, when 66 unique turtles (n = 55 *P. gorzugi*) were detected during the drone survey compared to 18 (n = 9 *P. gorzugi*) turtles during the visual survey and only one turtle (n = 0 *P. gorzugi*) trapped. The highest number of turtles ever detected during a visual survey was 28 (4 October 2019; Del Rio, San Felipe Golf Course, San Felipe Creek) and the highest number of turtles ever detected through trapping was 18 (1 July 2019; Eagle Pass Golf Course, settling pond along Rio Grande). At sites when total turtle detections are low, all survey methods appear to perform similarly.

It is not surprising that trapping resulted in the highest identification percentage (100%), as when turtles are in hand, a confident identification can consistently be made. However, what remains informative is that drone surveys had a significantly higher turtle identification percentage than visual surveys. This significant difference highlights the potential use of drones for turtle surveys and suggests that drone surveys may be superior to visual surveys in the detection of other wildlife. We continue to explore our data and which survey method may perform best under specific environmental or site conditions, such as when turbidity is high or when sites are associated with springs.

Figure 3.1. Drone and equipment used for drone surveys of *Pseudemys gorzugi*. A) DJI Matrice 600 Pro unmanned aerial vehicle with survey equipment attached used to survey for *Pseudemys gorzugi*; B) Gremsy T-3 gimbal with the MAIA multi-spectrometer and digital camera attached. This configuration ensured equal weight distribution to assist in keeping the cameras level during flight.

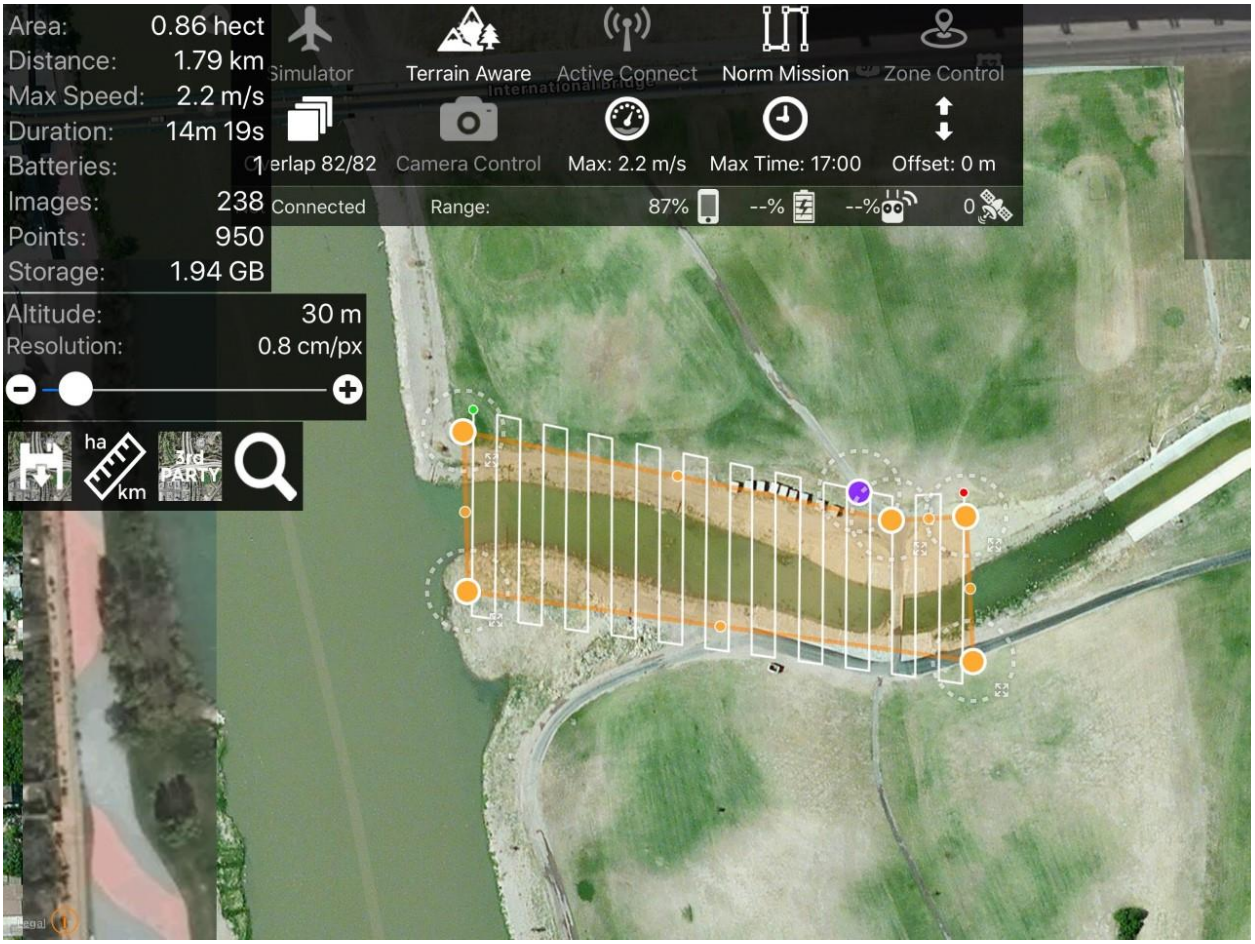

Figure 3.2. A screenshot MapsMadeEasy, the primary app used to conduct drone flights during this project. Basic flight parameters and the drone flight path are depicted for a flight conducted at Eagle Pass Golf Course, spillway into Rio Grande, Maverick County.

Figure 3.3. Drone image of a *Pseudemys gorzugi* and *Trachemys scripta* basking in the Rio Grande, near Salineño, Starr County. Inset: magnified view of two turtles basking, *P. gorzugi* on the top and *T. scripta* on the bottom.

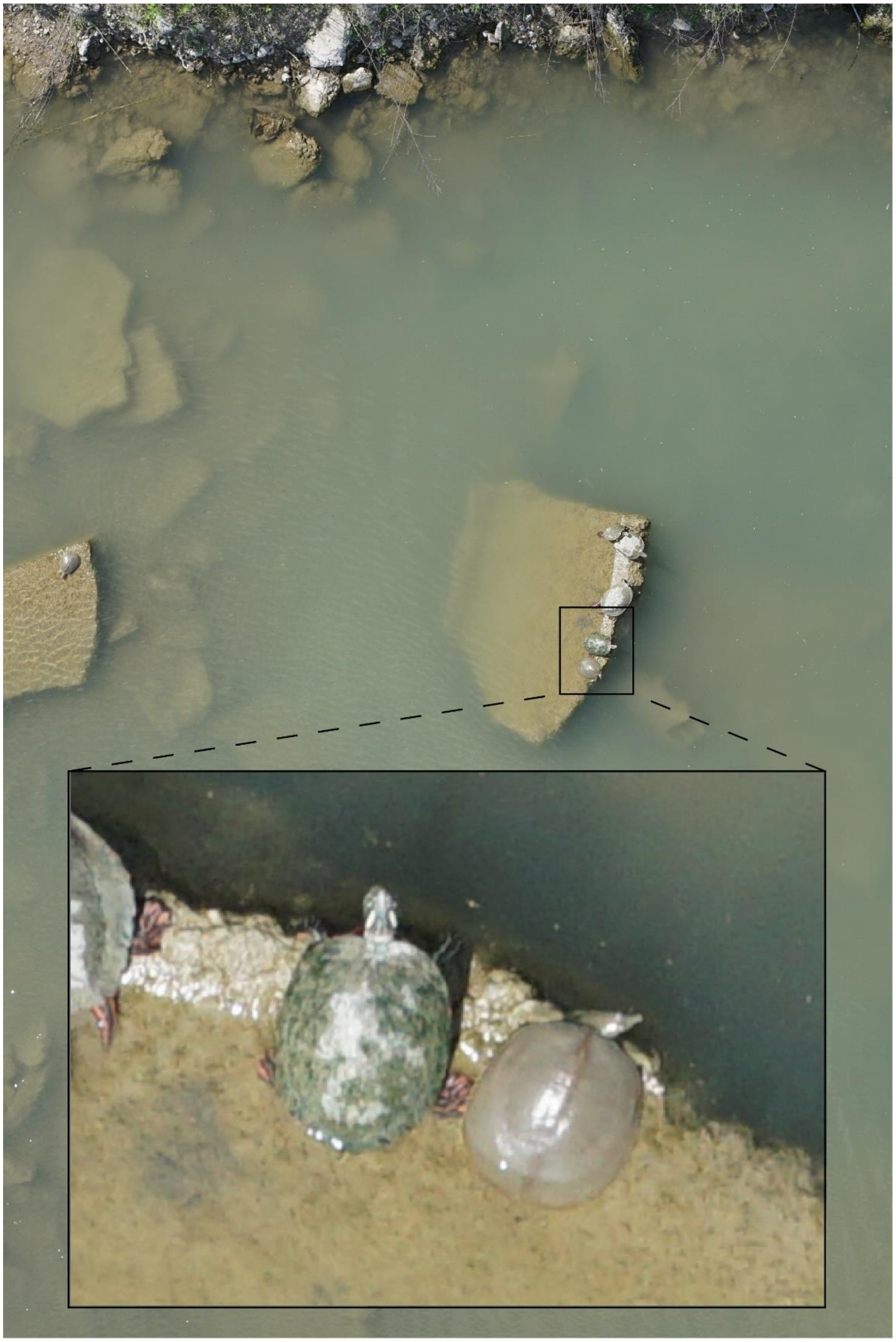

Figure 3.4. Drone image of a *Pseudemys gorzugi* and *Apalone spinifera* basking at Eagle Pass Golf Course, spillway into Rio Grande, Maverick County. Inset: magnified view of two turtles basking, *P. gorzugi* on the left and *A. spinifera* on the right.

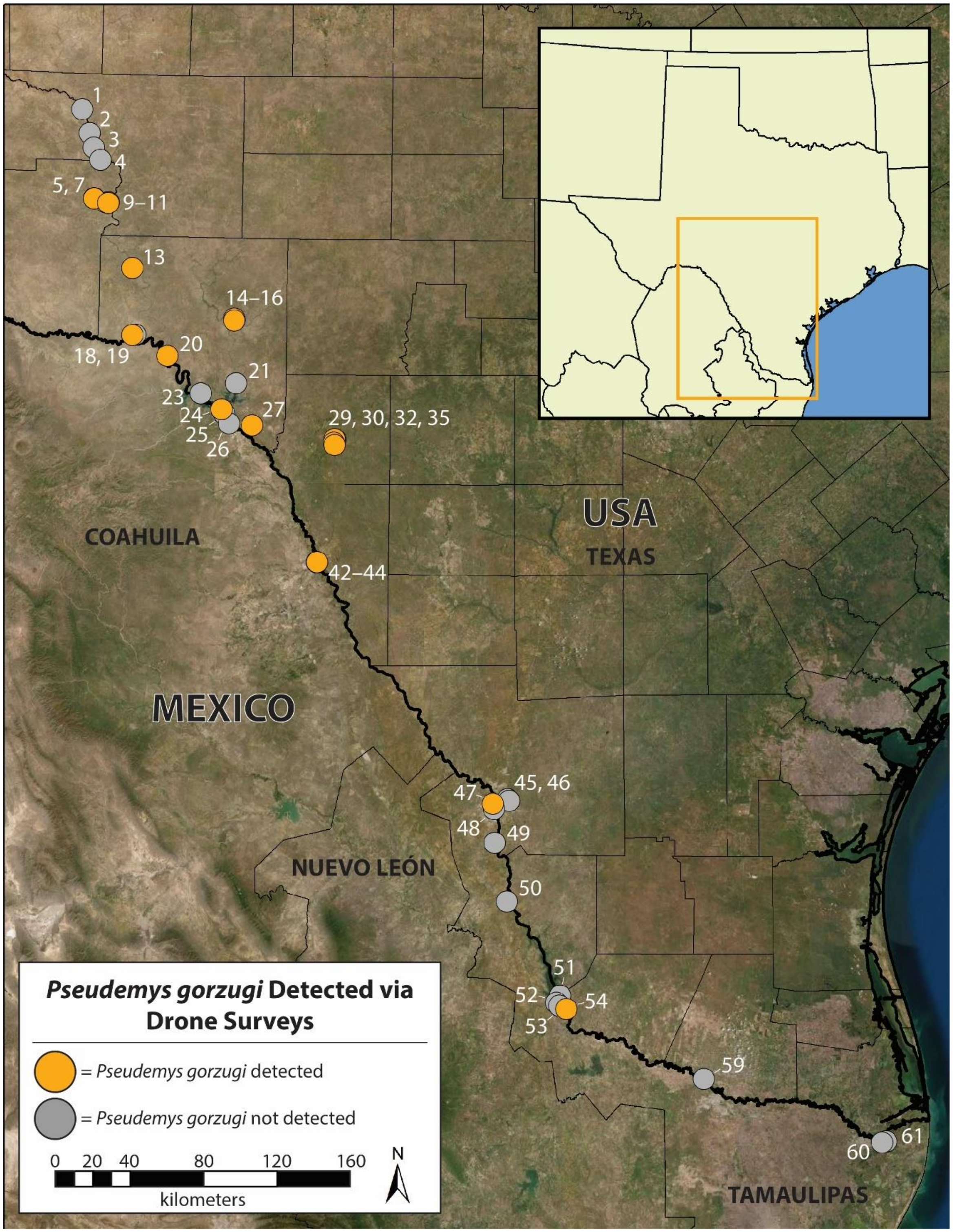

Figure 3.5. Map of 42 sites where drone surveys were conducted for *Pseudemys gorzugi* in southwestern Texas, USA. Sites where *P. gorzugi* was positively detected are indicated in orange. Sites where *P. gorzugi* was not detected are indicated in gray. Site numbers correspond to those used in Table 3.1.

Figure 3.6. Drone image from Eagle Pass Golf Course, spillway into Rio Grande, Maverick County showing mass basking of *Pseudemys gorzugi*. The photograph captured 27 *Pseudemys gorzugi*, of which 26 were basking on a single rock.

Figure 3.7. Drone image showing subaerial basking of several *Pseudemys gorzugi* on dense aquatic vegetation at Del Rio, San Felipe Springs Golf Course, San Felipe Creek, Val Verde County.

Figure 3.8. Drone image showing courtship behaviors between two pairs of *Pseudemys gorzugi* in the Rio Grande, spillway below Amistad Dam, Val Verde County.

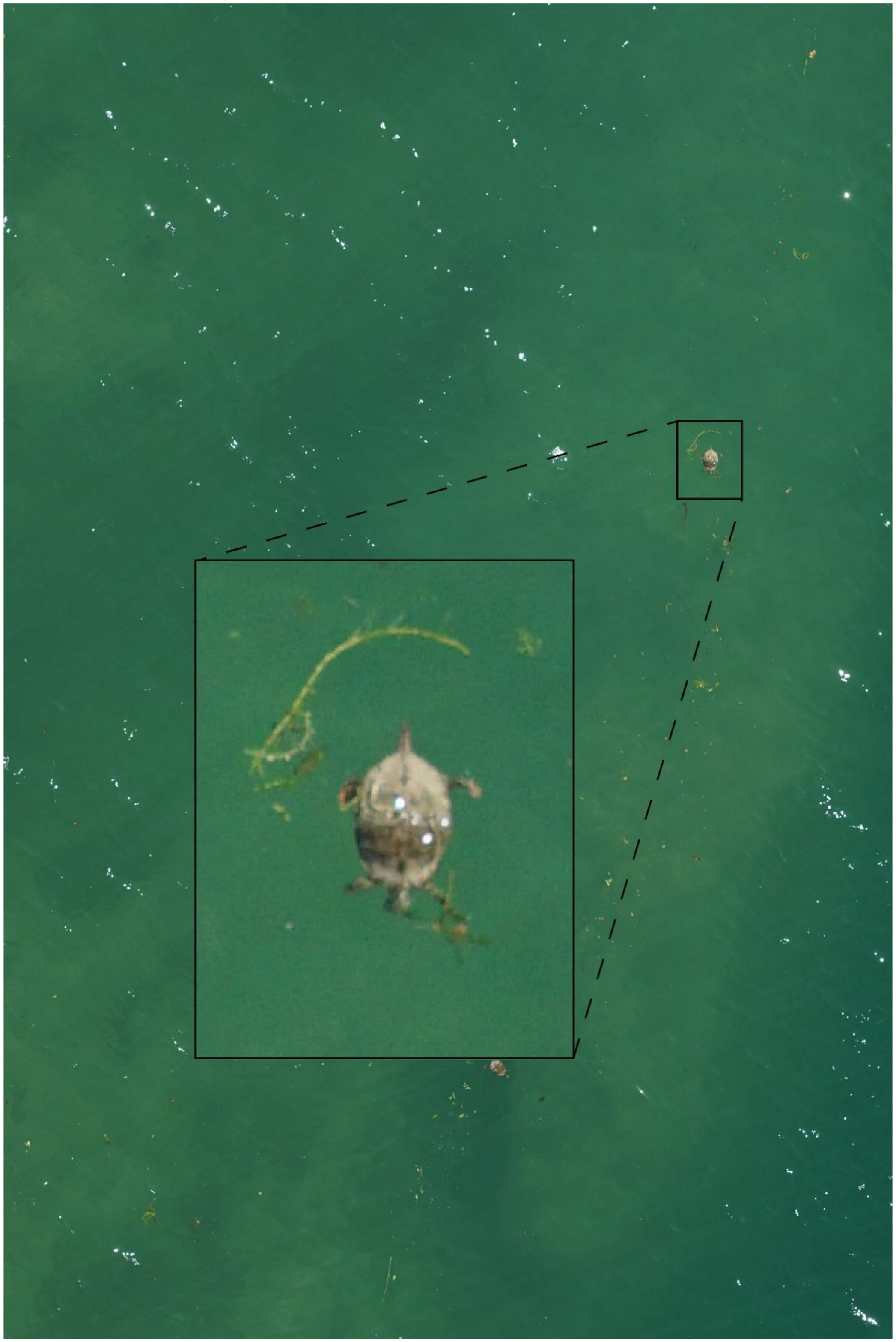

Figure 3.9. Drone image showing an adult *Pseudemys gorzugi* foraging on a piece of aquatic vegetation at TNC Dolan Falls Preserve, Devils River, Dolan Falls, Val Verde County. Inset: magnified view of foraging *P. gorzugi*.

Figure 3.10. Drone image of three adult *Pseudemys gorzugi* (white circles) and one unidentified turtle (black circle) from the Rio Grande, Laredo, near water treatment center, Webb County. These turtles were not visible from shore and this drone image was our first record of *P. gorzugi* at this site.

Figure 3.11. Drone image of an adult *Pseudemys gorzugi* (white circle) from Fort Clark Springs, Las Moras Creek, Buzzard Roost, Kinney County, that was under water and not visible from the shoreline during visual surveys.

Figure 3.12. Drone image of the first *Pseudemys gorzugi* (white circle) documented in Crockett County, Texas. This individual was observed in the Pecos River, 0.8 river km upstream of confluence with Independence Creek. This drone image allowed us increase trapping efforts in this area, which resulted in additional detections of *P. gorzugi* in subsequent sampling trips.

Figure 3.13. Drone image of five Black-bellied Whistling Ducks (*Dendrocygna autumnalis*) perched on a log at Fort Clark Springs, Las Moras Creek, Buzzard Roost, Kinney County, which were undisturbed by the drone flying directly overhead.

Figure 3.14. Drone image of native and introduced fish (Cypriniformes) during a survey at Fort Clark Springs, Headwater Pond, Kinney County, showing the potential of drone surveys to target different species.

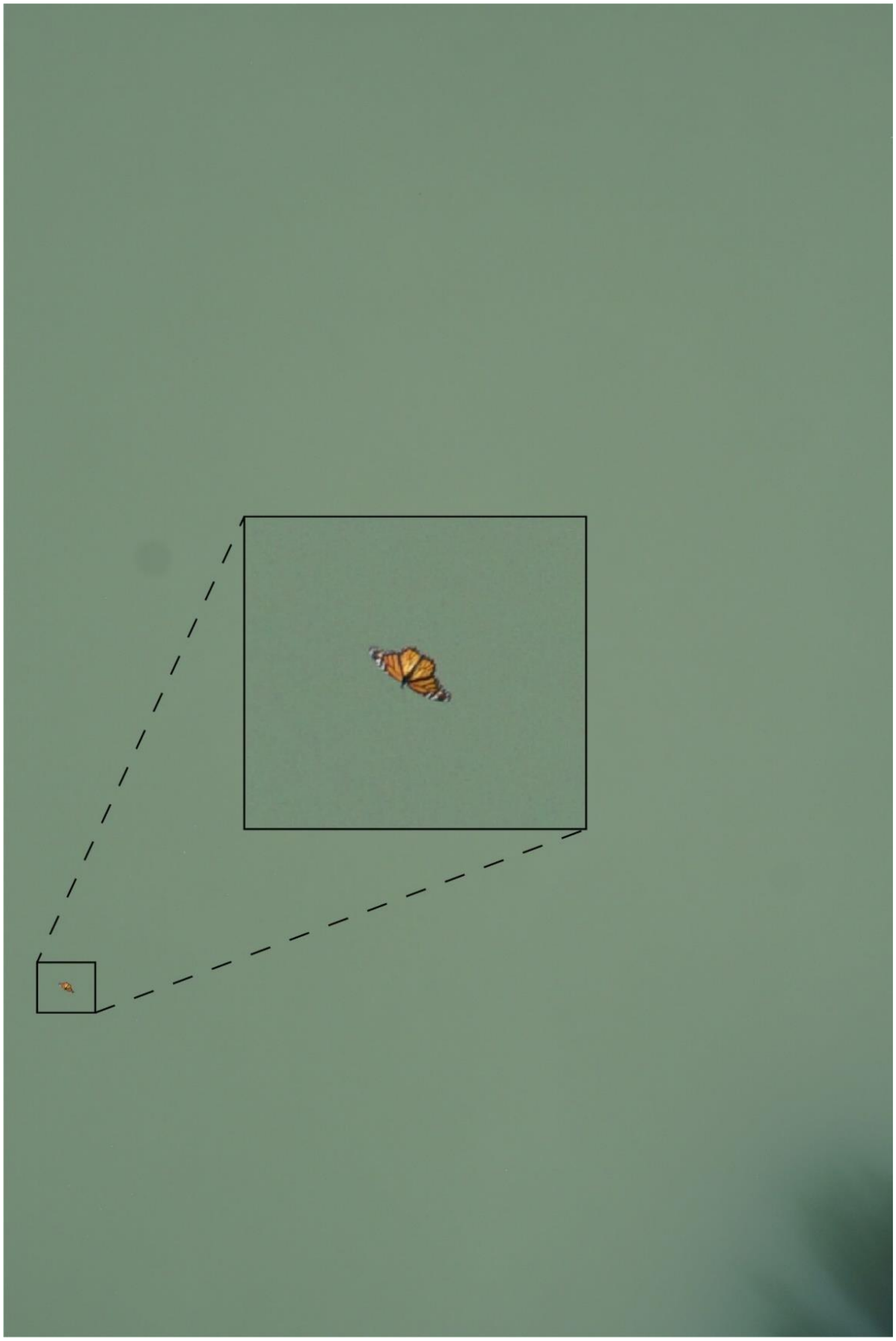

Figure 3.15. Drone surveys resulted in images of insects that were identifiable to species such as this Monarch Butterfly (*Danaus plexippus*) from drone surveys along the Pecos River, 0.3 river km upstream of confluence with Independence Creek, Crockett County. Inset: magnified view of the Monarch Butterfly.

Figure 3.16. Drone image of five basking and swimming adult *Pseudemys gorzugi* (white circles) and one unidentified turtle (black circle) from the Eagle Pass Golf Course, spillway into Rio Grande, Maverick County. This site, like several other sites where *P. gorzugi* was observed, was littered with trash, and occurs along degraded habitat (manicured lawns of a golf course).

Figure 3.17. Drone image of visible turtle tracks left in the muddy bottom of Pump Canyon, Langtry, Val Verde County showing the potential use of drone surveys to locate habitats used by turtles.

Figure 3.18. Environmental DNA (eDNA) filtering equipment, including a plastic pitcher on the end of a telescoping pole, 47-mm filter cup, and a hand-powered automotive fluid evacuator.

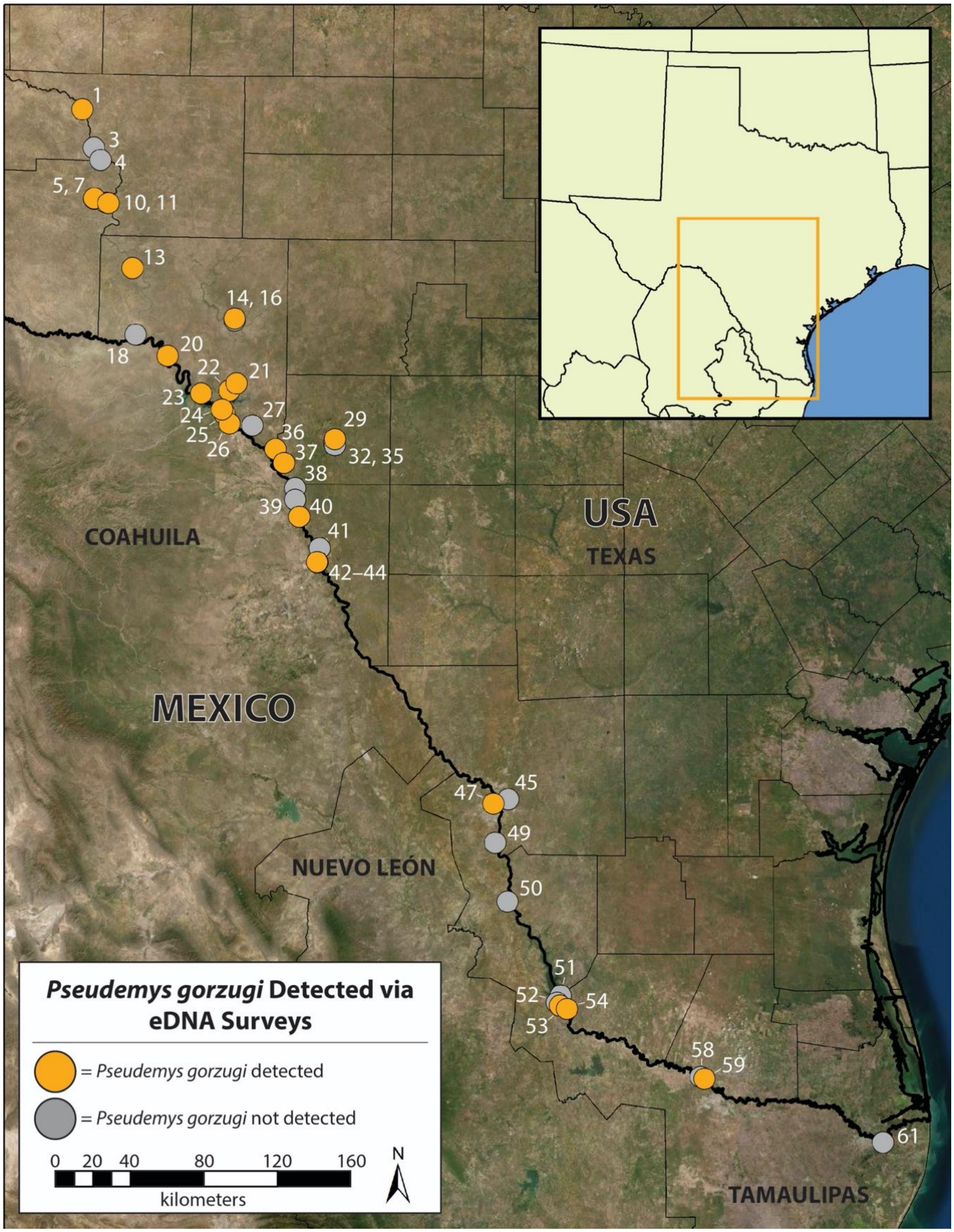

Figure 3.19. Map of 42 sites where samples were analyzed for *Pseudemys gorzugi* environmental DNA (eDNA) in southwestern Texas, USA. Sites where *P. gorzugi* was positively detected are indicated in orange. Sites where *P. gorzugi* was not detected are indicated in gray. Site numbers correspond to those used in Table 3.3.

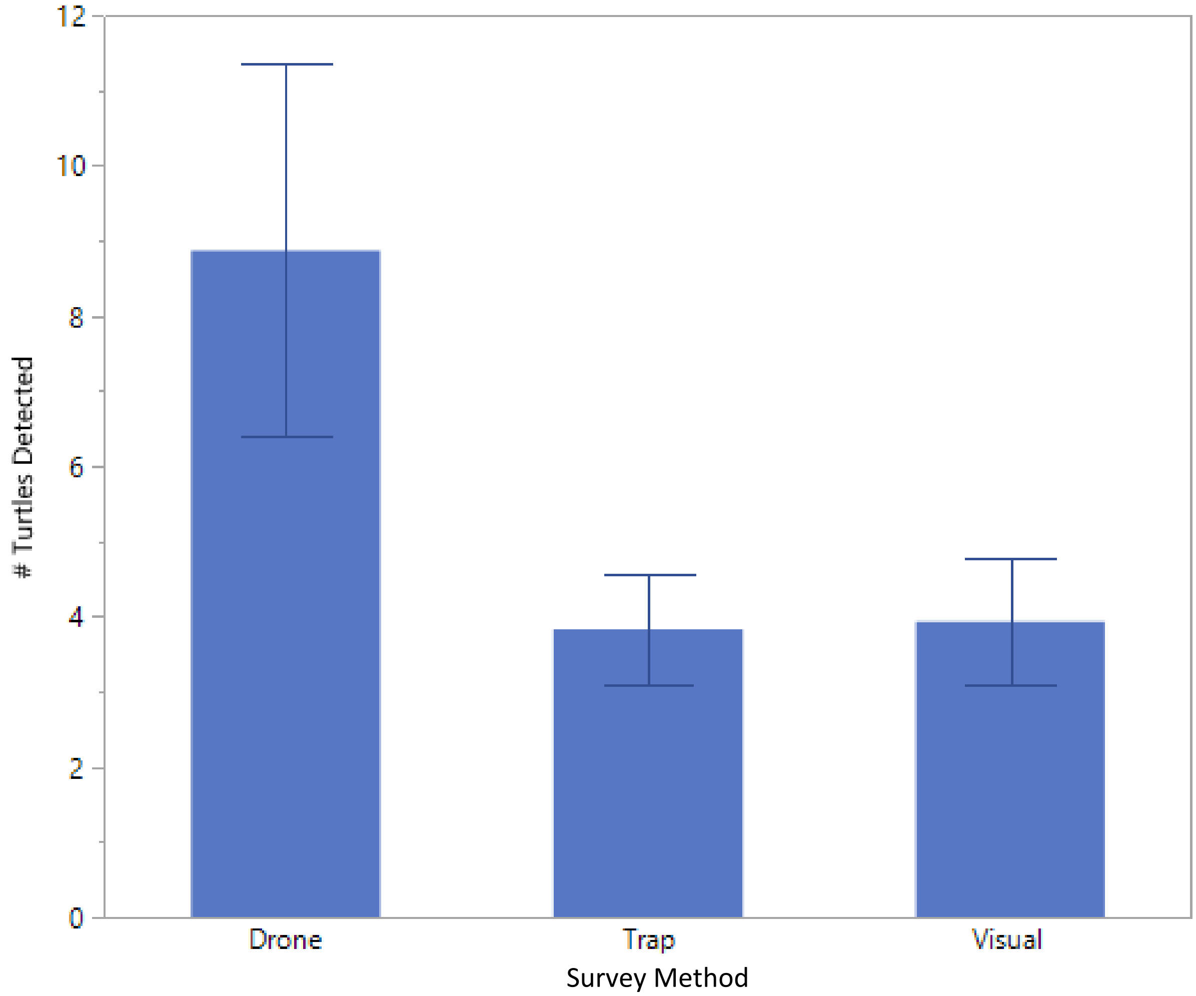

Figure 3.20. Mean (± 1 SE) number of total turtles detected during drone, trap, and visual surveys.

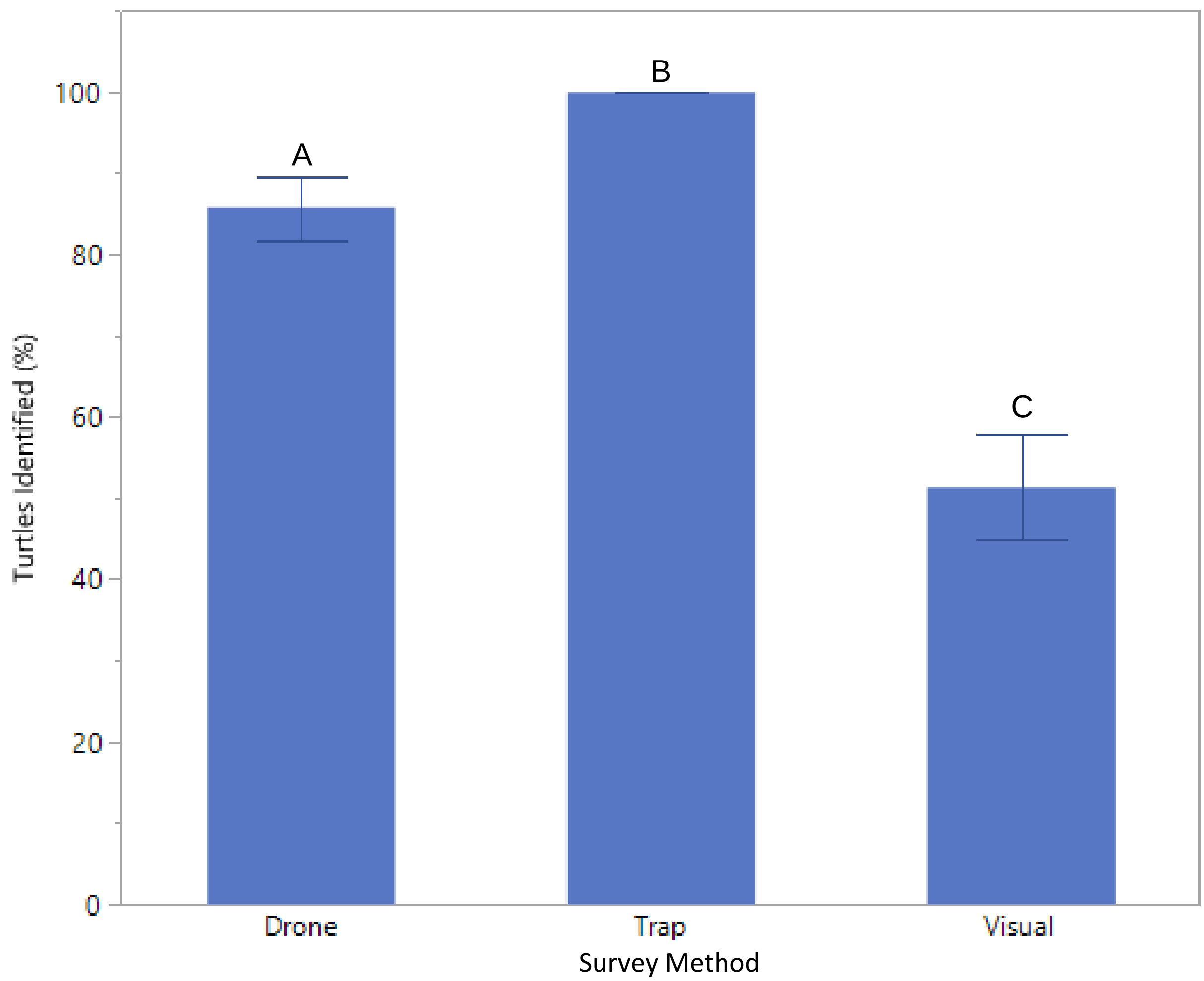

Figure 3.21. Mean (± 1 SE) turtle identification percentage during drone, trap, and visual surveys. Letters indicate groupings from Wilcoxon multiple comparison tests ($\alpha = 0.05$).

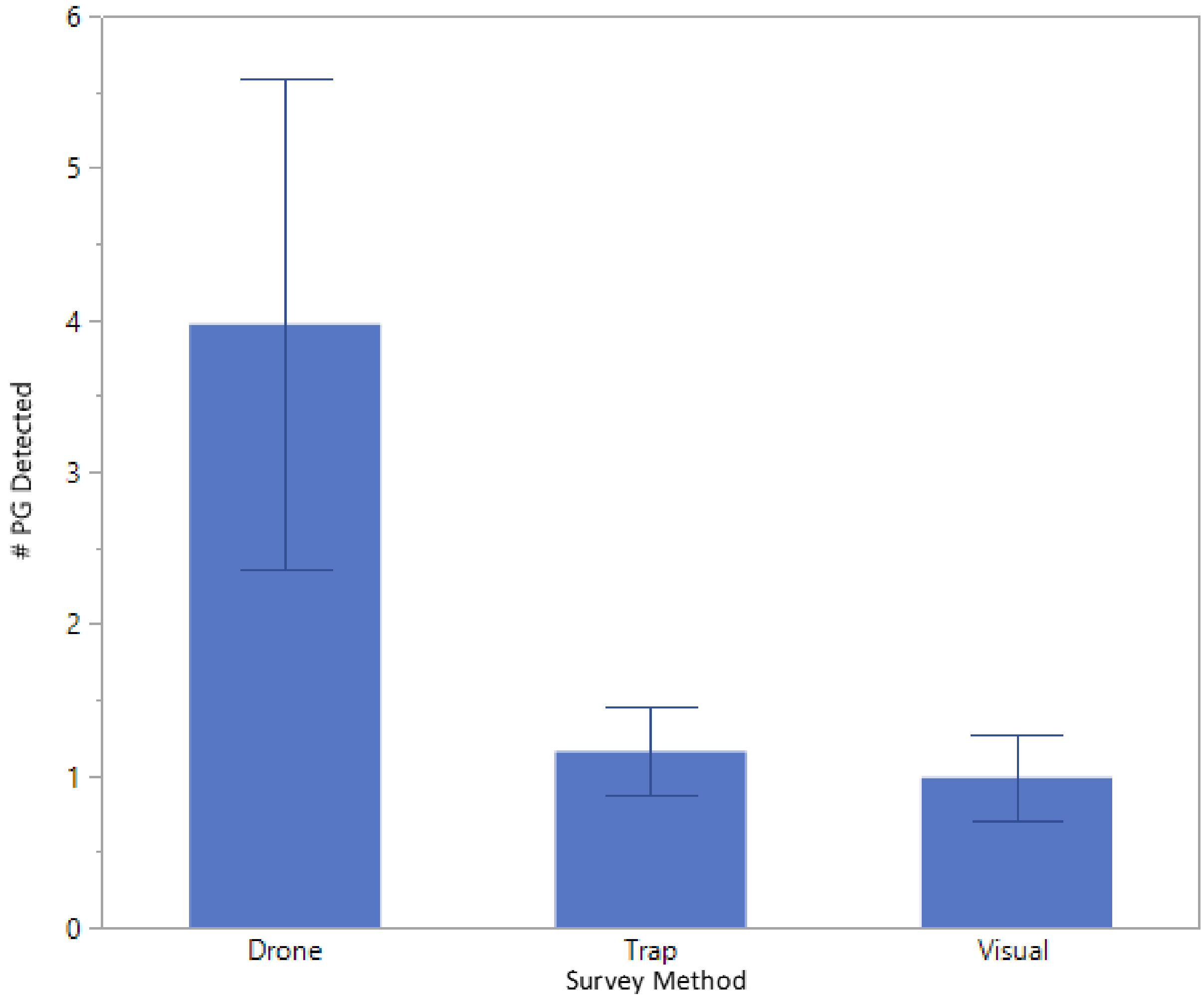

Figure 3.22. Mean (± 1 SE) number of *Pseudemys gorzugi* detected during drone, trap, and visual surveys.

Table 3.1. Mean (± 1 SD) number of three species of turtles (*Pseudemys gorzugi*, *Trachemys scripta*, *Apalone spinifera*), as well as unidentified turtles, observed in drone surveys at sampled sites. Site locality information, number of visits, *Pseudemys gorzugi* (PG) detection, and mean (± 1 SD) percent identification of observed turtles is also provided (ID %). Site numbers correspond to those used in Table 2.1.

Site #	County	Site	Latitude	Longitude	# of Visits	PG Detected	*Pseudemys gorzugi*	*Trachemys scripta*	*Apalone spinifera*	Unidentified	ID %
1	Pecos	Pecos River, at US Hwy 190 crossing	30.90516	-101.88080	2	no	0 (± 0)	1.0 (± 0)	0.5 (± 0.7)	9.3 (± 1.5)	71.3 (± 13.2)
2	Pecos	Pecos River, at Texas Rock Rd (Crockett Co Rd 306)	30.78851	-101.83502	2	no	0 (± 0)	0.5 (± 0.7)	0 (± 0)	10.0 (± N/A)	73.0 (± N/A)
3	Pecos	Pecos River, at I-10 crossing	30.71808	-101.80954	1	no	0 (± N/A)	2.0 (± N/A)	0 (± N/A)	4.5 (± 3.5)	86.3 (± 5.3)
4	Pecos	Pecos River, at TX Hwy 290 crossing	30.65960	-101.77020	1	no	0 (± N/A)	1.0 (± N/A)	0 (± N/A)	1.0 (± 0)	41.7 (± 58.9)
5	Terrell	TNC Independence Creek Preserve, Lower Lake	30.46955	-101.80131	2	yes	4.5 (± 3.5)	1.0 (± 1.4)	2.0 (± 1.4)	12.0 (± 8.0)	53.4 (± 24.6)
7	Terrell	TNC Independence Creek Preserve, raceway below Upper Lake	30.46736	-101.80181	1	yes	3.0 (± N/A)	0 (± N/A)	0 (± N/A)	2.0 (± 0)	82.6 (± 6.9)
9	Crockett	Pecos River, 0.8 river km upstream of confluence with Independence Creek	30.45259	-101.71940	1	yes	1.0 (± N/A)	1.0 (± N/A)	4.0 (± N/A)	0 (± N/A)	100.0 (± N/A)
10	Terrell	Independence Creek, at County Road crossing	30.45026	-101.73124	2	no	0 (± 0)	0 (± 0)	0.5 (± 0.7)	2.3 (± 2.1)	78.0 (± 22.2)
11	Crockett	Pecos River, 0.3 river km upstream of confluence with Independence Creek	30.44767	-101.72119	2	yes	1.0 (± 0)	0 (± 0)	2.5 (± 0.7)	0 (± 0)	100.0 (± N/A)
13	Val Verde	Pecos River, at Pandale crossing	30.13120	-101.57450	2	yes	3.0 (± 1.4)	0 (± 0)	0 (± 0)	0 (± 0)	–
14	Val Verde	TNC Dolan Falls Preserve, Devils River, upstream of confluence with	29.89387	-100.99561	2	yes	1.5 (± 2.1)	0.5 (± 0.7)	0 (± 0)	1.0 (± 1.4)	0 (± N/A)
15	Val Verde	TNC Dolan Falls Preserve, Dolan Creek, near confluence with Devils	29.88591	-100.99292	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± N/A)	100.0 (± N/A)
16	Val Verde	TNC Dolan Falls Preserve, Devils River, Dolan Falls	29.88385	-100.99397	3	yes	29.0 (± 22.5)	0 (± 0)	0 (± 0)	0 (± N/A)	100.0 (± N/A)
18	Val Verde	Rio Grande, near Langtry	29.80564	-101.55088	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± 0)	100.0 (± 0)
19	Val Verde	Pump Canyon, Langtry	29.80343	-101.56750	1	yes	4.0 (± N/A)	0 (± N/A)	2.0 (± N/A)	0 (± N/A)	100.0 (± N/A)
20	Val Verde	Pecos River, near confluence with Rio Grande	29.70431	-101.36667	1	yes	1.0 (± N/A)	0 (± N/A)	5.0 (± N/A)	0 (± N/A)	–
21	Val Verde	Lake Amistad, Rough Canyon	29.57490	-100.97809	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	100.0 (± 0)
23	Val Verde	Lake Amistad, Box Canyon	29.52420	-101.17585	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	100.0 (± N/A)
24	Val Verde	Rio Grande, spillway below Amistad Dam	29.44737	-101.05667	1	yes	56.0 (± N/A)	0 (± N/A)	8.0 (± N/A)	0 (± N/A)	100.0 (± N/A)
25	Val Verde	Rio Grande, weir below Amistad Dam	29.42455	-101.04118	1	no	0 (± N/A)	3.0 (± N/A)	0 (± N/A)	0 (± 0)	100.0 (± 0)

26	Val Verde	Rio Grande, near Lugo property	29.37719	-101.01348	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± N/A)	100.0 (± N/A)
27	Val Verde	Del Rio, San Felipe Springs Golf Course, San Felipe Creek	29.37029	-100.88526	3	yes	11.7 (± 9.3)	5.3 (± 3.2)	10.7 (± 4.6)	13.0 (± N/A)	31.6 (± N/A)
29	Kinney	Fort Clark Springs, Headwater Pond	29.30944	-100.42125	3	yes	9.0 (± 1.7)	3.0 (± 3.0)	0 (± 0)	0 (± N/A)	100.0 (± N/A)
30	Kinney	Fort Clark Springs, Las Moras Creek, near guard station	29.30740	-100.41750	1	no	0 (± N/A)	1.0 (± N/A)	0 (± N/A)	0 (± N/A)	100.0 (± N/A)
32	Kinney	Fort Clark Springs, Las Moras Creek, upstream of golf pro shop	29.29043	-100.42386	3	yes	5.0 (± 3.6)	1.0 (± 0)	0.3 (± 0.6)	0 (± 0)	100.0 (± N/A)
35	Kinney	Fort Clark Springs, Las Moras Creek, Buzzard Roost	29.28034	-100.42076	2	yes	6.0 (± 4.2)	3.5 (± 0.7)	1.0 (± 0)	0.7 (± 1.2)	80.0 (± 28.3)
42	Maverick	Eagle Pass Golf Course, spillway into Rio Grande	28.70416	-100.51046	2	yes	19.0 (± 14.1)	2.5 (± 2.1)	4.0 (± 1.4)	0 (± N/A)	–
43	Maverick	Rio Grande, along Eagle Pass Golf Course	28.70294	-100.51089	1	no	0 (± N/A)	0 (± N/A)	2.0 (± N/A)	0 (± 0)	–
44	Maverick	Eagle Pass Golf Course, settling pond along Rio Grande	28.70146	-100.50979	1	yes	1.0 (± N/A)	19.0 (± N/A)	7.0 (± N/A)	0 (± N/A)	–
45	Webb	Lake Casa Blanca International State Park, Casa Blanca Lake, near El	27.54447	-99.44098	1	no	0 (± N/A)	1.0 (± N/A)	1.0 (± N/A)	0 (± 0)	–
46	Webb	Lake Casa Blanca International State Park, Casa Blanca Lake, fishing pier	27.53861	-99.43475	1	no	0 (± N/A)	1.0 (± N/A)	1.0 (± N/A)	0 (± 0)	–
47	Webb	Rio Grande, Laredo, near water treatment center	27.52372	-99.52431	3	yes	2.0 (± 1.7)	0 (± 0)	0 (± 0)	0 (± 0)	100.0 (± 0)
48	Webb	Rio Grande, Laredo, near international railroad bridge crossing	27.49835	-99.51674	2	no	0 (± 0)	0 (± 0)	1.0 (± 1.4)	0 (± N/A)	–
49	Webb	Rio Grande, near El Cenizo	27.33117	-99.51195	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	100.0 (± N/A)
50	Zapata	Rio Grande, near San Ygancio	27.04330	-99.44496	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	16.0 (± N/A)	80.0 (± N/A)
51	Starr	Falcon State Park, Falcon Lake	26.58179	-99.15259	2	no	0 (± 0)	2.0 (± 2.8)	0.5 (± 0.7)	1.0 (± 1.7)	57.1 (± N/A)
52	Starr	Rio Grande, spillway below Falcon Dam	26.54608	-99.17093	3	no	0 (± 0)	1.3 (± 2.3)	0 (± 0)	0 (± N/A)	100.0 (± N/A)
53	Starr	Rio Grande, near Chapeno	26.53233	-99.15546	1	no	0 (± N/A)	0 (± N/A)	0 (± N/A)	5.7 (± 4.6)	83.9 (± 0.96)
54	Starr	Rio Grande, near Salineño	26.51429	-99.11662	2	yes	0.5 (± 0.7)	4.0 (± 2.8)	1.0 (± 1.4)	0.5 (± 0.7)	80.0 (± N/A)
59	Hidalgo	Rio Grande, near National Butterfly Center	26.16934	-98.36742	2	no	0 (± 0)	0 (± 0)	0 (± 0)	0.5 (± 0.7)	0 (± N/A)
60	Cameron	Rio Grande, downstream of TNC Southmost Preserve	25.85462	-97.37676	1	no	0 (± N/A)	2.0 (± N/A)	0 (± N/A)	0 (± 0)	100.0 (± 0)
61	Cameron	Rio Grande, near TNC Southmost Preserve Office	25.85008	-97.39865	2	no	0 (± 0)	0.5 (± 0.7)	0 (± 0)	0 (± N/A)	100.0 (± N/A)

Table 3.2. Primer sequences used in analyses to detect *Pseudemys gorzugi* environmental DNA (eDNA), annealing temperature (°C), and product size (bp).

PCR	Primer Set	Sequence (5' to 3')	Annealing Temperature	Product Size
Initial	PG_CO1_FW1	CAGAACTAAGCCAACCAGGTA	57	155
Initial	PG_CO1_RV1mod1	GGTGCTCCAATAATCAGTGG		
Nested	PG_CO1_FW1_nest	CTTTTAGGAGATGACCAAGTCTAT	57	118
Nested	PG_CO1_RV1_nest	TCAGTGGTACAAGTCAATTTCCA		

Table 3.3. Sites that were screened for *Pseudemys gorzugi* environmental DNA (eDNA). Also included is the sampling date, mean (± 1 SD) volume of site water (ml) filtered through three cellulose filters, total DNA concentrations in samples 1–3 (ng/µl), and whether we detected *P. gorzugi* (PG) eDNA. Two of the three samples had to be successfully sequenced as *P. gorzugi* (indicated in bold) in order for a successful detection at a site. Site numbers correspond to those used in Table 2.1.

Site #	Date	County	Site	Latitude	Longitude	Volume Filtered	Sample 1	Sample 2	Sample 3	PG Detected
1	18 May 2019	Pecos	Pecos River, at US Hwy 190 crossing	30.90516	-101.88080	1600.0 (± 173.2)	**2.40**	**3.02**	**1.78**	yes
3	18 May 2019	Pecos	Pecos River, at I-10 crossing	30.71808	-101.80954	833.3 (± 144.3)	too low	too low	too low	no
4	18 May 2019	Pecos	Pecos River, at TX Hwy 290 crossing	30.65960	-101.77020	1000.0 (± 0)	0.11	**0.39**	too low	no
5	5 June 2019	Terrell	TNC Independence Creek Preserve, Lower Lake	30.46955	-101.80131	2000.0 (± 0)	**13.40**	**5.12**	**9.66**	yes
7	7 June 2019	Terrell	TNC Independence Creek Preserve, raceway below Upper Lake	30.46736	-101.80181	2000.0 (± 0)	0.20	too low	too low	no
10	6 June 2019	Terrell	Independence Creek, at County Road crossing	30.45026	-101.73124	2000.0 (± 0)	**5.44**	0.89	**1.07**	yes
11	5 June 2019	Crockett	Pecos River, 0.3 river km upstream of confluence with Independence Creek	30.44767	-101.72119	1916.7 (± 144.3)	**0.51**	**1.12**	**0.58**	yes
13	6 June 2019	Val Verde	Pecos River, at Pandale crossing	30.13120	-101.57450	900.0 (± 91.7)	**0.45**	**0.22**	0.24	yes
14	20 July 2019	Val Verde	TNC Dolan Falls Preserve, Devils River, upstream of confluence with Dolan Creek	29.89387	-100.99561	2000.0 (± 0)	too low	**0.14**	**0.38**	yes
16	20 July 2019	Val Verde	TNC Dolan Falls Preserve, Devils River, Dolan Falls	29.88385	-100.99397	2000.0 (± 0)	too low	too low	too low	no
18	22 June 2019	Val Verde	Rio Grande, near Langtry	29.80564	-101.55088	250.0 (± 0)	too low	1.04	2	no
20	23 June 2019	Val Verde	Pecos River, near confluence with Rio Grande	29.70431	-101.36667	1333.3 (± 577.4)	**0.45**	too low	**0.88**	yes
21	21 June 2019	Val Verde	Lake Amistad, Rough Canyon	29.57490	-100.97809	2000.0 (± 0)	**2.22**	**0.43**	**1.03**	yes
22	21 June 2019	Val Verde	Lake Amistad, along Spur 406	29.54023	-101.01623	1916.7 (± 144.3)	**0.41**	**4.04**	**0.35**	yes
23	21 June 2019	Val Verde	Lake Amistad, Box Canyon	29.52420	-101.17585	2000.0 (± 0)	**0.70**	**0.42**	**0.49**	yes
24	2 October 2019	Val Verde	Rio Grande, spillway below Amistad Dam	29.44737	-101.05667	2000.0 (± 0)	**0.20**	**0.15**	**0.35**	yes
25	21 August 2019	Val Verde	Rio Grande, weir below Amistad Dam	29.42455	-101.04118	2000.0 (± 0)	**0.24**	**0.10**	**0.2**	yes
26	3 October 2019	Val Verde	Rio Grande, near Lugo property	29.37719	-101.01348	2000.0 (± 0)	**0.88**	too low	**0.55**	yes
27	31 July 2019	Val Verde	Del Rio, San Felipe Springs Golf Course, San Felipe Creek	29.37029	-100.88526	1000.0 (± 0)	**0.45**	0.11	too low	no
29	26 June 2019	Kinney	Fort Clark Springs, Headwater Pond	29.30944	-100.42125	2000.0 (± 0)	17.60	**2.28**	**0.20**	yes

32	29 June 2019	Kinney	Fort Clark Springs, Las Moras Creek, upstream of golf pro shop	29.29043	-100.42386	1916.7 (± 144.3)	too low	too low	too low	no
35	31 July 2019	Kinney	Fort Clark Springs, Las Moras Creek, Buzzard Roost	29.28034	-100.42076	2000.0 (± 0)	too low	too low	too low	no
36	11 March 2019	Val Verde	Sycamore Creek, at US Hwy 277 crossing	29.25473	-100.75216	2000.0 (± 0)	**2.90**	**5.20**	**10.40**	yes
37	11 March 2019	Kinney	Pinto Creek, at US Hwy 277 crossing	29.18898	-100.70340	2000.0 (± 0)	**1.57**	**0.97**	**0.64**	yes
38	11 March 2019	Maverick	Tequesquite Creek, at US Hwy 277 crossing	29.06453	-100.63899	2000.0 (± 0)	too low	0.49	too low	no
39	11 March 2019	Maverick	irrigation canal along US Hwy 277, near Las Moras Creek	29.00785	-100.63817	1416.7 (± 381.9)	too low	too low	0.14	no
40	11 March 2019	Maverick	Quemado Creek, along US Hwy 277	28.92578	-100.61490	666.7 (± 144.3)	**0.19**	**0.16**	too low	yes
41	10 March 2019	Maverick	Elm Creek, near US Hwy 277	28.77016	-100.49828	2000.0 (± 0)	too low	too low	too low	no
42	9 March 2019	Maverick	Eagle Pass Golf Course, spillway into Rio Grande	28.70416	-100.51046	1916.7 (± 144.3)	too low	too low	too low	no
43	29 June 2019	Maverick	Rio Grande, along Eagle Pass Golf Course	28.70294	-100.51089	1883.3 (± 202.1)	**0.30**	**0.18**	**0.14**	yes
44	1 July 2019	Maverick	Eagle Pass Golf Course, settling pond along Rio Grande	28.70146	-100.50979	600.0 (± 173.2)	0.41	0.41	**0.50**	no
45	6 September 2019	Webb	Lake Casa Blanca International State Park, Casa Blanca Lake, near El Ranchito	27.54447	-99.44098	1750.0 (± 250.0)	0.16	0.18	0.10	no
47	6 September 2019	Webb	Rio Grande, Laredo, near water treatment center	27.52372	-99.52431	333.3 (± 144.3)	**1.52**	too low	**0.15**	yes
49	5 September 2019	Webb	Rio Grande, near El Cenizo	27.33117	-99.51195	583.3 (± 144.3)	**0.99**	0.44	0.89	no
50	7 July 2019	Zapata	Rio Grande, near San Ygancio	27.04330	-99.44496	443.3 (± 268.6)	0.14	too low	0.25	no
51	4 September 2019	Starr	Falcon State Park, Falcon Lake	26.58179	-99.15259	666.7 (± 144.3)	0.10	3.18	0.11	no
52	7 July 2019	Starr	Rio Grande, spillway below Falcon Dam	26.54608	-99.17093	1633.3 (± 321.5)	0.27	**0.39**	0.24	no
53	6 July 2019	Starr	Rio Grande, near Chapeno	26.53233	-99.15546	2000.0 (± 0)	**2.68**	**0.52**	**0.11**	yes
54	6 July 2019	Starr	Rio Grande, near Salineño	26.51429	-99.11662	2000.0 (± 0)	0.51	**0.38**	**0.81**	yes
58	12 March 2019	Hidalgo	Bentsen-Rio Grande Valley State Park, La Parida Banco	26.17906	-98.38716	1500.0 (± 500.0)	**1.24**	too low	0.38	no
59	24 September 2019	Hidalgo	Rio Grande, near National Butterfly Center	26.16934	-98.36742	2000.0 (± 0)	**2.12**	1.58	**2.18**	yes
61	24 September 2019	Cameron	Rio Grande, near TNC Southmost Preserve Office	25.85008	-97.39865	1666.7 (± 577.4)	too low	too low	too low	no

LITERATURE CITED

American Rivers. 2003. America's most endangered rivers of 2003. Available at www. americanrivers.org.

Bailey LA, Dixon JR, Forstner MRJ. 2005. *Pseudemys gorzugi* (Rio Grande Cooter). Color pattern. *Herpetological Review* 36:313.

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

Bailey LA, Forstner MRJ, Dixon JR, Hudson R. 2014. Contemporary status of the Rio Grande cooter (Testudines: Emydidae: *Pseudemys gorzugi*) in Texas: phylogenetic, ecological, and conservation considerations, pp. 320–334, *in* Hoyt CA, Karges J (eds.), Proceedings of the Sixth Symposium on the Natural Resources of the Chihuahuan Desert Region. Chihuahuan Desert Research Institute, Fort Davis, Texas.

Beauvais GP, Buskirk SW. 1999. Modifying estimates of sampling effort to account for sprung traps. *Wildlife Society Bulletin* 27:39–43.

Bevan E, Whiting S, Tucker T, Guinea M, Raith A, Douglas R. 2018. Measuring behavioral responses of sea turtles, saltwater crocodiles, and crested terns to drone disturbance to define ethical operating thresholds. *PLoS ONE* 13:e0194460.

Biserkov VY, Lukanov SP. 2017. Unmanned aerial vehicles (UAVs) for surveying freshwater turtle populations: methodology adjustment. *ACTA Zoologica Bulgarica* 10:161–163.

Bogolin AP, Davis DR, Rahman AF. 2019a. *Pseudemys gorzugi* (Rio Grande cooter). Predation. *Herpetological Review* 50:775.

Bogolin AP, Davis DR, Ruppert KM, Kline RJ, Rahman AF. 2019b. Geographic distribution: USA, Texas, Crockett Co.: *Pseudemys gorzugi* (Rio Grande cooter). *Herpetological Review* 50:745.

Bonner TH, Littrell BM. 2016. Aquatic surveys of Delaware River of Texas. Final report to Texas Comptroller of Public Accounts.

Buckland ST, Goudie IBJ, Borchers DL. 2000. Wildlife population assessment: past developments and future directions. *Biometrics* 56:1–12.

Candiago S, Remondino F, De Giglio M, Dubbini, M, Gattelli M. 2015. Evaluating multispectral images and vegetation indices for precision farming applications from UAV images. *Remote Sensing* 7:4026–4047.

Christman BL, Kamees LK. 2007. Current distribution of the blotched watersnake (*Nerodia erythrogaster transversa*) and the Rio Grande cooter (*Pseudemys gorzugi*) in the Lower Pecos River system Eddy County, New Mexico 2006–2007. Final report to New Mexico Department of Game and Fish.

Collins JT. 1991. Viewpoint: a new taxonomic arrangement for some North American amphibians and reptiles. *Herpetological Review* 22:42–43.

Degenhardt WG, Painter CW, Price AH. 1996. Amphibians and Reptiles of New Mexico. University of New Mexico Press, Albuquerque, New Mexico. 431 pp., 123 plates.

Dixon JR. 2013. Amphibians and Reptiles of Texas with Keys, Taxonomic Synopses, Bibliography, and Distribution Maps. Third Edition. Texas A&M University Press, College Station, Texas. 447 pp.

Ernst CH. 1990. *Pseudemys gorzugi*. *Catalogue of American Amphibians and Reptiles* 461:1–2.

Ernst CH, Hershey MF, Barbour RW. 1974. A new coding system for hardshelled turtles. *Transactions of the Kentucky Academy of Science* 35:27–28.

Ficetola GF, Miaud C, Pompanon F, Taberlet P. 2008. Species detection using environmental DNA from water samples. *Biology Letters* 4:423–425.

Forstner MRJ, Bailey L, Ferrell S, Dixon JR, Hudson R. 2004. Population status, genetic structure, and ecological aspects of the Rio Grande river cooter (*Pseudemys gorzugi*) in Texas. Final report to Texas Parks and Wildlife Department.

Frazer NBK, Gibbons KP, Owens TJ. 1990. Turtle trapping: preliminary tests of conventional wisdom. *Copeia* 1990:1150–1152.

Gibbons JW. 1990. Life History and Ecology of the Slider Turtle. Smithsonian Institution Press, Washington, D.C. 384 pp.

Global Scan Technologies LLC. 2019. WorldView-2 Satellite Imagery and Sensor Specifications. Available at http://www.gstdubai.com/satelliteimagery/worldview-2.html [accessed 15 May 2019].

Goldberg CS, Turner CR, Deiner K, Klymus KE, Thomsen PF, Murphy MA, Spear SF, McKee A, Oyler-McCance SJ, Cornman RS, Laramie MB, Mahon AR, Lance RF, Pilliod DS, Strickler KM, Waits LP, Fremier AK, Takahara T, Herder JE, Taberlet P. 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution* 7:1299–130.

Goldberg CS, Strickler KM, Pilliod DS. 2015. Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. *Biological Conservation* 183:1–3.

Goldberg CS, Pilliod DS, Arkle RS, Waits LP. 2011. Molecular detection of vertebrates in stream water; a demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. *PLoS ONE* 6:e22746

Goncalves J, Henriques R, Paulo Alves R, Sousa-Silva Monteiro AT, Angela Lomba, Marcos B, Honrado J. 2016. Evaluating an unmanned aerial vehicle-based approach for assessing habitat extent and condition in fine-scale early successional mountain mosaics. *Applied Vegetation Science* 19:132–146.

Grenzdörffer GJ, Engel A, Teichert B. 2008. The photogrammetric potential of low-cost UAVs in forestry and agriculture. *The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences* 37(B1):1207–1214.

Gu W, Swihart RK. 2004. Absent or undetected? Effect on non-detection of species occurrence on wildlife-habitat models. *Biological Conservation* 116:195–203.

Hibbitts TD, Hibbitts TL. 2016.Texas Turtles and Crocodilians. A Field Guide. University of Texas Press, Austin, Texas. 257 pp.

Hodgson A, Kelly N, Peel D. 2013. Unmanned aerial vehicles (UAVs) for surveying marine fauna: a dugong case study. *PLoS ONE* 8:e79556.

Hoffmann C, Schubert G, Calvignac-Spencer S. 2016. Aquatic biodiversity assessment for the lazy. *Molecular Ecology* 25:846–848.

Hofreiter M, Mead JI, Martin P, Poinar HN. 2003. Molecular caving. *Current Biology* 13:R693–R695.

Iverson JB. 1992a. A Revised Checklist with Distribution Maps of the Turtles of the World. Privately printed, Richmond, Indiana. 363 pp.

Iverson JB. 1992b. Correlates of reproductive output in turtles (order Testudines). *Herpetological Monographs* 6:25–42.

Jerde CL, Mahon AR, Chadderton WL, Lodge DM. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters* 4:150–187.

Jones IV, Pearlstine LG, and Percival HF. 2006. An assessment of small unmanned aerial vehicles for wildlife research. *Wildlife Society Bulletin* 34:750–758.

Koski WR, Allen T, Ireland D, Buck G, Smith PR, Macrander AM, Halick MA, Rushing C, Sliwa DJ, McDonald TL. 2009. Evaluation of an unmanned airborne system for monitoring marine mammals. *Aquatic Mammals* 35:347–357.

Lanica RA, Kendall WL, Pollock KH, Nichols JD. 2005. Estimating the number of animals in wildlife populations, pp. 106–153 *in* Braun CE (ed.), Techniques for Wildlife Investigations and Management. 6th Edition. The Wildlife Society, Bethesda, Maryland.

Letter AW, Waldon KJ, Pollock DA, Mali I. 2019. Dietary habits of Rio Grande cooters (*Pseudemys gorzugi*) from two sites within the Black River, Eddy County, New Mexico, USA. *Journal of Herpetology* 53:204–208.

Levings GW, Healy DF, Richey SF, Carter LF. 1998. Water Quality in the Rio Grande Valley, Colorado, New Mexico, and Texas, 1992-95. United States Geological Survey Circular 1162.

Lindeman PV. 2007. Diet, growth, body size, and reproductive potential of the Texas river cooter (*Pseudemys texana*) in the South Llano River, Texas. *Southwestern Naturalist* 52:586–594.

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

MacLaren AR, Foley DH, Sirsi S, Forstner MRJ. 2017. Updating methods of satellite transmitter attachment for long-term monitoring of the Rio Grande cooter (*Pseudemys gorzugi*). *Herpetological Review* 48:48–52.

Mali I, Brown DJ, Ferrato JR, Forstner MRJ. 2014. Sampling freshwater turtle populations using hoop nets: testing potential biases. *Wildlife Society Bulletin* 38:580–585.

Mali I, Brown DJ, Jones MC, Forstner MRJ. 2012. Switching bait as a method to improve freshwater turtle capture and recapture success with hoop net traps. *Southeastern Naturalist* 11:311–318.

Mali I and Forstner MRJ. 2017. Survey of western river cooter (*Pseudemys gorzugi*) in New Mexico within the Black River Drainage. Final report to New Mexico Department of Game and Fish.

Mali I, Letter AW, and Suriyamongkol T. 2018. Phase II: Demography of western river cooter (*Pseudemys gorzugi*) populations within the Black River Drainage. Final report to New Mexico Department of Game and Fish.

Mulero-Pázmány M, Stopler R, Essen LV, Negro JJ, Sassen T. 2014. Remotely piloted aircraft systems as a rhinoceros anti-poaching tool in Africa. *PLoS ONE* 9:e83873.

Ouédraogo MM, Degré A, Debouche C, Lisein J. 2014. The evaluation of unmanned aerial system-based photogrammetry and terrestrial laser scanning to generate DEMs of agricultural watersheds. *Geomorphology* 214:339–355.

Pierce LJS, Stuart JN, Ward JP, Painter CW. 2016. *Pseudemys gorzugi* Ward 1984 – Rio Grande Cooter, Western River Cooter, Tortuga de Oreja Amarilla, Jicotéa del Río Bravo, pp. 100.1–100.12 *in* Rhodin AGJ, Iverson JB, van Dijk PP, Saumure RA, Buhlmann KA, Pritchard PCH, Mittermeier RA [eds.], Conservation Biology of Freshwater Turtle and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. Chelonian Research Monographs 5, Lunenburg, Massachusetts.

Plummer MV. 1977. Activity, habitat and population structure in the turtle, *Trionyx muticus*. *Copeia* 1977:431–440.

Rees AF, Avens L, Ballorain K, Bevan E, Broderick AC, Carthy RR, Mangel JC. 2018. The potential of unmanned aerial systems for sea turtle research and conservation: a review and future directions. *Endangered Species Research* 35: 81–100.

Reid BN, Le M, McCord JWP, Iverson B, Georges A, Bergmann T, Amato G, Desalle R, Naro-Maciel E. 2011. Comparing and combining distance-based and character-based approaches for barcoding turtles. *Molecular Ecology Resources* 11:956–967.

Renshaw MA, Olds BP, Jerde CL, McVeigh MM, Lodge DM. 2015. The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol-chloroform-isoamyl alcohol DNA extraction. *Molecular Ecology Resources* 15:168–176.

Takara T, Minamoto T, Doi H. 2013. Using environmental DNA to estimate the distribution of an invasive fish species in ponds. *PLoS ONE* 8:e56584.

Thomas RB, Nall IM, House WJ. 2008. Relative efficacy of three different baits for trapping pond-dwelling turtles in east-central Kansas. *Herpetological Review* 39:186–188.

Thomsen PF, Kielgast J, Iversen LL, Moller PR, Rasmussen M, Willerslev, E. 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS ONE* 7:e41732.

Turner CR, Barnes MA, Xu CC, Jones SE, Jerde CL, Lodge DM. 2014. Particle size distribution and optimal capture of aqueous macrobial eDNA. *Methods in Ecology and Evolution* 5:676–684.

[USDOI] United States Department of the Interior. 1998. Water-resources issues in the Rio Grande—Rio Conchos to Amistad Reservoir subarea. United States Department of the Interior, Washington, D.C.

Van Gemert JC, Veenman CJ, Geusebroek JM. 2009. Episode-constrained cross-validation in video concept retrieval. *IEEE Transactions on Multimedia* 11:780–786.

Vermeulen C, Lejeune P, Lisein J, Sawadogo P, Bouché P. 2013. Unmanned aerial survey of elephants. *PLoS ONE* 8:e54700.

Ward JP. 1984. Relationship of chrysemyd turtles of North America (Testudines: Emydidae). Special Publications, the Museum Texas Technical University 21:1–50.

Westoby MJ, Brasington J, Glasser NF, Hambrey MJ, Reynolds JM. 2012. “Structure-from-motion” photogrammetry: a low-cost, effective tool for geoscience applications. *Geomorphology* 179:300–314.

Appendix 1. List of water quality parameters measured during each sampling visit. Temperature (°C), dissolved oxygen (DO; mg/L), conductivity (µS/cm), oxygen-reduction potential (ORP; mV), nitrite (NO_2^-; ppm), nitrate (NO_3^-; ppm), ammonia (NH_3; ppm), hardness (ppm), and alkalinity (ppm) are all provided. Site numbers correspond to those used in Table 2.1.

Site #	Date	County	Latitude	Longitude	Temp	pH	DO	Conductivity	ORP	Nitrite	Nitrate	Ammonia	Hardness	Alkalinity
1	18 May 2019	Pecos	30.90516	-101.88083	27.4	8.95	10.98	26000	153.5	0	0	0.1	425	30
1	10 August 2019	Pecos	30.90516	-101.88083	29.0	7.94	6.27	20590	162.3	0	0	0	425	40
1	17 October 2019	Pecos	30.90516	-101.88083	19.1	9.22	9.63	23000	242.7	0	0	0.25	425	120
2	10 August 2019	Pecos	30.78851	-101.83502	32.8	6.34	7	19440	101.8	0	0	0.25	425	40
2	17 October 2019	Pecos	30.78851	-101.83502	20.2	9.10	11.76	19820	340.3	0	0	0	425	–
3	18 May 2019	Pecos	30.71808	-101.80954	28.9	8.29	11.53	12260	219.8	0	0	0	425	80
4	18 May 2019	Pecos	30.65960	-101.77022	26.2	7.90	6.64	10750	214.8	0	0	0	425	80
5	5 June 2019	Terrell	30.46955	-101.80131	28.3	8.41	12.05	781	123	0	0	0	250	240
5	11 August 2019	Terrell	30.46955	-101.80131	32.0	7.34	0.23	785	-333.9	0	0	0	425	180
7	7 June 2019	Terrell	30.46736	-101.80181	26.1	8.01	11.74	922	88.6	0	2	0	425	240
9	10 August 2019	Crockett	30.45259	-101.71940	33.6	7.90	–	6060	124.7	0	0	0	425	240
10	6 June 2019	Terrell	30.45026	-101.73124	28.4	8.51	8.09	1038	75.7	0	0	0	425	240
10	11 August 2019	Terrell	30.45026	-101.73124	28.5	8.00	8.35	1035	99.2	0	0	0	425	160
11	5 June 2019	Crockett	30.44767	-101.72119	29.3	8.51	7.3	11440	113	0	0	0.5	425	180
11	18 October 2019	Crockett	30.44767	-101.72119	20.7	8.3	7.34	314.9	–	0	0	0	425	–
13	30 March 2019	Val Verde	30.13120	-101.57450	19.5	8.19	7.96	5270	123.9	–	–	–	–	–
13	6 June 2019	Val Verde	30.13120	-101.57450	26.8	8.27	6.87	4250	118.6	0.15	1	0.25	425	180
14	27 April 2019	Val Verde	29.89387	-100.99561	26.2	8.42	10.92	469	119.7	–	–	–	–	–
14	20 July 2019	Val Verde	29.89387	-100.99561	30.0	8.42	9.12	436	220.9	0	2	0	425	220
14	19 September 2019	Val Verde	29.89387	-100.99561	26.9	7.97	7.27	–	9.6	0	2	0	425	160

15	20 July 2019	Val Verde	29.88591	-100.99292	24.8	8.43	12.54	457	222.1	0	2	0.25	425	240
15	19 September 2019	Val Verde	29.88591	-100.99292	27.0	7.89	9.2	470	11.9	0	2	0.25	425	240
16	28 April 2019	Val Verde	29.88385	-100.99397	22.2	8.34	8.9	462	103.5	–	–	–	–	–
16	20 July 2019	Val Verde	29.88385	-100.99397	27.9	8.42	8.1	445	248.3	0	0	0	250	240
16	19 September 2019	Val Verde	29.88385	-100.99397	29.8	8.27	–	–	93.5	0	0	0	425	240
17	22 June 2019	Val Verde	29.80829	-101.54893	28.4	8.34	6.95	1338	164.4	0	0	0.25	400	210
18	22 June 2019	Val Verde	29.80564	-101.55088	29.1	8.38	6.76	1434	117	0	0	0.15	425	240
18	23 August 2019	Val Verde	29.80564	-101.55088	30.4	8.17	7.16	877	122.8	0	0	0	425	240
20	23 June 2019	Val Verde	29.70431	-101.36667	30.5	8.32	9.82	2600	180.8	–	–	–	400	160
20	23 August 2019	Val Verde	29.70431	-101.36667	28.2	7.91	7.22	2134	99.8	0	0	0	425	240
21	21 June 2019	Val Verde	29.57490	-100.97809	28.5	8.79	9.77	598	160.9	0	0	0	250	180
21	22 August 2019	Val Verde	29.57490	-100.97809	32.2	8.24	8.2	556	77.4	0	0	0	425	180
21	3 October 2019	Val Verde	29.57490	-100.97809	26.7	8.48	6.91	570	130.4	0	0	0.25	250	240
22	21 June 2019	Val Verde	29.54023	-101.01623	31.4	8.29	5.54	1052	101.8	0	0	0.1	400	180
23	21 June 2019	Val Verde	29.52420	-101.17585	28.4	8.57	8.74	1080	100.1	0	0	0	425	120
23	22 August 2019	Val Verde	29.52420	-101.17585	29.5	8.29	7.69	868	117	0	0	0	425	120
23	3 October 2019	Val Verde	29.52420	-101.17585	30.1	8.51	7.53	988	111.7	0	0	0	425	160
24	20 June 2019	Val Verde	29.44737	-101.05667	22.1	8.44	5.07	1094	187.3	0	1	0.25	425	160
24	2 October 2019	Val Verde	29.44737	-101.05667	25.9	8.36	2.67	969	-159.8	0	0	0	425	100
25	21 June 2019	Val Verde	29.42455	-101.04118	27.2	8.50	7.89	1042	123.3	0	1	0.25	425	160
25	21 August 2019	Val Verde	29.42455	-101.04118	27.0	7.69	5.04	996	143.7	0	0	0	425	160
26	17 May 2019	Val Verde	29.37719	-101.01348	21.1	8.26	7.75	1117	188.4	0	0	0	425	180
26	31 July 2019	Val Verde	29.37719	-101.01348	28.2	8.36	8.06	1020	236.3	0	0	0	425	240
26	3 October 2019	Val Verde	29.37719	-101.01348	26.1	8.00	6.14	1004	130.6	0	0	0	425	240
27	16 May 2019	Val Verde	29.37029	-100.88526	26.0	7.81	11.68	550	182.8	0	0	0	425	240

27	31 July 2019	Val Verde	29.37029	-100.88526	28.4	7.94	0.2	640	143.4	0	0	0	350	240
27	4 October 2019	Val Verde	29.37029	-100.88526	27.0	7.78	0.21	559	–	0	2	0	425	240
29	9 November 2018	Kinney	29.30944	-100.42125	22.7	7.29	–	503	209.9	0	0	0	250	240
29	11 May 2019	Kinney	29.30944	-100.42125	23.4	7.70	10.47	436	182.7	–	–	–	–	–
29	26 June 2019	Kinney	29.30944	-100.42125	26.2	7.87	8.67	430	175.3	0	2	0.15	425	210
29	30 July 2019	Kinney	29.30944	-100.42125	26.3	8.12	6.84	–	252.8	0	3	0.25	250	220
30	11 May 2019	Kinney	29.30740	-100.41745	23.1	7.90	8.64	442	203.6	–	–	–	–	–
31	10 November 2018	Kinney	29.29273	-100.42075	21.4	7.77	–	505	184.9	0	2	0	250	240
32	11 May 2019	Kinney	29.29043	-100.42386	24	8.34	10.46	440	203.3	–	–	–	–	–
32	29 June 2019	Kinney	29.28638	-100.42263	25.5	8.20	9.26	437	121.2	0	3	0.2	200	240
32	30 July 2019	Kinney	29.28638	-100.42263	28.9	8.31	8.9	432	214.4	0	0	0.25	425	240
34	10 November 2018	Kinney	29.28238	-100.42325	21.0	7.87	–	505	181.3	0	2	0	425	240
35	10 November 2018	Kinney	29.28034	-100.42076	20.3	7.97	–	507	200.4	0	2	0	250	240
35	29 June 2019	Kinney	29.28034	-100.42076	25.0	8.29	8.12	454	172.9	0	2	0	300	210
35	31 July 2019	Kinney	29.28034	-100.42076	24.9	8.77	7.48	431	279.6	0	1	0.25	425	240
36	11 March 2019	Val Verde	29.25473	-100.75216	17.5	7.81	–	452	132.3	–	–	–	250	180
37	11 March 2019	Kinney	29.18898	-100.70340	19.4	8.24	–	518	105.8	–	–	–	–	–
38	11 March 2019	Maverick	29.06453	-100.63899	18.2	7.79	–	1748	134.3	–	–	–	425	180
39	11 March 2019	Maverick	29.00785	-100.63817	18.5	8.35	–	986	106.6	–	–	–	250	180
40	11 March 2019	Maverick	28.92578	-100.6149	20.3	8.01	–	1777	130.1	–	–	–	425	240
41	10 March 2019	Maverick	28.77016	-100.49828	20.6	8.26	–	–	83	–	–	–	250	240
42	9 March 2019	Maverick	28.70416	-100.51046	18.8	8.04	7.56	2430	118.3	0.1	3	0.2	423	180
42	30 June 2019	Maverick	28.70416	-100.51046	28.3	8.43	7.05	1065	99.7	0	0	0.25	425	140
43	10 March 2019	Maverick	28.70294	-100.51089	19.1	8.51	8.55	901	101	–	–	0	250	230
43	29 June 2019	Maverick	28.70294	-100.51089	33.4	8.79	10.73	889	144.7	0	1	0	425	160

44	11 March 2019	Maverick	28.70146	-100.50979	20.7	10.48	–	1592	52.4	–	–	–	250	180
44	1 July 2019	Maverick	28.70146	-100.50979	29.5	9.03	7.78	1463	129.6	0	1	0	425	100
45	9 July 2019	Webb	27.54447	-99.44098	35.0	9.00	9.4	1293	134.8	0	0	0	375	–
45	6 September 2019	Webb	27.54447	-99.44098	34.6	8.65	9.43	1427	118	0	0	0	425	240
46	9 July 2019	Webb	27.53861	-99.43475	31.9	9.03	9.92	1289	128.1	0	0	0	425	
46	6 September 2019	Webb	27.53861	-99.43475	31.0	8.67	7.78	1030	95.9	0	0	0	425	240
47	14 April 2019	Webb	27.52372	-99.52431	21.6	8.14	6.94	990	86.9	–	–	–	–	–
47	9 July 2019	Webb	27.52372	-99.52431	30.4	8.49	7.43	804	174.8	0	0	0.25	425	–
47	6 September 2019	Webb	27.52372	-99.52431	30.6	8.11	5.96	894	119.3	0	0	0	425	180
48	14 April 2019	Webb	27.49835	-99.51674	22.8	8.18	6.72	964	109.4	–	–	–	–	–
48	9 July 2019	Webb	27.49835	-99.51674	32.0	8.39	7.44	813	157.4	0	0	0	425	
49	14 April 2019	Webb	27.33117	-99.51195	24.0	8.17	0.36	987	175.1	–	–	–	–	–
49	5 September 2019	Webb	27.33117	-99.51195	30.0	7.87	4.62	1079	101.8	0	0	0.25	425	180
50	7 July 2019	Zapata	27.04330	-99.44496	30.3	8.55	7.8	924	153.6	0	0	0	425	120
51	28 May 2019	Starr	26.58179	-99.15259	30.0	8.94	9.28	986	65.3	0	0	0	–	80
51	7 July 2019	Starr	26.58179	-99.15259	31.3	8.89	10.3	986	111.1	0	0	0	250	120
51	4 September 2019	Starr	26.58179	-99.15259	28.1	8.74	8.02	1022	53.6	0	0	0	425	150
52	28 May 2019	Starr	26.54608	-99.17093	28.0	8.66	7.76	982	68.3	0	0	0	–	80
52	7 July 2019	Starr	26.54608	-99.17093	32.5	8.76	10.02	983	104.5	0	0	0.25	350	180
52	4 September 2019	Starr	26.54608	-99.17093	28.5	8.38	7.34	985	61.2	0	0	0	375	150
53	6 July 2019	Starr	26.53233	-99.15546	31.1	9.02	9.57	992	94.2	0	0	0.25	425	100
54	11 November 2018	Starr	26.51429	-99.11662	20.0	8.66	–	909	125.4	0	0	0	250	180
54	28 May 2019	Starr	26.51429	-99.11662	27.7	8.60	9.12	993	5.1	0	0	0	–	120
54	6 July 2019	Starr	26.51429	-99.11662	29.1	8.59	7.86	991	129	0	1	0.25	425	100
54	4 September 2019	Starr	26.51429	-99.11662	28.3	7.69	1.94	961	-210.8	0	0	0	250	180

58	12 March 2019	Hidalgo	26.17906	-98.38716	24.8	8.33	9.85	6910	132.1	–	–	–	–	–
59	24 September 2019	Hidalgo	26.16934	-98.36742	32.2	8.53	7.65	1101	207	0	0	0	425	120
59	23 October 2019	Hidalgo	26.16934	-98.36742	27.7	8.45	7.93	1134	580	0	0	0	425	–
60	23 October 2019	Cameron	25.85462	-97.37676	28.4	8.33	6.24	1346	530	0	2	0	–	–
61	24 May 2019	Cameron	25.85008	-97.39865	29.3	8.68	0.008	1245	-6.6	–	–	–	–	–
61	24 September 2019	Cameron	25.85008	-97.39865	32.5	8.76	11.5	1349	169.7	0	2	0	425	180

Appendix 2. List of habitat characters scored during each sampling visit. Sites characteristics including turbidity, presence of flow, algae mats, woody debris, and trees, percentage of floating, submerged, and emergent vegetation and substrate, and surrounding land use are described. Site numbers correspond to those used in Table 2.1.

Site #	Date	County	Latitude	Longitude	Turbidity	Flow	Algae Mats	Woody Debris	Trees	Floating Vegetation	Submerged Vegetation	Emergent Vegetation	Substrate	Adjacent Land Use
1	18 May 2019	Pecos	30.90516	-101.88083	moderate	yes	yes	yes	yes	5	55	0	40	road, rangeland, undeveloped
1	10 August 2019	Pecos	30.90516	-101.88083	clear	yes	yes	yes	yes	40	30	5	25	road, undeveloped
1	17 October 2019	Pecos	30.90516	-101.88083	clear	yes	no	yes	yes	5	40	5	50	road, undeveloped
2	10 August 2019	Pecos	30.78851	-101.83502	slight-moderate	yes	yes	yes	no	20	60	0	20	road, undeveloped
2	17 October 2019	Pecos	30.78851	-101.83502	clear	yes	yes	yes	yes	20	65	5	10	road, undeveloped
3	18 May 2019	Pecos	30.71808	-101.80954	moderate	yes	no	yes	yes	0	15	5	80	road, rangeland, undeveloped
4	18 May 2019	Pecos	30.65960	-101.77022	moderate-heavy	yes	no	yes	yes	0	5	5	90	rangeland, road, undeveloped
5	5 June 2019	Terrell	30.46955	-101.80131	none	yes	no	yes	yes	0	60	5	35	undeveloped
5	11 August 2019	Terrell	30.46955	-101.80131	clear	yes	yes	yes	yes	15	35	0	50	undeveloped
7	7 June 2019	Terrell	30.46736	-101.80181	none	yes	yes	no	yes	10	70	0	20	undeveloped
9	10 August 2019	Crockett	30.45259	-101.71940	moderate	yes	no	yes	yes	0	0	10	90	road, undeveloped
10	6 June 2019	Terrell	30.45026	-101.73124	slight	yes	no	no	no	0	10	10	80	undeveloped
10	11 August 2019	Terrell	30.45026	-101.73124	clear	yes	no	no	yes	0	10	5	85	road, undeveloped
11	5 June 2019	Crockett	30.44767	-101.72119	slight-moderate	yes	yes	yes	no	10	10	20	60	undeveloped
11	18 October 2019	Crockett	30.44767	-101.72119	moderate-high	yes	no	yes	yes	0	0	5	95	undeveloped
13	30 March 2019	Val Verde	30.13120	-101.57450	slight-moderate	yes	no	no	yes	0	0	20	80	road, undeveloped
13	6 June 2019	Val Verde	30.13120	-101.57450	slight-moderate	yes	no	yes	yes	0	10	30	60	undeveloped, road
14	27 April 2019	Val Verde	29.89387	-100.99561	clear	yes	no	no	yes	0	10	20	70	undeveloped
14	20 July 2019	Val Verde	29.89387	-100.99561	clear	yes	yes	no	yes	5	10	5	80	undeveloped
14	19 September 2019	Val Verde	29.89387	-100.99561	clear	yes	yes	no	yes	5	10	5	80	undeveloped

15	20 July 2019	Val Verde	29.88591	-100.99292	clear	yes	no	yes	yes	0	10	5	85	undeveloped
15	19 September 2019	Val Verde	29.88591	-100.99292	clear	yes	yes	no	yes	10	20	10	60	undeveloped
16	28 April 2019	Val Verde	29.88385	-100.99397	clear	yes	no	no	yes	0	0	0	100	undeveloped
16	20 July 2019	Val Verde	29.88385	-100.99397	clear	yes	no	no	yes	0	0	5	95	undeveloped
16	19 September 2019	Val Verde	29.88385	-100.99397	clear	yes	yes	no	yes	5	0	5	90	undeveloped
17	22 June 2019	Val Verde	29.80829	-101.54893	heavy	yes	no	no	yes	0	0	5	95	undeveloped
18	22 June 2019	Val Verde	29.80564	-101.55088	heavy	yes	no	no	yes	0	0	10	90	undeveloped
18	23 August 2019	Val Verde	29.80564	-101.55088	heavy	yes	no	no	yes	0	0	10	90	undeveloped
20	23 June 2019	Val Verde	29.70431	-101.36667	heavy	yes	no	yes	no	0	0	10	90	undeveloped, road
20	23 August 2019	Val Verde	29.70431	-101.36667	heavy	yes	no	yes	no	0	10	10	80	undeveloped
21	21 June 2019	Val Verde	29.57490	-100.97809	clear	yes	no	yes	no	0	10	10	80	undeveloped
21	22 August 2019	Val Verde	29.57490	-100.97809	slight	yes	no	yes	no	0	20	5	75	undeveloped
21	3 October 2019	Val Verde	29.57490	-100.97809	slight-moderate	yes	no	yes	no	0	20	0	80	undeveloped
22	21 June 2019	Val Verde	29.54023	-101.01623	slight	yes	no	yes	no	5	10	30	55	undeveloped
23	21 June 2019	Val Verde	29.52420	-101.17585	clear	yes	no	yes	no	0	10	10	80	undeveloped
23	22 August 2019	Val Verde	29.52420	-101.17585	moderate	yes	no	yes	no	10	10	0	80	undeveloped
23	3 October 2019	Val Verde	29.52420	-101.17585	slight-moderate	yes	no	yes	no	10	40	0	50	undeveloped
24	20 June 2019	Val Verde	29.44737	-101.05667	clear	yes	no	no	no	0	0	5	95	undeveloped
24	2 October 2019	Val Verde	29.44737	-101.05667	slight	yes	no	no	yes	0	5	10	85	undeveloped
25	21 June 2019	Val Verde	29.42455	-101.04118	clear	yes	no	no	yes	0	10	10	80	undeveloped
25	21 August 2019	Val Verde	29.42455	-101.04118	moderate	yes	no	no	yes	0	40	10	50	undeveloped
26	17 May 2019	Val Verde	29.37719	-101.01348	slight	yes	no	yes	yes	0	0	20	80	residential, undeveloped
26	31 July 2019	Val Verde	29.37719	-101.01348	clear	yes	no	no	yes	0	40	0	60	undeveloped, residential, road
26	3 October 2019	Val Verde	29.37719	-101.01348	slight	yes	no	yes	yes	5	10	10	75	residential, undeveloped
27	16 May 2019	Val Verde	29.37029	-100.88526	clear	yes	yes	no	yes	0	50	10	40	residential, golf course

27	31 July 2019	Val Verde	29.37029	-100.88526	clear	yes	yes	yes	yes	40	40	5	15	residential, road
27	4 October 2019	Val Verde	29.37029	-100.88526	clear	yes	yes	yes	yes	45	40	5	10	road, residential
29	9 November 2018	Kinney	29.30944	-100.42125	clear	yes	no	no	yes	0	75	0	25	residential, road
29	11 May 2019	Kinney	29.30944	-100.42125	clear	yes	yes	no	yes	20	75	0	5	residential, road
29	26 June 2019	Kinney	29.30944	-100.42125	clear	yes	yes	no	yes	25	50	0	25	residential, road
29	30 July 2019	Kinney	29.30944	-100.42125	clear	yes	yes	no	yes	15	70	0	15	residential, road
30	11 May 2019	Kinney	29.30740	-100.41745	clear	yes	no	no	yes	0	25	70	5	residential, road
31	10 November 2018	Kinney	29.29273	-100.42075	clear	yes	no	yes	yes	0	10	0	90	residential, road
32	11 May 2019	Kinney	29.29043	-100.42386	clear	yes	no	yes	yes	0	20	40	40	residential, road
32	29 June 2019	Kinney	29.28638	-100.42263	moderate	yes	no	yes	yes	5	10	20	65	residential, road
32	30 July 2019	Kinney	29.28638	-100.42263	slight-moderate	yes	no	yes	yes	5	15	30	50	residential, road
34	10 November 2018	Kinney	29.28238	-100.42325	clear	yes	no	yes	yes	0	10	0	90	residential, road
35	10 November 2018	Kinney	29.28034	-100.42076	clear	yes	no	yes	yes	0	0	50	50	road, undeveloped
35	29 June 2019	Kinney	29.28034	-100.42076	slight-moderate	yes	yes	yes	yes	10	10	60	20	residential, undeveloped
35	31 July 2019	Kinney	29.28034	-100.42076	moderate	yes	no	yes	yes	10	10	55	25	residential, undeveloped,
36	11 March 2019	Val Verde	29.25473	-100.75216	clear	yes	no	yes	yes	20	10	0	70	road, rangeland
37	11 March 2019	Kinney	29.18898	-100.70340	clear	yes	no	yes	yes	0	5	15	80	road, rangeland
38	11 March 2019	Maverick	29.06453	-100.63899	clear	yes	yes	yes	yes	35	25	20	20	road, rangeland
39	11 March 2019	Maverick	29.00785	-100.63817	slight-moderate	yes	no	no	yes	0	0	0	100	road, rangeland
40	11 March 2019	Maverick	28.92578	-100.6149	moderate	yes	no	yes	yes	0	0	0	100	road, rangeland
41	10 March 2019	Maverick	28.77016	-100.49828	slight-moderate	yes	no	yes	yes	0	10	20	70	residential, road
42	9 March 2019	Maverick	28.70416	-100.51046	moderate	yes	yes	no	yes	0	0	0	100	residential, road
42	30 June 2019	Maverick	28.70416	-100.51046	moderate	yes	no	no	yes	0	10	10	80	residential, road
43	10 March 2019	Maverick	28.70294	-100.51089	clear	yes	no	yes	yes	0	25	0	75	residential, road
43	29 June 2019	Maverick	28.70294	-100.51089	moderate	yes	no	yes	no	5	15	20	60	residential, road

44	11 March 2019	Maverick	28.70146	-100.50979	slight-moderate	no	no	no	no	0	0	0	100	road, residential
44	1 July 2019	Maverick	28.70146	-100.50979	moderate	no	no	no	no	0	10	0	90	residential, road
45	9 July 2019	Webb	27.54447	-99.44098	moderate-high	no	no	no	yes	5	5	20	70	undeveloped
45	6 September 2019	Webb	27.54447	-99.44098	moderate	yes	no	no	no	0	20	5	75	undeveloped
46	9 July 2019	Webb	27.53861	-99.43475	moderate	no	no	no	no	5	5	20	70	undeveloped
46	6 September 2019	Webb	27.53861	-99.43475	moderate	yes	no	no	no	0	20	20	60	undeveloped
47	14 April 2019	Webb	27.52372	-99.52431	high	yes	no	yes	yes	0	0	25	75	road, residential,
47	9 July 2019	Webb	27.52372	-99.52431	moderate	yes	no	no	yes	5	10	5	80	residential, undeveloped,
47	6 September 2019	Webb	27.52372	-99.52431	high	yes	no	yes	yes	0	20	10	70	undeveloped, residential
48	14 April 2019	Webb	27.49835	-99.51674	high	yes	no	yes	yes	0	0	25	75	road, residential
48	9 July 2019	Webb	27.49835	-99.51674	moderate	yes	no	no	yes	5	5	10	80	residential, undeveloped,
49	14 April 2019	Webb	27.33117	-99.51195	high	yes	no	yes	yes	0	0	25	75	residential, undeveloped
49	5 September 2019	Webb	27.33117	-99.51195	moderate	yes	no	no	yes	0	0	5	95	undeveloped, residential
50	7 July 2019	Zapata	27.04330	-99.44496	moderate	yes	no	yes	yes	5	10	5	80	residential, undeveloped
51	28 May 2019	Starr	26.58179	-99.15259	moderate	no	no	no	no	0	20	20	60	undeveloped
51	7 July 2019	Starr	26.58179	-99.15259	moderate	yes	no	yes	no	0	10	0	90	undeveloped
51	4 September 2019	Starr	26.58179	-99.15259	moderate	yes	no	no	no	0	5	5	90	undeveloped
52	28 May 2019	Starr	26.54608	-99.17093	moderate	yes	no	no	yes	0	20	20	60	undeveloped
52	7 July 2019	Starr	26.54608	-99.17093	moderate	yes	no	no	yes	5	10	5	80	undeveloped
52	4 September 2019	Starr	26.54608	-99.17093	moderate	yes	no	no	yes	0	20	5	75	undeveloped
53	6 July 2019	Starr	26.53233	-99.15546	moderate	yes	yes	yes	yes	10	20	10	60	residential
54	11 November 2018	Starr	26.51429	-99.11662	clear	yes	no	no	yes	0	0	0	100	residential, road
54	28 May 2019	Starr	26.51429	-99.11662	moderate	yes	no	no	yes	0	20	20	60	residential, undeveloped
54	6 July 2019	Starr	26.51429	-99.11662	moderate	yes	yes	no	yes	10	20	20	50	undeveloped, residential
54	4 September 2019	Starr	26.51429	-99.11662	moderate	yes	yes	no	yes	0	25	5	70	undeveloped, residential

58	12 March 2019	Hidalgo	26.17906	-98.38716	moderate	no	no	no	yes	0	5	5	90	undeveloped
59	24 September 2019	Hidalgo	26.16934	-98.36742	slight-moderate	yes	yes	yes	yes	0	10	5	85	undeveloped
59	23 October 2019	Hidalgo	26.16934	-98.36742	slight-moderate	yes	no	yes	yes	5	10	5	80	undeveloped
60	23 October 2019	Cameron	25.85462	-97.37676	moderate-high	yes	no	yes	yes	0	10	10	80	undeveloped
61	24 May 2019	Cameron	25.85008	-97.39865	heavy	yes	no	yes	yes	0	30	10	60	undeveloped
61	24 September 2019	Cameron	25.85008	-97.39865	moderate-heavy	yes	no	no	yes	0	10	10	80	undeveloped