5-2020

Comparing Novel and Traditional Sampling Methodologies to Analyze the Population Status of the Rio Grande Cooter (Pseudemys gorzugi)

Amy P. Bogolin
The University of Texas Rio Grande Valley

COMPARING NOVEL AND TRADITIONAL SAMPLING METHODOLOGIES TO ANALYZE THE POPULATION STATUS OF THE RIO GRANDE COOTER (*PSEUDEMYS GORZUGI*)

A Thesis

by

AMY P. BOGOLIN

Submitted to the Graduate College of
The University of Texas Rio Grande Valley
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2020

Major Subject: Agricultural, Environmental, and Sustainability Sciences

COMPARING NOVEL AND TRADITIONAL SAMPLING METHODOLOGIES TO ANALYZE THE POPULATION STATUS OF THE RIO GRANDE COOTER (*PSEUDEMYS GORZUGI*)

A Thesis
by
AMY P. BOGOLIN

COMMITTEE MEMBERS

Dr. Abdullah F. Rahman
Chair of Committee

Dr. Richard J. Kline
Committee Member

Dr. MD Saydur Rahman
Committee Member

Dr. Drew R. Davis
Committee Member

May 2020

ABSTRACT

Bogolin, Amy P., Comparing Novel and Traditional Sampling Methodologies to Analyze the Population Status of the Rio Grande Cooter (Pseudemys gorzugi). Master of Science (MS), May 2020, 178 pp., 18 tables, 44 figures, references 225 titles.

The Rio Grande Cooter, *Pseudemys gorzugi*, is an understudied aquatic turtle species of conservation concern in the Rio Grande, Pecos, and Devils river systems. Traditional sampling methodologies for aquatic turtle species face numerous challenges, but novel sampling methodologies, such as drone-based surveys and environmental DNA analysis, may address these issues. This study compared novel sampling methodologies to traditional sampling methodologies in mean detections and identifications of aquatic turtle species, developed and implemented an environmental DNA assay to detect *P. gorzugi*, and characterized *P. gorzugi* habitat. Following an introductory chapter, each task is addressed by chapter and formatted to meet *Biological Conservation* guidelines. Novel sampling technologies were successful in detecting and identifying aquatic turtle species and we recommend implementation of these methodologies as a survey tool for aquatic turtle species. Additional studies should be undertaken to further evaluate *P. gorzugi* populations to better inform conservation and management decisions.

DEDICATION

I would like to dedicate the completion of my thesis to my family who has supported me every step throughout this unpredictable journey, and to my study subjects for providing continued inspiration.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my family, in particular my mother, who has stood with me every step of the way and has provided more strength and support than words can describe; and to my father, who has instilled his love of science in me. It was through your direction and example that has created the hard-working, motivated, and passionate scientist that I am today. I will be forever grateful for all these intangible gifts you have bestowed.

I would like to thank committee member Dr. Drew Davis, whose guidance through virtually every step of the project has made this study possible. Without your assistance and direction, this project would not have been completed. Additionally, I would like to thank the chair of my committee Dr. Abdullah F. Rahman, and committee members Dr. Richard Kline and Dr. MD Saydur Rahman for providing the opportunity to pursue my dream of studying turtles. Furthermore, I am forever gratefully to Krista Ruppert and Padraic Robinson, who really stepped up in order to produce valuable data in a limited time frame, and Ivy Hinson, who provided much-needed drone support.

I would also like to thank the Texas Comptroller Office for providing the funding for this project and the numerous outside organizations and individuals who granted us permission to sample on their property. This includes 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 (FAA) for issuing a waiver to conduct drone flights

in a no-fly zone. Several organizations generously granted us sampling permission including Fort Clark Springs Association, City of Del Rio, Eagle Pass Golf Course, San Felipe Springs Golf Course, The National Butterfly Center, and the United States Border Patrol, as well as private landowners, including J. Chandler and R. Jasso (Chandler Ranch), J. Lugo, R. Skiles, and K. Bowden.

Acknowledgments extend to Clint Guadiana of the Gladys Porter Zoo for allowing the collection of samples for preliminary eDNA analysis and Diana Lucio, also from Gladys Porter Zoo, for providing information on zoo records.

Lastly, I would like to thank Katie Harris and Blake Arnold for selflessly giving me the push I needed to accept this position. Katie, you have been a constant source of knowledge, support, and friendship, which I continue to value. It was through your example that I learned how to navigate graduate school. Blake, you have perhaps sacrificed the most of all, and I will never forget all that you have done to make my dreams come true. It would be fruitless to try and list all ways that you have supported me, but someday I hope to return the favor.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

The order Testudines is composed of fourteen extant families of turtles, with origins dating to over 200 million years ago. This ancient group is struggling to survive in modern times as seen in the drastic decreases in turtle populations worldwide, with 61% of the 356 turtle species considered threatened or extinct (Lovich et al., 2018). Several factors explain this alarming trend, including habitat destruction, collection for pet trade and food, and climate change, leading turtles to receive the designation as one of the most threatened of vertebrate groups (Lovich et al., 2018). Before the Anthropocene, turtles had large population sizes and as a result, compromised a large biomass across the landscape which increased their ability to provide many ecological services, such as seed dispersal and germination, nutrient cycling, and bioturbation of soils (Lovich et al., 2018). Their role in seed dispersal is especially important in the case of rare and endangered plants, and for some plants such as the Mayapple (*Podopyllum peltatum*), where the Eastern Box Turtle (*Terrapene carolina carolina*) is the only known seed dispersal agent (Rust and Roth, 1981). Several species are even considered to be ecosystem engineers or keystone species such as the Gopher Tortoise (*Gopherus polyphemus*) and the Mohave Desert Tortoise (*Gopherus agassizii*), providing habitats that are used by over 350 species (Kinlaw and Grasmueck, 2012; Catano and Stout, 2015; Johnson et al., 2017). Additionally, due to their longevity and role in mineral cycles, some turtle species have been found to be environmental indicators of mercury (Golet and Haines, 2001), radioactivity (Hinton

and Scott, 1990), and other pollutants (Herbert et al., 1993). With their important ecological roles and recent declines, it is essential that turtle species are studied in order to preserve this ancient order.

The Rio Grande Cooter (*Pseudemys gorzugi*) is a large, freshwater aquatic turtle species found in the Rio Grande, Pecos, and Devils river systems in southwestern Texas, southeastern New Mexico, and northeastern Mexico, including the Mexican states of Tamaulipas, Nuevo León, and Coahuila (Figure 1; Iverson, 1992a; Degenhardt et al., 1996; Dixon, 2013). The population in New Mexico is disjunct from the population in Texas and Mexico, with a 160 km gap separation likely due to anthropogenic changes of the Pecos River such as water extractions, pollution, and modification of flow rates (Ward, 1984; United States Department of the Interior, 1998). However, despite this separation both populations are genetically similar suggesting that this separation is recent (Bailey et al., 2008). *Pseudemys gorzugi* was recently classified as a unique species (Collins, 1991), with Ernst (1990) breaking from previous classification as a subspecies of River Cooter, *P. concinna*. Changes in classification were due to the lack of genetic exchange noted between these subspecies, different morphological characteristics, and an allopatric distribution from other *Pseudemys* (Ernst, 1990).

Pseudemys gorzugi reaches an average of 198 mm (male) and 243 mm (female) in carapace length with an elongate oval carapace covered in black, yellow, and green concentric circles (Figure 2; Ernst, 1990; Degenhardt et al., 1996; Bailey et al., 2014). Older males often become melanistic with an overall darkening of their carapace that obscures the patterning (Figure 2C; Bailey et al., 2005). Sexual dimorphism is pronounced, with females reaching larger adult sizes and males having a broader tail and longer foreclaws (Ernst, 1990; Degenhardt et al., 1996). The nesting season is assumed to last from April through August (Bohannon, 2019) with

a potentially later start to the nesting season in the New Mexico population (Suriyamongkol and Mali, 2019). *Pseudemys gorzugi* is omnivorous and opportunistic, consuming dicot and monocot vegetation, filamentous algae, and arthropods (Lindeman, 2007; Mali et al., 2018a; Letter, 2019). Juveniles exhibit a more specialized and more omnivorous diet, while adult males consume greater amounts of dicot vegetation and adult females consume more filamentous algae (Letter et al., 2019). They are active year-round and can be found in a range of habitats including shallow, clear streams, turbid waterways, and large, deep pools, inhabiting both lentic and lotic water bodies (Degenhardt et al., 1996; Pierce et al., 2016).

Few studies have been conducted on *P. gorzugi* due to its limited range, recent species designation, and elusive behavior. This has resulted in only one other turtle species receiving fewer citations in published literature out of all other turtle species in the United States and Canada (Lovich and Ennen, 2013). *Pseudemys gorzugi* is considered locally abundant in a few locations, though overall, low population densities are observed, and it is unknown if this is a natural characteristic of this species (Bailey et al., 2008; Dixon, 2013). Recent studies by Bailey et al. (2008) and Forstner et al. (2004) have shown that populations are patchily distributed and concentrated to only a few stretches of U.S. tributaries, with a concerning lack of juvenile *P. gorzugi* noted in Texas. In recent years, *P. gorzugi* populations have been subjected to numerous threats such as habitat degradation and collection for the pet trade (Bailey et al., 2014; Mali and Forstner, 2017). Modifications to river flow rates, flood control practices including construction of dams and channels, as well as water pollution from untreated sewage inflows, runoff from agriculture and mining, and atmospheric deposits, all place *P. gorzugi* populations at risk (Bailey et al., 2008), and have led to the designation of the Rio Grande as one of the top ten most endangered rivers in the United States (United States Department of the Interior, 1998; American

Rivers, 2003). Fishing bycatch and wanton killing of *P. gorzugi* by commercial and recreational river users have further threatened populations (Bailey et al., 2008; MacLaren et al., 2017). These concerns have led to a state designation of Threatened in New Mexico (New Mexico Department of Game and Fish [NMDGF], 2006) and Mexico (Secretaríade Medio Ambiente y Recursos Naturales, 2010), Near Threatened by the IUCN (Pierce et al., 2016), and a Species of Greatest Conservation Need in Texas (Texas Parks and Wildlife Department, 2012). Currently, its status is under review by the United States Fish and Wildlife Service in regard to a potential federal listing, with a decision to be made by the end of the fiscal year of 2021 (USFWS, 2015).

The multitude of threats facing *P. gorzugi*, in combination with the overall lack of knowledge of this species, highlights the need for data collection to ensure its survival (Pierce et al., 2016). With turtle species disappearing at an alarming rate, it has become imperative to understand the threats these species are facing to enact conservation measures to prevent further biodiversity loss (Lovich et al., 2018). It is essential that a thorough survey effort be undertaken throughout the Rio Grande and its tributaries to determine the current distribution and population health 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. This study was developed and conducted to address these needs and provide data on this understudied species. Chapter II compares the effectiveness of the novel sampling methodology of drone-based surveys against the traditional survey methods of visual and trapping surveys while locating and quantifying *P. gorzugi* populations. Chapter III discusses the development and implementation of another novel survey methodology of environmental DNA (eDNA) analyses in *P. gorzugi* detection. Finally, Chapter IV includes habitat characterization and water quality analysis of *P. gorzugi* habitat.

REFERENCES

American Rivers. (2003). *America's most endangered rivers of 2003*. Retrieved March 16, 2020, from https://s3.amazonaws.com/american-rivers-website/wp-content/uploads/2016/02/24220916/2003-mer-report.pdf.

Bailey, L. A., Dixon, J. R., & Forstner, M. R. J. (2005). Natural history note. Color pattern. Reticulate melanism in *Pseudemys gorzugi*. *Herpetological Review, 36*(3), 313.

Bailey, L. A., Dixon, J. R., Hudson, R., & Forstner, M. R. (2008). Minimal genetic structure in the Rio Grande cooter (*Pseudemys gorzugi*). *The Southwestern Naturalist*, *53*(3), 406–411.

Bailey, L. A., Forstner, M. R., Dixon, J. R., & Hudson, R. (2014). Contemporary status of the Rio Grande cooter (Testudines: Emydidae: *Pseudemys gorzugi*) in Texas: phylogenetic, ecological and conservation consideration. In *Proceedings of the Sixth Symposium on the Natural Resources of the Chihuahuan Desert Region. Texas: Chihuahuan Desert Research Institute* (pp. 320–334).

Bohannon, A. M. (2019). Reproductive biology of the Rio Grande cooter (*Pseudemys gorzugi*) in Texas. Final report to Texas Comptroller of Public Accounts.

Catano, C. P., & Stout, I. J. (2015). Functional relationships reveal keystone effects of the gopher tortoise on vertebrate diversity in a longleaf pine savanna. *Biodiversity and conservation, 24*(8), 1957–1974.

Collins, J. T. (1991). Viewpoint: a new taxonomic arrangement for some North American amphibians and reptiles. *Herpetological Review*, *22*(2), 42–43.

Degenhardt, W. G., Painter, C. W., & Price, A. H. (1996). *Amphibians and Reptiles of New Mexico*. University of New Mexico Press, Albuquerque, New Mexico. 431 pp., 123 plates.

Dixon, J. R. (2013). *Amphibians and Reptiles of Texas: With Keys, Taxonomic Synopses, Bibliography, and Distribution Maps.* Third Edition. College Station, Texas: Texas A&M University Press, 447 pp.

Ernst, C. H. (1990). *Pseudemys gorzugi*. *Catalogue of American Amphibians and Reptiles (CAAR), 461,* 1–2.

Forstner, M. R. J., Bailey, L., Ferrell, S., Dixon, J. R., & Hudson, R. (2004). *Population status, genetic structure, and ecological aspects of the Rio Grande River Cooter (Pseudemys gorzugi) in Texas*. Final technical report to the Non-Game Division of Texas Parks and Wildlife Department.

Golet, W. J., & Haines, T. A. (2001). Snapping turtles (*Chelydra serpentina*) as monitors for mercury contamination of aquatic environments. *Environmental Monitoring and Assessment, 71*(3), 211–220.

Hebert, C. E., Glooschenko, V., Haffner, G. D., & Lazar, R. (1993). Organic contaminants in snapping turtle (*Chelydra serpentina*) populations from southern Ontario, Canada. *Archives of Environmental Contamination and Toxicology*, *24*(1), 35–43.

Hinton, T. G., & Scott, D. E. (1990). Radioecological techniques for herpetology, with an emphasis on freshwater turtles. In: J. Whitfield Gibbons (ed.), *.Life History and Ecology of the Slider Turtle.* Smithsonian Institution Press, Washington, DC, pp. 267–287.

Iverson, J. B. (1992a). *A Revised Checklist with Distribution Maps of the Turtles of the World*. Privately printed, Richmond, Indiana. 363 pp.

Johnson, S. A., Ober, H. K., & Adams, D. C. (2017). Are keystone species effective umbrellas for habitat conservation? A spatially explicit approach. *Journal for Nature Conservation*, *37*, 47–55.

Kinlaw, A., & Grasmueck, M. (2012). Evidence for and geomorphologic consequences of a reptilian ecosystem engineer: the burrowing cascade initiated by the gopher tortoise. *Geomorphology*, *157*, 108–121.

Letter, A. W., Waldon, K. J., Pollock, D. A., & 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*(3), 204–208.

Lindeman, P. V. (2007). Diet, growth, body size, and reproductive potential of the Texas river cooter (*Pseudemys texana*) in the South Llano River, Texas. *The Southwestern Naturalist*, *52*(4), 586–594.

Lovich, J. E., & Ennen, J. R. (2013). A quantitative analysis of the state of knowledge of turtles of the United States and Canada. *Amphibia-Reptilia*, *34*(1), 11–23.

Lovich, J. E., Ennen, J. R., Agha, M., & Gibbons, J. W. (2018). Where have all the turtles gone, and why does it matter? *Bioscience*, *68*(10), 771–781.

MacLaren, A. R., Foley, D., Sirsi, S., & Forstner, M. R. (2017). Updating methods of satellite transmitter attachment for long-term monitoring of the Rio Grande Cooter (*Pseudemys gorzugi*). *Herpetological Review*, *48*(1), 48–52.

Mali, I. & Forstner, M. R. J. (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, A. W., & Suriyamongkol, T. (2018a). 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.

New Mexico Department of Game and Fish [NMDGF]. 2006. The comprehensive wildlife conservation strategy for New Mexico. Santa Fe: New Mexico Department of Game and Fish.

Pierce, L. J. S., Stuart, J. N., Ward, J. P., & Painter, C. W. (2016). *Pseudemys gorzugi* Ward 1984 - Rio Grande Cooter, Western River Cooter, Tortuga de Oreja Amarilla, Jicotéa del Río Bravo. In: Rhodin, A. G. J., Iverson, J. B., van Dijk, P. P., Saumure, R. A., Buhlmann, K. A., Pritchard, P. C. H., Mittermeier R. A., 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: Chelonian Research Foundation*, 1–12.

Rust, R. W., & Roth, R. R. (1981). Seed production and seedling establishment in the mayapple, *Podophyllum peltatum* L. *American Midland Naturalist*, *105*(1), 51–60.

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

Suriyamongkol, T., & Mali, I. (2019). Aspects of the reproductive biology of the Rio Grande Cooter (*Pseudemys gorzugi*) on the Black River, New Mexico. *Chelonian Conservation and Biology*, *18*(2), 187–194.

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

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.

USFWS [United States Fish and Wildlife Service]. (2015). Endangered and threatened wildlife and plants; 90-day findings on 31 species. *Federal Register, 80,* 37568–37579.

Ward, J. P. (1984). Relationships of Chrysemyd Turtles of North America (Testudines: Emydidae). Special Publications, the Museum Texas Technical University 21:1–50.

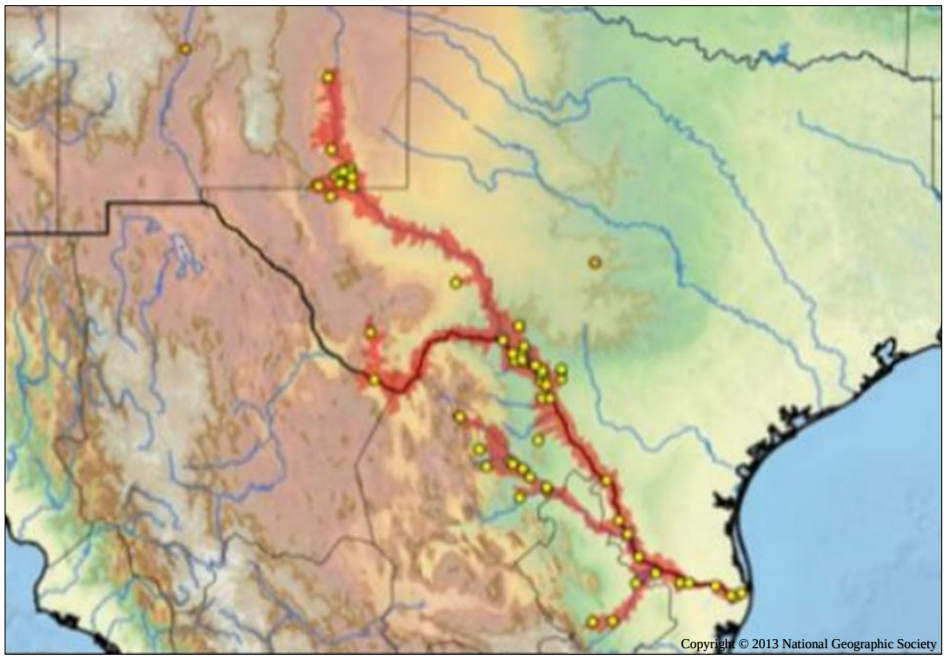

Figure 1. Historical range of *Pseudemys gorzugi* in southwestern USA and northeastern Mexico. Adapted from Pierce et al. (2016). Yellow dots indicate museum occurrence records of native populations and orange dots indicate introduced or misidentified specimens. The red shading is the projected historic distribution of *P. gorzugi*.

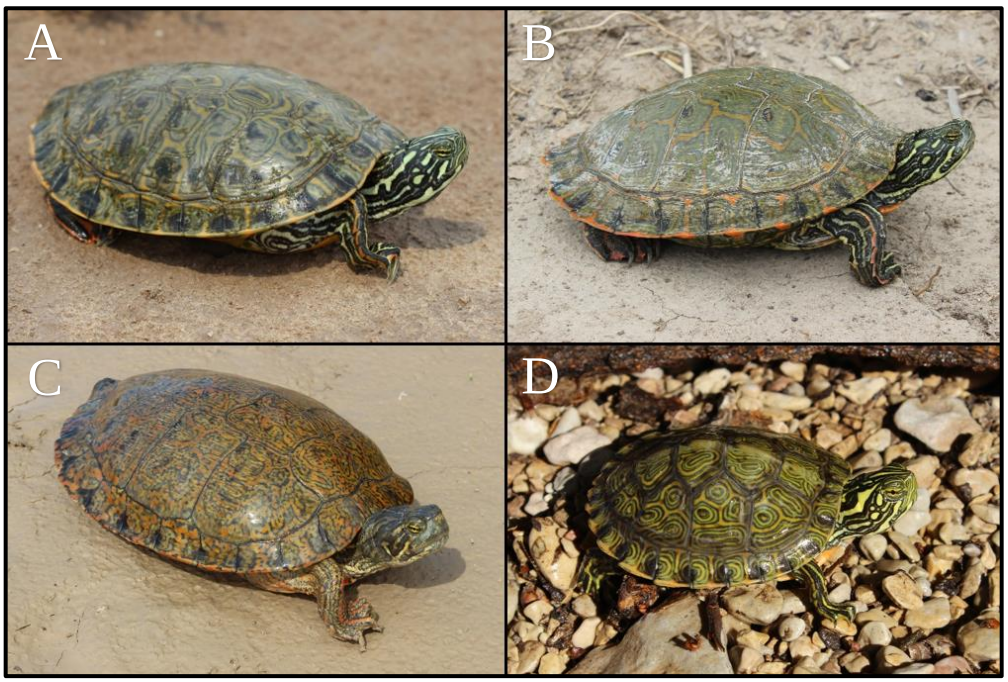

Figure 2. Representative photographs of *Pseudemys gorzugi* captured during this study. These photographs depict the variation amongst individuals in this species. (A) adult male (DRD 5628); (B) adult female (DRD 6101); (C) adult male showing the changes in coloration that occurs with some older males with reticulate melanism (DRD 6080); and (D) juvenile (iNaturalist 35863373). All photos by Drew R. Davis.

CHAPTER II

COMPARISON OF SAMPLING METHODOLOGIES TO INFORM CONSERVATION OF THE RIO GRANDE COOTER (*PSEUDEMYS GORZUGI*)

Introduction

Aquatic freshwater turtle species are often surveyed with traditional sampling methodologies, such as trapping with basking traps, seining, visual surveys, snorkeling and SCUBA surveys, with baited hoop-net traps being the most prominent (Beauvais and Buskirk, 1999; Buckland et al., 2000; Lancia et al., 2005). However, 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 differences in bait (Mali et al., 2012), sex (Ream and Ream, 1966), and trap design (Mali et al., 2012) influencing whether a turtle will enter a trap, and the presences of turtles in a trap can impact whether a turtle enters or not (Thomas et al., 1999). Trap happy (Deforce et al., 2004) and trap shy (Mali et al., 2012) turtles have been documented, as well as escapes of turtles from the traps (Mali et al., 2013). Less invasive sampling methodologies such as visual surveys are often less effective than trapping, especially for elusive species, and limited to areas where water access is available (Akre et al., 2012; Davy et al., 2015; Mali and Forstner, 2017).

For elusive species, such as *Pseudemys gorzugi*, traditional methodologies have resulted in mixed success (Christman and Kamees, 2007; Bailey et al., 2008; Mali et al., 2014; Mali et al.,

2018a). Particularly amongst Texas populations, survey efforts have been largely unsuccessful (Degenhardt et al. 1996; Bailey et al., 2014; Bonner and Littrell, 2016) resulting in *P. gorzugi* receiving the designation as one of the least studied turtle species in North America (Lovich and Ennen, 2013). Facing several threats such as habitat degradation, collection for the pet trade, and intentional destruction (Bailey et al., 2008; Bailey et al., 2014; Mali and Forstner, 2017), concerns over this species' conservation status has led to the designation of Threatened in New Mexico (New Mexico Department of Game and Fish [NMDGF], 2006) and Mexico (Secretaríade Medio Ambiente y Recursos Naturales, 2010), Near Threatened by the IUCN (Pierce et al., 2016), and a Species of Greatest Conservation Need in Texas (Texas Parks and Wildlife Department, 2012) with its status under review by the United States Fish and Wildlife Service in regard to a potential federal listing. A decision is to be determined by the end of the fiscal year of 2021 (USFWS, 2015). With the need for more information regarding this species, novel sampling methodologies such as small unmanned aerial vehicle (drone) surveys may demonstrate the potential to fill in information gaps.

With increasing familiarity and affordability of drones, conservation workers and wildlife biologists have embraced drone technology to light prescribed fires (Twidwell et al., 2016), map water sources (Su, 2015), search for invasive plants (Alvarez-Taboada et al., 2017), and to conduct wildlife surveys (Jones et al., 2006; Hodgson et al., 2013). To date, numerous species have been successfully surveyed including Bornean Orangutans (Burke et al., 2019), African Bush Elephants (Vermeulen et al., 2013), rhinoceroses (Mulero-Pázmány et al., 2014), Gentoo Penguins (Ratcliffe et al., 2015), geese (Chabot and Bird, 2012), Dugongs (Hodgson et al., 2013), whales (Aniceto et al., 2018), and sea turtles (Bevan et al., 2018) using drone-based surveys. Recently, freshwater aquatic turtle species have been added to the list of species

surveyed using drones, in three recent studies (Biserkov and Lukanov, 2017; Daniels, 2018; Karcher, 2019).

Drones can conduct programmed flights over a survey area and camera attachments take photographs or obtain video feed to be analyzed for population counts, abundance, threats, tracks, nesting sites, and multiple other types of data (van Germart et al., 2015; Christie et al., 2016; Jiménez Lopez and Mulero-Pázmány, 2019). They are relatively inexpensive when compared to traditional sampling methodologies, are less labor intensive, and drones can often survey areas where ground access is limited (Koh et al, 2012; Ratcliffe et al., 2015; van Gemert et al., 2015). Drones also have the benefit of being less invasive, and documented wildlife response to drone flights has been minimal (Linchant et al., 2015; Christie et al., 2016; Bevan et al., 2018) with flights as low as 7 m failing to disturb seabirds (Kudo et al., 2012). With technology and efficiency being continually improved, drones are expected to become widely incorporated into wildlife surveys (Koh et al., 2012; Christie et al., 2016; Rees et al., 2018).

Drone-based surveys for wildlife is a novel sampling methodology that has tremendous potential for wildlife management. The potential use of drone surveys was shown by Biserkov and Lukanov (2017) in a preliminary proof-of concept study and expanded upon by Daniels (2018) who compared drone surveys to visual surveys conducted with spotting scopes. This study, however, adds to the literature by increasing sample size, camera resolution, and number of comparisons to other methodologies.

While some improvements would greatly enhance the feasibility of drone surveys, it seems likely that they will be continually incorporated into freshwater turtle surveys. Limitations such as battery life are being continually addressed, with newer models offering longer flight times than previous models (Moon, 2017). Increased flight time permits larger survey areas,

increasing the practicality of these surveys. Decreasing payload can also increase flight time (DJI, 2020), and we recommend using smaller drones and cameras when possible. This study required a large drone to accommodate the payload of both a digital and multispectral camera, but in instances where habitat assessment is not needed, a smaller drone could support the weight of just the digital camera. Other restrictions such as weather, as the drone used in this study could not be flown in rain or wind over 17 mph, are more difficult to address and may need to be accepted as a limitation of this methodology.

In this study, we developed and implemented a drone-based survey to quantify *P. gorzugi* populations throughout the southwestern Texas portion of its range and compared these results to the traditional survey methods of visual and trapping surveys by examining detection and identification percentages. To the best of our knowledge, we are the first to compare these three sampling methodologies while surveying for a freshwater aquatic turtle species. Specifically, our objectives were to (1) determine the current range and population status of *P. gorzugi* to inform conservation efforts (2) develop and implement a drone survey protocol for aquatic turtle species and (3) compare the effectiveness of drone surveys against visual and trapping methodologies.

Materials and Methods

Study Sites

Study sites were located in southwestern Texas along the Rio Grande, Pecos, and Devils river watersheds, focusing on both the mainstem rivers and their tributaries. Research encompassed 61 unique localities from a most northwestern point near Iraan, Texas in Pecos County to a southeastern point near Brownsville, Texas in Cameron County (Figure 3; Table 1). Locations were chosen based upon *P. gorzugi* historical distribution (Figure 1), current

distribution records, and visual scouting events. A sizeable gap between our sampling sites existed along the Rio Grande between Eagle Pass and Laredo, Texas which was due to a lack of river access in this area. Substantial habitat variation occurred throughout the study sites, with differences in water body size, depth, flow rate, algal cover, and water source. Additionally, differences in the surrounding habitat occurred, with variances in human disturbance, riverbank height, topography, and vegetation cover and type (Figure 4).

In total, 61 unique localities were visited from November 2018 to October 2019 (Figure 3; Table 1). Some of these locations were opportunistic site additions upon discovery of *P. gorzugi* in the area. These opportunistic sites were often the result of turtles captured on land (Site 8), crossing roads (Site 28), or informal snorkel surveys (Site 6; Figure 3; Table 1). Additional scouting trips inspired from conversations with local residents produced photographic observations of *P. gorzugi* at Sites 55, 56, and 57, the furthest *P. gorzugi* have been recorded downstream in the Rio Grande in recent decades (Figure 3; Table 1). Due to logistical, financial, and time constraints, some of these localities as well as some of our traditional method comparison sites did not undergo every sampling methodology. Efforts were made to have a minimum of two sampling visits to each site throughout the study period, however, some variability existed, with one to three visits for each locality having occurred.

Drone Surveys

A DJI Matrice 600 Pro unmanned aerial vehicle (cat. # CP.SB.000308, SZ DJI Technology Co., Ltd, Shenzhen, Guangdong, China) was used to conduct drone surveys (Figure 5A). A Gremsy T-3 gimbal (cat. # Gremsy T3V3, Gremsy.com, Ho Chi Minh City, Vietnam) was attached and slightly modified to accommodate the digital and multispectral cameras that were used (Figure 5B). Flights were programmed using the Maps Made Easy App (Drones Made

Easy, San Diego, CA, USA) 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 6). This overlap was calculated using pre-set settings within this app, and are not accurate for the camera that we used. In reality, our overlap between transects was less than the app calculated, but was sufficient to cover the entire survey area. These flight parameters were chosen after a series of test flights to determine the optimal photograph resolution possible with minimal disturbance on turtle behavior. Flights were conducted in linear transects and perpendicular to the direction of flow in lotic systems to assist in photo-stitching. The entire study area was surveyed when possible, amounting to ca. 1.2 ha with a 10 m border around the water body. This area was determined as it 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 (SZ DJI Technology Co., Ltd, Shenzhen, Guangdong, China) was also utilized to conduct flights. These flights had a frontal 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. In order to minimize any potential impact that drone surveys could have on turtle detection (i.e., startled turtles seeking cover or leaving survey area) the order of drone and visual surveys was determined randomly using a random number set generated in Excel. All drone flights were conducted by Amy P. Bogolin and under a Federal Aviation Administration remote pilot license (certificate # 4189203).

High Resolution Digital Camera. A SONY ILCE α6000 E-mount camera with APS-C sensor (cat. # ILCE-6000, SONY, Kōnan, Minato, Tokyo) was attached to the drone via the gimbal

(Figure 5B). 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 RAW 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^2 with a pixel size of 1.4 mm.

After completing the flight, images were individually analyzed to detect and identify turtles. In order to differentiate between species, turtles were examined for distinctive markings characteristic of each species. *Pseudemys gorzugi* usually has distinctive yellow bands on top of the head and red-orange color webbing between the toes. Occasionally, the concentric circles on their carapace were visible in drone imagery as well (Figure 7). The Red-eared Slider, *Trachemys scripta elegans,* has red bands on the head by the tympana and often has yellow bands extending down the sides of their carapace (Figure 7A). However, these red markings are often faded in melanistic males, which likely led to the categorization of some of these turtles in the unknown grouping. The Spiny Softshell, *Apalone spinifera,* is a solid light gray or tan color, and the vertebrae of their backbone is visible through their leathery carapace (Figure 7B). The head of *A. spinifer*a is also much narrower than the other species, with an elongated protruding snout, the presence of which was used in photo identification (Figure 7B). A combination of these characteristics was used to determine the identification of each photographed turtle. In cases where a turtle's species was uncertain, these turtles were classified as unknown, but still counted. With a size of 1.4 mm/pixel, the photograph resolution was sufficient to detect species-

specific characteristics, and instances where species classification could not be assigned were likely due to turtles being obscured in water, vegetation, or shade, and from wind, which can move the camera during flight and reduce focus.

Photographs containing turtles were uploaded onto Google Maps utilizing their GPS stamps to determine their locations relative to other photographs containing turtles (Figure 8). Adjacent photographs were analyzed to determine whether any of the turtles present were duplicates from other photographs. This was accomplished by looking at individual characteristics of the turtles such as size, sex, unique markings, as well as their activity and location relative to their surroundings. Duplicates were likely, due to the large overlap between transects and high photograph interval rate, but most were easily identified. Challenges mostly arose in locations where large numbers of *P. gorzugi* were swimming in open water such as TNC Dolan Falls Preserve, Devils River, Dolan Falls (Site 16; Figure 3; Table 1) and Rio Grande, spillway below Amistad Dam (Site 24; Figure 3; Table 1). After accounting for duplicate turtles, final counts were determined for each species. encountered.

Visual Surveys

Visual surveys were conducted from the shore using Eagle Optics Ranger 10×42 binoculars (Eagle Optics, Middleton, WI, USA). All turtles visible from the shoreline were counted, noting species and behavior (basking, swimming, or in a trap). Turtles that could not be identified were marked as unknown, but their behavior was still noted. During the survey, the observer moved up and down the shoreline to gain additional vantage points when possible, but remained 3 m from the shoreline to minimize the observer's impact on turtle behavior. Survey durations were 15 min to coincide with average drone flight duration and an attempt was made to match the drone survey areas. This time frame was determined more than adequate to accurately

assess an area, with the majority of detections occurring in the beginning of the survey, and few, if any resulting in the final minutes. Once again, in order to minimize any potential impact that the survey could have on turtle detection, the order of the visual and drone methodologies was determined randomly from the random number set generated in Excel. Fifteen minutes was allowed between the two methodologies to give any startled turtles a chance to return to their former locations. One observer (Amy P. Bogolin) was used to minimize detection and identification variability that could occur due to differences in skill level and experience. The observer had prior experience with turtle identification and was familiar with the three species that were encountered.

Trapping Surveys

Three standard hoop-net traps (length: 182.88 cm, width: 121.92 cm, mesh size: 4.45 cm) 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 mouth of the trap, but also allowed a pocket of air to prevent drowning of trapped turtles as suggested in Lagler (1943). A combination of stakes and string was used to secure the traps to the shore and prevent trap collapse, and occasionally PVC piping was secured along the length of the trap to help keep the trap open. PVC piping was used primarily in sites with rocky substrates, which were difficult to drive stakes into. Traps were baited with canned sardines in oil, and the trap mouths were set facing downstream to allow for turtles following the scent from downstream to swim into the trap. Some localities were not suitable for trapping due to shoreline characteristics which prevented the traps from being secured to shore, fluctuating water depths from upstream dams, and lack of shoreline access.

To remedy this, floating traps were designed and implemented in the latter portion of this study. Floating traps used the same hoop-net traps used in our previous trapping efforts with the PVC piping holding the trap open, but additionally had swimming pool noodles secured lengthwise along the outside of the trap to keep them afloat (Figure 9). Pool noodles were secured approximately three quarters up the trap, which ensured an air pocket was still present in each trap to prevent trapped turtles from drowning. These traps were placed further away from shore in deeper water, and closer to known basking areas. Weighted kayak anchors (Brybelly Holdings, Inc., Greenfield, IN, USA), measuring 5.9 kg were tied to the end of the trap, preventing the trap from drifting downstream.

All traps were checked ca. 24 hr after deployment, and trapped turtles were removed for processing before being returned to the water (described below). Traps and bait were also checked during sampling to ensure that trap repairs were not needed, such as repairing tears in the mesh, and that the bait did not need replacing. Upon ca. 48 hr in the water, traps were pulled, with trapped turtles removed, processed, and released.

Turtle Processing. Trapped turtles, as well as a few opportunistic hand and snorkel captures, underwent a brief processing procedure. Measurements including straight carapace length (SCL) and width (CW), shell height (SH), and plastron length (PL) and width (PW) were obtained with Mantax Blue calipers (cat. # 11-100-1101, Haglöf Sweden AB, Långsele, Sweden) in millimeters. For the purposes of this study, plastron width was measured between the junction of the marginal, pectoral, and abdominal scutes on each side of the turtle. Turtles were then weighed on either an iBalance i2600 (cat. # SCM2600BLACK, HBI Technologies Phoenix, AZ, USA) or an iBalance i5500 (cat. # SCM5500BLACK, HBI Technologies Phoenix, AZ, USA) digital scale with turtles too heavy for the scale weighed with a fishing scale. Additionally,

turtles were sexed using secondary sexual characteristics according to Gibbons and Lovich (1990) and notched with a unique identification number on their marginals following a modified version of the system presented by Ernst et al. (1974; Figure 10). The first identification number assigned was 1001 to reduce the likelihood that we would issue a duplicate number from any previous studies that may have occurred in these areas. Additionally, turtles were numbered in succession regardless of species. Turtles that were already notched in previous studies maintained their previous identification numbers when possible and were assigned a new number if their previous identification number was already used. This was typically done by adding notches on their marginals corresponding to the thousands values.

Photographs were obtained of the carapace and plastron to help identify recaptures and provide photo vouchers of the individuals trapped. Additionally, a cloacal swab was obtained by rotating a cotton tipped swab three times inside the turtle's cloaca. Swabs were stored in test tubes containing 500 μL of DNA/RNA shield (cat. # R1100-250, Zymo Research, Irvine, CA, USA). A tissue clip from the webbing of the turtle's left hind foot was obtained and divided into two test tubes, one containing DNAzol (cat. # 10503027, Molecular Research Center, Inc., Cincinnati, OH, USA) and the other 95% ethanol. Both cloacal swabs and tissue samples were stored for use in future studies.

All turtle species captured underwent processing, however, plastron width was not obtained for *A. spinifera* and this species was not notched due to their morphology. Instead, photographs were used to identify these individuals, and the left hind foot was examined to look for missing tissue which could identify a recapture. *Apalone spinifera* was still assigned an identification number starting at AS1.

Methodology Comparison

One of the goals of this project was to determine which methodology was the most effective at surveying for *P. gorzugi*. This was determined through comparing the average number of total turtles detected, identified, and identification percentages amongst drone, visual, and trap surveys. Further analysis compared the average numbers of *P. gorzugi* identified amongst these methodologies. Environmental DNA (eDNA) analysis could not be included since this data is not quantitative and is described separately in Chapter III.

Sampling effort and units varied between methodologies, which made an equal comparison of these methodologies difficult. In order to determine which constituted an equal comparison we determined our ideal sampling efforts for each methodology. This was based upon our assessment of what sampling effort would be generally acceptable and logistically possible for each method. Any data that was collected from a sampling event in which sampling effort deviated greatly from our ideal was excluded. We determined our ideal sampling effort for drone surveys to be a flight that covered an area of ca. 1.25 ha, which was the maximum area that could be covered in one set of batteries in the Maps Made Easy app with our flight parameters. Visual surveys were 15 min, a duration based upon drone survey duration and preliminary tests, and ideal trapping events were three traps set at 48 trap h per trap for a total of 144 trap h per survey. Additionally, site visits that detected no turtles were excluded from analysis. This was to prevent our data from becoming zero-heavy and since a detection comparison is not possible when there are no detections. The resulting abundance and identification percentages for site visits were averaged to avoid pseudoreplication in our final dataset, which produced one value for each site and ensured that our data were independent from one another.

Statistical Analyses

Analyses were performed to test for differences in mean turtle detections, identifications, *P. gorzugi* detections, and identification percentages. Additionally, sites were sorted into several different categories based upon habitat characteristics, with the above analyses ran for each category to determine if differences existed between drone and trap data (Appendix A). The categories were spring-fed (yes or no), waterbody type (mainstem, tributary, or reservoir), turbidity (low, mid-level, high), flow (yes or no), connectivity (yes or no), algal mats (presence or absence), woody debris (presence or absence), trees (presence or absence), and shoreline vegetation (presence or absence).

The data for this project was non-normally distributed and groups had unequal variance as determined by Shapiro-Wilk and Welch's t-tests. Due to this, non-parametric tests, primarily Kruskal-Wallis and Wilcoxon multiple comparisons tests, were used for analysis. Means for all analyses are reported as mean (± 1 SD). All analyses were conducted in JMP v14 statistical software (SAS Institute, Cary, NC, USA).

Ethical Statement

All research was conducted under a Texas Parks and Wildlife (TPWD) Scientific Research Permit (permit # SPR-1018-294), TPWD State Park Scientific Study Permit (permit # 2019_R2_RGV_02), TPWD Aerial Wildlife and Exotic Animal Management Permit (permit # M-1603), NPS Scientific Research and Collecting Permit (permit # AMIS-2018-SCI-0007), The Nature Conservancy (Texas Chapter) Scientific Investigation and Collection Permit, International Boundary and Water Commission (IBWC) U.S. Section Permit (permit # USIBWC-19-2-0011), Certificate of Waiver or Authorization (certificate # 2019-P107-CSA-10089), and a University of Texas Rio Grande Valley IACUC protocol (protocol # AUP-18-28).

Results

Drone Surveys

Seventy-three drone surveys were conducted at 42 unique localities throughout the sampling period (Figure 11; Table 2). Drone flights conducted with the Maps Made Easy app were on average 14 min 32 sec (± 1 min 1 sec) in duration and both apps covered on average a survey area of 1.18 (± 0.21) ha. A total of 84,441 photographs from drone surveys were collected, resulting in 640 detections of turtles including *P. gorzugi* (n = 307), *T. s. elegans* (n = 93), *A. spinifera* (n = 89), and unidentifiable turtles (n = 151). The average identification percentage of turtles depicted in drone-based imagery throughout this study was 82.3% (± 27.8). *Pseudemys gorzugi* was detected at 18 (42.8%) of these unique localities (Figure 11; Table 2). Our average detections for each species at the conclusion of the study (n = 42 sites) included 4.21 (± 10.18) *P. gorzugi*, 1.29 (± 2.74) *T. s. elegans*, 1.22 (± 2.66) *A. spinifera*, and 2.07 (± 4.22) unidentifiable turtles (Table 2). The average detections for each species per site can be found on Table 2. The site with the highest number of *P. gorzugi* detections was the Rio Grande, spillway below Amistad dam, with 56 (± N/A) *P. gorzugi* identified on the one drone survey completed at that site **(**Site 24; Figure 11; Table 2). Including only sites where *P. gorzugi* was documented through drone-based surveys (n = 18 sites), the average detection was 9.59 (± 13.68) *P. gorzugi*.

Visual Surveys

In total, 84 visual surveys were conducted at 44 unique localities during the survey period (Figure 12; Table 3). Visual surveys resulted in 315 turtle detections with *P. gorzugi* (n = 91), *T. s. elegans* (n = 20), and *A. spinifera* (n = 25), identified, as well as turtles that were unidentifiable (n = 171). *Pseudemys gorzugi* was identified at 15 (34.1%) of the 44 localities surveyed (Figure

12; Table 3). Our average detections from visual surveys for each species at the conclusion of the study (n = 44 sites) included 1.1 (± 2.3) *P. gorzugi*, 0.3 (± 0.1) *T. s. elegans*, 0.3 (± 1.0) *A. spinifera*, and 2.0 (± 3.71) turtles that were unidentifiable (Table 3). The site with the highest average detections was TNC Dolan Falls Preserve, Devils River, Dolan Falls with an average of 7.7 (± 4.2) *P. gorzugi* identified per visit (Site 16; Figure 12; Table 3). The average detections of *P. gorzugi* when only including sites where *P. gorzugi* was ever visually detected (n = 15 sites) was 2.8 (± 2.2) individuals. Identification percentages varied among sites, with an overall average identification percentage of 50.8% (± 35.1) for all visual surveys conducted (Table 3).

Trapping Surveys

Trapping surveys occurred at 39 unique localities for a total of 8096 trapping h, constituting 66 trapping events (Figure 13; Table 4). This produced an average overall trap effort of 43.87 (± 7.09) h per trap. Some trapping efforts were less than our ideal trapping effort of 48 h due to issues with trap collapse, trap theft, tears in the traps, and variable water levels from dam releases. All trapped turtles were identified, leading to an identification percentage of 100%, with *A. spinifera, T. s. elegans*, and *P. gorzugi* detected throughout the study period. *Pseudemys gorzugi* was trapped at 18 (46.2%) of the 39 localities sampled **(**Figure 13; Table 4). At the conclusion of this study, the average turtles trapped per species was as follows (n = 39 sites): 1.03 ± 1.82 *P. gorzugi*, 1.65 ± 3.31 *T. s. elegans*, and 0.80 ± 1.29 *A. spinifera*. The highest average number of *P. gorzugi* trapped was 7.0 (± N/A) individuals that occurred during the one trapping event at the Pecos River, 0.3 river km upstream of confluence with Independence Creek (Site 11; Figure 13; Table 4). The highest number of *P. gorzugi* trapped per hour (± 1 SD) occurred at Fort Clark Springs, Las Moras Creek, upstream of golf pro shop with 0.03 (± 0.03) *P.*

gorzugi per h (Site 32, Figure 13; Table 4). When only including sites where *P. gorzugi* was ever successfully trapped (n = 18 sites), the average detections amounts to 2.96 (± 1.97) *P. gorzugi*.

A few *P. gorzugi* were taken as vouchers throughout the course of this study and deposited in the Biodiversity Collections, University of Texas at Austin (TNHC). This included two individuals from Crockett County, both adult male *P. gorzugi* (TNHC 114131 [DRD 6080]; TNHC 114132 [DRD 6081]). Additionally an adult male *P. gorzugi* was vouchered from Val Verde County (TNHC 114465 [DRD 5628]).

Turtle Processing. Overall, 242 unique turtles were processed, including 86 *P. gorzugi*, 101 *T. s. elegans*, and 55 *A. spinifera.* Trapping resulted in the capture of 219 of these turtles, and 23 (19 *P. gorzugi*, 2 *T. s. elegans*, and 2 *A. spinifera*) turtles were captured by hand and during opportunistic snorkel surveys. Only adult turtles were trapped; the two juveniles processed, both *P. gorzugi*, were a result of hand captures. Seven turtles were recaptured throughout the course of the study. Measurements of processed turtles can be found in Table 5. The average SCL for *P. gorzugi* was 193.8 mm (± 43.3) for males and 233.3 mm (± 55.1) for females, with the largest *P. gorzugi* a female of 304 mm SCL (Table 5). The average mass for *P. gorzugi* was 1026.1 g (± 573.6) for males and 1886.3 g (± 1066.4) for females, with the largest *P. gorzugi* a female with a mass of 3964.0 g (Table 5). While the turtle with the longest SCL may have been larger than the latter turtle, issues with the scale prevented an accurate measurement from being obtained.

Most turtles processed throughout the course of the study appeared outwardly healthy. A few individuals had leeches present on their soft tissue. One hand captured adult female *P. gorzugi* (DRD 5884) from TNC Independence Creek Preserve, raceway below Upper Lake, Terrell County (Site 7; Figure 3; Table 1) had sustained severe damage to the limbs, most likely the result of a predator attack. Due to the severity of this injury, this individual was euthanized

and vouchered. A trapped *T. s. elegans* (DRD 5649) from Rio Grande, weir below Amistad Dam (Site 25; Figure 3; Table 1) also had sustained severe injury to the limbs. It was found partially drowned in a collapsed trap. This site was located below Amistad Dam and unbeknownst at the time experienced fluctuating water levels. Most likely a predator collapsed the trap when the water level was low and partially consumed the turtle, leaving once the water level began to rise at the next dam release. This individual was also euthanized and vouchered. Additionally, predation of a juvenile *P. gorzugi* was observed while scouting sampling sites in Del Rio, Texas, immediately adjacent to Del Rio, San Felipe Springs Golf Course, San Felipe Creek (Site 27; Figure 3; Table 1). The individual (ca. 5 cm) was seen being manipulated in the bill of a Yellow-crowned Night Heron (*Nyctanassa violacea*) and while actual consumption was not observed before the heron flew off, the turtle appeared unresponsive by that time (Bogolin et al., 2019a).

Photographs of all processed turtles were uploaded to iNaturalist as part of the Herps of Texas project (https://www.inaturalist.org/projects/herps-of-texas) and given the tag "TX Comptroller – UTRGV – Pseudemys gorzugi" in order to group these records together.

Methodology Comparison

Our actual average flight area was 1.18 ha (± 0.21) ha. Anything that deviated more than two standard deviations from the mean was not included in the analysis (n =1), with the exception of two flights that were conducted on smaller bodies of water, in which case the whole water body was surveyed, as a larger survey area would not have resulted in more turtle detections. This resulted in the elimination of a single aborted flight that occurred when the drone lost connectivity with the controller. One 10-min visual survey, which was shortened to coincide with a shorter flight time over a small body of water, was removed from analysis to eliminate any inconsistencies in this sampling methodology. Our average trap hours was 43.87 h

(± 7.09). Any trapping effort more than two standard deviations away from this mean was excluded (n = 3). This excluded shortened trap effort that resulted from trap theft, fluctuating water levels from dam release, and preliminary trapping efforts that occurred before our sampling protocol was finalized. Turtles were not detected via any sampling methodology on 17 sampling events which included three unique sites: (1) Pecos River, ca. 0.4 river km below confluence with Independence Creek (Site 12; Figure 3; Table 1), (2) Lake Amistad, along Spur 406 (Site 22; Figure 3; Table 1), and (3) Fort Clark Springs, Las Moras Creek, near guard station (Site 30; Figure 3; Table 1).

The mean number of turtle detections across sampling methodologies was not found to be significantly different ($H = 2.55$, $df = 2$, $p = 0.28$; Figure 14). Analysis of *P. gorzugi* detections among survey types also were not significantly different ($H = 1.93$, $df = 2$, $p = 0.38$; Figure 15). The mean number of turtle identifications across sampling methodologies was found to be significantly different ($H = 9.70$, $df = 2$, $p = 0.008$) with both drone surveys ($p = 0.004$) and trapping surveys ($p = 0.019$) resulting in more identifications than visual surveys (Figure 16). Mean turtle identifications between drone and trapping surveys were not found to be significantly different ($p = 0.58$; Figure 16). A significant difference in identification percent was found among survey methods ($H = 42.73$, $df = 2$, $p < 0.001$; Figure 17). The identification percent for trapping surveys was significantly higher than both drone ($p = 0.001$) and visual surveys ($p < 0.001$; Figure 17). The identification percent was also higher for drone surveys compared to visual surveys ($p < 0.001$; Figure 17).

Variance in count data for each methodology was observed in several sites with large turtle populations such as Rio Grande, spillway below Amistad Dam (Site 24; Figure 3; Table 1), where on 2 October 2019, the drone survey detected 80 unique turtles (n = 56 *P. gorzugi*) while

only 10 turtles (n = 0 *P. gorzugi*) were detected during the visual survey, and trapping resulted in only six turtles (n = 0 *P. gorzugi*). Similar results were observed on 19 September 2019 at TNC Dolan Falls Preserve, Devils River, Dolan Falls (Site 14, Figure 3; Table 1) where the drone survey documented 66 unique turtles (n = 55 *P. gorzugi*), the visual survey produced 18 turtles detections (n = 9 *P. gorzugi*) and only one turtle (n = 0 *P. gorzugi*) was trapped. With the highest number of turtle detections for a visual survey being 28 (4 October 2019 in Del Rio, San Felipe Golf Course, San Felipe Creek; Site 27, Figure 3; Table 1) and 18 for trapping (1 July 2019 in Eagle Pass Golf Course, settling pond along Rio Grande; Figure 3; Table 1), drone surveys showed their potential to outcompete the other methodologies in turtle detections. However, at low density sites where turtle detections were low overall, all survey methodologies appeared to perform similarly.

When examining differences between drone and trap data, there was no significant difference in mean identification percentage between drone and trap methods at sites that had no connectivity ($p = 1.00$), no flow ($p = 0.07$), and no trees ($p = 0.11$). Additionally, there was no significant difference in mean identification percentage between drone and trap methods at sites with high turbidity ($p = 0.29$) and reservoir sites ($p = 0.19$). In the rest of the analyses, mean trapping identification percentages were higher than drone identification percentages, which matched the overall comparison between mean identification percentages of drone, visual, and trapping surveys. Additionally, pairwise comparisons failed to detect a significant difference in overall number of turtle detections between the three methodologies in all categories.

Discussion

Drone Surveys

Once the drone protocol was established, surveys resulted in high-quality imagery with minimal disturbance to turtles and other wildlife. With its unique aerial viewpoint, the drone was able to document several turtles than were not visible from shore (Figure 18). The superiority of an aerial vantage to that of a ground viewpoint for collecting population count data was also noted in Hodgson et al. (2018). A high number of overall detections demonstrates the ability of drone-based surveys to locate turtles in their natural environment. High identification percentages further demonstrated the ability to determine the species of turtles detected, which is essential for species-specific surveys. These characteristics are crucial for wildlife surveys (Morrison et al., 2008), and drone-based surveys were able to meet these requirements of high overall abundance and identification percentages demonstrating its applicability. Identification percentages could likely be even further improved upon with fine-tuning the camera settings.

Drone surveys exceeded expectation, producing an abundance of additional data to supplement the quantification and identification which was originally sought. Numerous identifiable behaviors were documented, including mass basking, with 26 *P. gorzugi* sharing a single basking rock (Figure 19) at Eagle Pass Golf Course, spillway into Rio Grande (Site 42; Figure 11; Table 2) on 9 March 2019. This observation supports previous observations of *P. gorzugi* basking in large numbers as noted by Mali et al. (2018b). 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 algal mats and other aquatic vegetation (Figure 19). This has been previously observed,

particularly in the hot summer months, when high temperatures discourage aerial basking (Mali et al., 2018b).

Courting behaviors were captured multiple times throughout this study and were more prevalent at sites with large *P. gorzugi* populations such as Eagle Pass Golf Course, spillway into Rio Grande (Site 42; Figure 11; Table 2) and Rio Grande spillway below Amistad Dam (Site 24, Figure 11; Table 2; Figure 19). Courting was observed throughout the sampling period (March–October), suggesting that reproduction could occur during a large portion of the year. Past literature only includes one instance of courting, which was documented on 12 July 2016, and as such, our findings greatly expand upon the timing of reproductive ecology for this species (Mali and Forstner, 2017).

Limited research exists on *P. gorzugi* diet and feeding habits, and we were able to document foraging behaviors of *P. gorzugi* with drone imagery (Figure 19). Throughout a series of photographs, the drone documented an adult male *P. gorzugi* approach and begin to consume a piece of aquatic vegetation floating on the surface of the water at TNC Dolan Falls Preserve, Devils River, Dolan Falls on 27 April 2019 (Site 16; Figure 11; Table 2). This confirms the suggestion in Letter et al. (2019) that at least some foraging occurs at the water's surface. The documentation of all these behaviors shows the potential uses of drone surveys for wildlife documentation, going beyond quantification by including descriptions of *P. gorzugi* natural history.

Confirming occupancy and noting behaviors are two potential aspects of drone-based surveys that could be applicable to other species as well. Throughout the study, numerous species of non-target wildlife were documented in drone imagery including several species of birds, fish, and invertebrates (Figure 20) confirming the potential of drones to survey for some of

these species as identified by McEvoy et al. (2016). Animals seemed undisturbed by the presence of the drone flying overhead, with little impact observed on their behavior supporting the findings of Kudo et al., 2012. On one occasion an Osprey (*Pandion haliaetus*) caught and consumed a fish while the drone was flying overhead. The fine resolution of the imagery allows for even butterflies to identified by species (Figure 20). Additionally, drone-based surveys gathered valuable habitat data. On several occasions, *P. gorzugi* was observed basking near trash, showing that pollution or degraded habitats may not prevent their occurrence (Figure 21). Drone imagery was also able to document tracks in the mud created from turtles crawling through shallow water (Figure 21) confirming that drones can be used to detect turtle tracks (van Gemert et al., 2014). The detection of tracks and other signs of wildlife highlights the potential of drone-based surveys to target potentially suitable habitats used by species, even when they are not directly observed.

While drone-based surveys have many benefits, several challenges have yet to be fully addressed. Throughout our study, drone surveys faced numerous implementation challenges. The U.S. Federal Aviation Administration requires drone operators to obtain a Remote Pilot License, which requires passing an aeronautical knowledge exam (Federal Aviation Association, 2020). Federal agencies require an additional lengthy permitting process for drone aspects of studies, and the USFWS currently has a no-drone policy which denied us access to survey on their land (Legal Information Institute, 2020). Photographing wildlife through aerial methods requires additional permits as well (AWM Permits, 2020), and obtaining all these can be a time-consuming process which took us several months (Appendix B).

Discovering optimal camera and flight parameters was also a time and labor-extensive process. Joyce et al. (2019) had previously noted similar difficulties, particularly in marine and

freshwater environments, due to the complexities of working over water. As the movement of drone flight prevented the use of automatic camera settings, manual settings had to be determined. As it was not possible to adjust camera settings during a flight, a separate flight had to be conducted to test each camera setting. In order to limit the amount of flights required, camera tests were initially conducted on the ground. However, once the angle of the camera was changed 90° to obtain a nadir viewpoint, the position in which it was mounted on the gimbal, the optimal camera settings changed, and all ground trials were of no use. Overall, 216 different camera settings were tested for this study, but only a subset of these were tested in aerial flights. Likely, resolution could have been further enhanced by fine-tuning these settings further; however, due to time constraints, we were unable to conduct additional tests and instead used settings which still produced adequate results. Only one set of camera settings were used throughout the duration of this study, which produced lower quality imagery in low-light conditions. To alleviate these effects, drone flights were conducted between 0900 h and 1700 h to avoid periods of low light whenever this was possible. Future drone surveys could additionally benefit from determining optimal conditions in different levels of light.

Several drone equipment issues arose throughout the study which were the result of a few different factors. Novel technologies are likely to experience issues as time has not allowed for issues to be identified and addressed, and some of the technical issues we faced were likely a result of lack of historical precedent (Gregory et al, 2015). An unexplained crash, connectivity issues, and various error messages that occurred in the apps we used were likely due to instances such as these. Furthermore, the field conditions encountered throughout this study were very challenging for technological equipment. The average air temperature was 29°C with our warmest day at 39°C. The temperature threshold for the drone and digital cameras was 40°C

(DJI, 2018; SONY, 2014) however, the equipment often experienced temperatures above this threshold, with battery temperatures in the low 40°C range after several flights. Batteries would retain heat for an extended time, requiring cooling in air conditioning for several hours before they were cool enough to recharge. On several occasions the tablet used to conduct drone flights overheated and shut off, requiring the drown to be flown with the tablet in an airconditioned vehicle. The multispectral camera also overheated and became unresponsive on a handful of occasions, and it is likely that some of the technological issues and subpar performances were due to the high temperatures experienced. Similarly, Hui (2019) experienced issues with equipment overheating during his study, which was resolved by changing to another brand which could better tolerate the environmental conditions he encountered. Fortunately, in many locations temperature extremes will be infrequently experienced and this should improve performance. In areas where this cannot be avoided, however, we suggest limiting drone flights to cooler periods of the day and year when possible. Due to the time constraints of this study, this was not possible.

With time and experience, most drone issues were able to be addressed, and technological advancements should solve remaining issues as drones continue to be implemented in scientific studies. We acknowledge the potential of drone surveys to document wildlife and believe that implementation should be feasible as technology continually progresses. The drone protocol depicted in this study can be tailored to different environments, and we encourage further exploration into its different applications.

Visual Surveys

Visual surveys have been a mainstay of aquatic turtle surveys due to their low cost, low effort, and minimal time requirements (Weber and Layzer, 2011). This study corroborated these

qualities with visual surveys facing few implementation challenges. For this study, visual surveys were always able to be conducted once instated into the sampling protocol, proving visual survey's widespread applicability to different sampling areas. With its ease of use and low costs, visual surveys will likely remain as a quick and easy wildlife assessment tool.

Despite these advantages, however, numerous challenges had to be addressed during this study that became apparent during visual survey. Differences in shoreline habitat drastically affected the quality of the visual surveys, with areas with tall shoreline vegetation (mostly *Phragmites* sp.), including the majority of our Rio Grande sites, greatly reducing the amount of survey area that was observable from the shore. At certain locations, such as TNC Dolan Falls Preserve, Dolan Creek, near confluence with Devils River (Site 15; Figure 12; Table 3), Rio Grande, near Langtry (Site 18; Figure 12; Table 3), and Rio Grande, spillway below Falcon Dam (Site 52; Figure 12; Table 3), less than 30 m of river length was visible from shore, greatly reducing the number of possible turtle detections, which was similarly recognized by Davy et al. (2015).

Additionally, observer bias remains an intrinsic component of visual surveys, with results dependent upon the skill level and experience of the observer (Anderson et al., 2001). To keep this bias constant one observer can be used (Mali and Forstner, 2017) as was done for this study. Some detections, however, may have been missed that could have been detected by someone with more experience, and additionally more identifications may have been possible. On one sampling event at TNC Independence Creek Preserve, Lower Lake (Site 5; Figure 12; Table 3) three visual surveys were conducted simultaneously by observers with differing levels of experience. The results of these surveys were seven, six, and two turtle detections, highlighting the potential effects of observers on survey results. This variability in detection probability can

greatly misinform population models (Gu and Swihart, 2004) and questions the capability of visual surveys to accurately determine abundance (Lancia et al., 1994).

Identification proved to be challenging for visual surveys as throughout the study few turtles were observed basking out of the water, and turtles swimming in the water were often obscured by aquatic vegetation or glare from the sun, hindering the detection of species-defining characteristics. This resulted in a low identification percentage overall, with many turtles being listed as unknown species. As species-specific data is often required for wildlife surveys (Morrison et al., 2008), this is a considerable disadvantage for visual surveys. Previous studies that have utilized visual surveys for aquatic turtles have had much higher identification percentages than was observed in our study, with less than 10% of the observed turtles unidentified in one study (Lindeman, 2015) and 3% unidentified in another (Weber and Layzer, 2011). In both of these studies the majority of the turtles observed were aerially basking and this increased visibility likely led to the higher identification percentages than we observed throughout our study. Visual surveys may be more effective for other species in different environments, but the behavioral tendency of *P. gorzugi* to engage in subaerial basking may limit the effectiveness of this survey methodology for this species, at least during the hot summer months.

While identification percentages may have been less than desired, three species were identified throughout the study, *A. spinifera*, *T. s. elegans*, and *P. gorzugi*, all of which were expected to occur in the survey area. The higher amount of *P. gorzugi* detections compared to other species could be indicative of larger *P. gorzugi* populations, but also may be due to this species tendency to aggregate in aquatic vegetation to subaerial bask, increasing their visibility (Mali et al., 2018b). Average detections were also much higher when excluding sites where *P.*

gorzugi was never visually detected, which could be due to this apparent clustered distribution (Bailey et al., 2014). Only two previous studies have conducted formal visual surveys for *P. gorzugi*, each producing variable results. In one study, four *P. gorzugi* were documented through 29 h of active search (Christman and Kamees, 2007). Another study observed between 18 and 44 *P. gorzugi* in nine different surveys that were between 55 to 80 min in length (Mali et al., 2018b). Our average detections of *P. gorzugi* fall between these two studies, however, comparisons are difficult due to the variances in the methodologies of each visual survey, most notably the use of a boat in Mali et al. (2018b). For our study, the sites with the highest average detections of *P. gorzugi* were TNC Dolan Falls Preserve, Devils River, Dolan Falls (Site 16; Figure 12; Table 3), Fort Clark Springs, Headwater Pond (Site 29; Figure 12; Table 3), and Del Rio, San Felipe Springs Golf Course, San Felipe Creek (Site 27; Figure 12; Table 3) which is likely due to a combination of large *P. gorzugi* populations in these areas and favorable shoreline accessibility, resulting in increased visibility.

Trapping Surveys

Trapping surveys are often at the forefront of aquatic turtle surveys, with capture data supplementing detections. Offering a 100% identification percentage and the opportunity to collect population health data such as measurements and tissue samples, trapping was found to provide unique advantages by physically capturing turtles during this study as noted by Jgermano (2012). Additionally, captured turtles can be vouchered, providing long-lasting specimens to document species presence in an area. In this study, we were able to trap and voucher a *P. gorzugi* at Pecos River, 0.8 river km upstream of confluence with Independence Creek (Site 9; Figure 13; Table 4) providing the first documentation of *P. gorzugi* in Crockett County (Bogolin et al., 2019b). Due to the unique data generated from trapping surveys, the

continuation of trapping in aquatic turtle surveys will likely persist, especially when measurement or health data needs to be collected from individuals.

A handful of challenges and numerous implementation issues often surmount when utilizing trapping surveys. Throughout the study we had several instances where traps completely or partially collapsed, as was similarly seen in DonnerWright et al. (1999). This occurred most often in study sites that were downstream from dams and subject to variable water levels including Rio Grande, weir below Amistad Dam (Site 25; Figure 13; Table 4) and Rio Grande, near Lugo property (Site 26; Figure 13; Table 4). Additionally, sites downstream from dams that experienced variable water levels occasionally had water levels drop below the mouth of the trap, preventing turtles from entering. Waterbodies with high flow and shallow waters provided parallel challenges for baited hoop-net trapping efforts in Sharath and Hegde (2003). Floating traps appeared to be an effective solution for variable water levels in this study, allowing the trap to move with rising and falling water levels, and would be advantageous to implement more thoroughly in future studies. Trapping surveys also require a favorable river substrate. At a few sites traps could not be placed due to inadequate river substrate such as Pecos River, near confluence with Rio Grande (Site 20; Figure 13; Table 4) where thick sinking mud prevented water access for trap deployment and at Fort Clark Springs, Headwater Pond (Site 29; Figure 13; Table 4) where cement banks prevented trap securement and installation. Ream and Ream (1966) similarly noted how environmental factors dictated where traps could be placed; a restriction inherent to the trapping method.

Trap theft remains an unfortunate yet common problem in trapping surveys (Boundy and Kennedy, 2006; Valdeón et al., 2010; Brown et al., 2011) and on one occasion at Rio Grande, Laredo, near international railroad bridge crossing (Site 48; Figure 13; Table 4) our traps were

stolen. Additionally, traps appeared tampered with at Lake Amistad, Box Canyon (Site 23; Figure 13; Table 4) and Rio Grande, near El Cenizo (Site 49; Figure 13; Table 4), and it is likely our traps were disturbed more frequently than realized as many of our trapping sites were at public access points. At Rio Grande, near Salineño (Site 54; Figure 13; Table 4) our traps were once inadvertently removed by TPWD Game Wardens who thought they were illegally set. In these instances, it is unknown if turtles were removed from traps when they were tampered with, decreasing the credibility of the data collected.

While environmental issues often hinder the implementation of trapping surveys, other factors can discourage the use of this method as well. Overall, trapping is a time consuming and labor-intensive process, requiring installation of the traps, multiple checks to remove and process turtles, and trap removal, a process which occurs over several days. While our surveys were only 48 h, many trapping surveys occur over a longer time span, with several previous *P. gorzugi* studies including 6 d per trapping event (Mali et al., 2018b; Mali et al., 2014; Suriyamongkol and Mali, 2019). Brown et al. (2011) found that high-intensity, short-duration trapping events can be just as effective as low-intensity, long-duration sampling, but this shorter time requirement is still labor-intensive and more time-intensive than other methods used during this study. Even with our relatively short trapping period, trapping surveys generated a large time commitment, with its implementation as a survey methodology greatly changing our sampling schedule and constricting the number of sites we could sample. Increased labor and time requirements can increase costs as well, making this sampling methodology unfeasible for some organizations.

In this study, trapping success was mixed; however, trapping of *P. gorzugi* was largely unsuccessful at some high-density sites such as TNC Dolan Falls Preserve, Devils River, Dolan Falls (Site 16; Figure 13; Table 4) and Rio Grande, spillway below Amistad Dam (Site 24;

Figure 13; Table 4) suggesting that bait type or trap placement could prevent their capture. Adults are primarily herbivorous, so it is possible that they do not respond strongly to canned sardines, the type of bait that we used, but this does not explain our success during some trapping surveys and *P. gorzugi* attraction to fish-based baits that was noted by Degenhardt et al. (1996), Mali et al. (2018b) and Mirabal et al. (2018). We did notice an apparent seasonal trend in trapping surveys, with lower detection noted during the hotter summer months, which is in line with current literature for other turtle species (Plummer, 1977), and could have led to our decreased success during this time.

Turtle Processing. Turtle measurements fell within the expected ranges previously noted in literature (Pierce et al., 2016). Females were larger than males, a trend typically seen in many turtle species, 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 that were not used by juveniles, and thus juveniles are underrepresented in our data set. Measurements from juveniles were the result of opportunistic hand captures. A small number of turtles were recaptured during the course of our study (n = 7), as well as several turtles that were marked from previous studies. The duration between our sampling events was not long enough to note differences in size or health of the individuals, and unfortunately the turtles from previous, unrelated studies were not assigned unique identification numbers, so measurements could not be compared.

Methodology Comparison

While no significant differences were detected for total number of turtle detections and number of *P. gorzugi* detections across sampling methodologies, overall, the drone had higher mean values than trapping and visual surveys. The rank-based nature of non-parametric

comparisons obscure the difference in magnitude between values, which may have contributed to this result. Differences between sampling methods may have been further obscured by the high variance in count data, as locations with large turtle populations resulted in drone surveys having higher number of detections. On several occasions drone surveys showed their potential to outcompete the other methodologies in turtle detections. However, at low density sites where turtle detections were low overall, all survey methodologies appeared to perform similarly.

Mean number of turtles identified and mean turtle identification percentage were both significantly higher for drone sampling methodologies in comparison to visual surveys, suggesting that drone surveys are superior to visual surveys for wildlife identification. As wildlife surveys are often species-specific (Morrison et al., 2008), identification percentage may be more important than overall detection, which is true in this study. The overall number of turtles detected was not as important as the overall number of *P. gorzugi* detected, which was only possible to determine through correct identification of the species.

Notably, no significant difference was detected between mean turtles identified for drone and trapping surveys. As trapping always results in successful identification, no significant difference is desired in this instance, thus demonstrating the effectiveness of drone surveys for turtle identification. In certain habitat categories, no significant difference was observed in identification percentages for drone and trapping surveys. Many of the categories where this outcome occurred describe large reservoirs, such as no connectivity and no flow. While it may appear that drone surveys excel in these habitat types, these habitats typically had fewer detections overall. It could be the low turtle density at these sites that led to the methodologies performing similarly and further studies should be conducted to explore this.

Each sampling methodology had unique characteristics, which resulted in different levels of invasiveness, effort, cost, requirements, advantages and challenges (Table 6). Drone surveys offered a minimally invasive, reduced effort method that did well in turtle detection and identification, but faced limitations from weather, licensing and permitting restrictions, and technological issues. The unique aerial viewpoint provided exceptional imagery of the study site and was able to document turtles that were not visible from the shoreline. Visual surveys were low cost and low effort, but identification percentages were low. Issues of observer bias further question the accuracy of the data collected, but for organizations that are time, budget, and labor restricted, this could offer a quick assessment of an area. Trapping surveys were highly invasive, high effort, and subject to human and environmental interference, but offered the unique capability of capturing turtles, which allowed for 100% identification, population health data, tissue collection, and voucher specimens. Despite the labor costs involved with trapping, this methodology provides valuable data which cannot be collected otherwise.

Each method was found to be imperfect and we conclude that there may be no overall superior method for *P. gorzugi* detection. Ideally, a host of sampling methodologies would be used to thoroughly evaluate a turtle species as suggested in several previous studies (Ream and Ream, 1966; Sterrett et al., 2010; Jgermano, 2012; Tesche and Hodges, 2015). By combining detection data from our methodology comparison sites, with drone, visual, and trapping surveys included, we were able to create a more comprehensive range map for *P. gorzugi* in southwestern Texas (Figure 22), than what had resulted from the individual methods (Figure 11–13). As many organizations face monetary, labor, and time restrictions, however, it is important to identify the goal and limitations of the survey efforts to determine which sampling methodology would be best to use. Finally, we acknowledge the high potential of drone surveys

and encourage their implementation, as it appears to be a minimally invasive and viable survey methodology, which should only increase in capability as drone technology continues to advance.

REFERENCES

Akre, T. S., Willson, J. D., & Wilson, T. P. (2012). Alternative methods for sampling freshwater turtles and squamates. *Reptile Biodiversity: Standard Methods for Inventory and Monitoring.* University of California Press, Los Angeles, California, USA, 187–192.

Alvarez-Taboada, F., Paredes, C., & Julián-Pelaz, J. (2017). Mapping of the invasive species *Hakea sericea* using unmanned aerial vehicle (UAV) and WorldView-2 imagery and an object-oriented approach. *Remote Sensing*, *9*(9), 913.

Anderson, D. R., Burnham, K. P., Lubow, B. C., Thomas, L. E. N., Corn, P. S., Medica, P. A., & Marlow, R. W. (2001). Field trials of line transect methods applied to estimation of desert tortoise abundance. *The Journal of Wildlife Management*, *65*(3) 583–597.

Aniceto, A. S., Biuw, M., Lindstrøm, U., Solbø, S. A., Broms, F., & Carroll, J. (2018). Monitoring marine mammals using unmanned aerial vehicles: quantifying detection certainty. *Ecosphere*, *9*(3), e02122.

AWM Permits. (2020). *AWM Aerial Wildlife Management Permits.* Retrieved March 2, 2020, https://tpwd.texas.gov/business/permits/land/wildlife_management/aerial_wl_management/.

Bailey, L. A., Dixon, J. R., Hudson, R., & Forstner, M. R. (2008). Minimal genetic structure in the Rio Grande cooter (*Pseudemys gorzugi*). *The Southwestern Naturalist*, *53*(3), 406–411.

Bailey, L. A., Forstner, M. R., Dixon, J. R., & Hudson, R. (2014). Contemporary status of the Rio Grande cooter (Testudines: Emydidae: *Pseudemys gorzugi*) in Texas: phylogenetic, ecological and conservation consideration. In *Proceedings of the Sixth Symposium on the Natural Resources of the Chihuahuan Desert Region. Texas: Chihuahuan Desert Research Institute,* pp. 320–334.

Beauvais, G. P., & Buskirk, S. W. (1999). Modifying estimates of sampling effort to account for sprung traps. *Wildlife Society Bulletin*, *27*(1), 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*(3), e0194460.

Biserkov, V. Y., & Lukanov, S. P. (2017). Unmanned aerial vehicles (UAVs) for surveying freshwater turtle populations: Methodology adjustment. *Acta Zoologica Bulgarica*, *10*, 161–163.

Bogolin, A. P., Davis, D. R., Rahman, A. F. (2019a). *Pseudemys gorzugi* (Rio Grande cooter). Predation. *Herpetological Review, 50*(4), 775.

Bogolin, A. P., Davis, D. R., Ruppert, K. M., Kline, R. J., Rahman, A. F. (2019b). Geographic distribution: USA, Texas, Crockett Co.: *Pseudemys gorzugi* (Rio Grande cooter). *Herpetological Review, 50*(4), 745.

Bonner, T. H. & Littrell, B. M. (2016). Aquatic surveys of Delaware River of Texas. Final report to Texas Comptroller of Public Accounts.

Boundy, J., & Kennedy, C. (2006). Trapping survey results for the alligator snapping turtle (*Macrochelys temminckii*) in southeastern Louisiana, with comments on exploitation. *Chelonian Conservation and Biology*, *5*(1), 3–9.

Brown, D. J., Mali, I., & Forstner, M. R. (2011). No difference in short-term temporal distribution of trapping effort on hoop-net capture efficiency for freshwater turtles. *Southeastern Naturalist*, *10*(2), 245–250.

Buckland, S. T., Goudie, I. B. J., & Borchers, D. L. (2000). Wildlife population assessment: past developments and future directions. *Biometrics*, *56*(1), 1–12.

Burke, C., Rashman, M. F., Longmore, S. N., McAree, O., Glover-Kapfer, P., Ancrenaz, M., & Wich, S. A. (2019). Successful observation of orangutans in the wild with thermal-equipped drones. *Journal of Unmanned Vehicle Systems*, *7*(3), 235–257.

Chabot, D., & Bird, D. M. (2012). Evaluation of an off-the-shelf unmanned aircraft system for surveying flocks of geese. *Waterbirds*, *35*(1), 170–174.

Christie, K. S., Gilbert, S. L., Brown, C. L., Hatfield, M., & Hanson, L. (2016). Unmanned aircraft systems in wildlife research: current and future applications of a transformative technology. *Frontiers in Ecology and the Environment*, *14*(5), 241–251.

Christman, B. L. & Kamees, L. K. (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. Santa Fe, NM: Unpublished report to New Mexico Department of Game and Fish, 36pp.

Daniels, K. (2018). Inferences about the conservation utility of using unmanned aerial vehicles to conduct rapid assessments for basking freshwater turtles. Masters Theses.

Davy, C. M., Kidd, A. G., & Wilson, C. C. (2015). Development and validation of environmental DNA (eDNA) markers for detection of freshwater turtles. *PLOS ONE*, *10*(7), e0130965.

Deforce, E. A., Deforce, C. D., & Lindeman, P. V. (2004). *Phrynops gibbus* (Gibba turtle). Trap-happy behavior. *Herpetological Review*, *35*(1), 55–56.

Degenhardt, W. G., Painter, C. W., & Price, A. H. (1996). *Amphibians and Reptiles of New Mexico*. University of New Mexico Press, Albuquerque, New Mexico. 431 pp., 123 plates.

DonnerWright, D. M., Bozek, M. A., Probst, J. R., & Anderson, E. M. (1999). Responses of turtle assemblage to environmental gradients in the St. Croix River in Minnesota and Wisconsin, USA. *Canadian Journal of Zoology*, *77*(6), 989–1000.

DJI. (2020). *DJI Matrice 600 Pro - DJI*. Retrieved March 1, 2020, from https://www.dji.com/matrice600-pro.

Ernst, C. H., Hershey, M. F., & Barbour, R. W. (1974). A new coding system for hardshelled turtles. *Transactions of the Kentucky Academy of Science*, *35*(1–2), 27–28.

Federal Aviation Association. (2020). *Become a Drone Pilot*. United States Department of Transportation. Retrieved March 15, 2020, https://www.faa.gov/uas/commercial_operators/become_a_drone_pilot/.

Gibbons, J. W. Lovich. JE (1990). Sexual dimorphism in turtles with emphasis on the slider turtle (*Trachemys scripta*). *Herpetological Monographs*, *4*, 1–29.

Gregory, T. S., Tse, Z. T. H., & Lewis, D. (2015). Drones: Balancing risk and potential. *Science*, *347*(6228), 1323–1323.

Gu, W., & Swihart, R. K. (2004). Absent or undetected? Effects of non-detection of species occurrence on wildlife–habitat models. *Biological Conservation*, *116*(2), 195–203.

Hodgson, A., Kelly, N., & Peel, D. (2013). Unmanned aerial vehicles (UAVs) for surveying marine fauna: a dugong case study. *PLOS ONE*, *8*(11), e79556.

Hodgson, J. C., Mott, R., Baylis, S. M., Pham, T. T., Wotherspoon, S., Kilpatrick, A. D., ... & Koh, L. P. (2018). Drones count wildlife more accurately and precisely than humans. *Methods in Ecology and Evolution*, *9*(5), 1160–1167.

Hui, N. (2019). *Efficient Drone-based Radio Tracking of Wildlife*. (UC San Diego). Masters Theses and Doctoral Dissertations.

Iverson, J. B. (1992b). Correlates of reproductive output in turtles (Order Testudines). *Herpetological Monographs*, *6*, 25–42.

Jiménez López, J., & Mulero-Pázmány, M. (2019). Drones for conservation in protected areas: present and future. *Drones*, *3*(1), 10.

Jgermano, D. (2012). Sampling of turtles: trapping and snorkeling. *Northwest Fauna, 7,* 37-50.

Jones IV, G. P., Pearlstine, L. G., & Percival, H. F. (2006). An assessment of small unmanned aerial vehicles for wildlife research. *Wildlife Society Bulletin*, *34*(3), 750–758.

Joyce, K. E., Duce, S., Leahy, S. M., Leon, J., & Maier, S. W. (2019). Principles and practice of acquiring drone-based image data in marine environments. *Marine and Freshwater Research*, *70*(7), 952–963.

Karcher, J. M. (2019). A biotelemetric study comparing diving behavior and brumation sites of translocated and resident northern map turtles (*Graptemys geographica*) and their response to replica model turtles on artificial basking/nesting platforms in the Upper Niagara River. Biology Theses. 35.

Koh, L. P., & Wich, S. A. (2012). Dawn of drone ecology: low-cost autonomous aerial vehicles for conservation. *Tropical Conservation Science*, *5*(2), 121–132.

Kudo, H., Koshino, Y., Eto, A., Ichimura, M., & Kaeriyama, M. (2012). Cost-effective accurate estimates of adult chum salmon, *Oncorhynchus keta*, abundance in a Japanese river using a radio-controlled helicopter. *Fisheries Research*, *119*, 94–98.

Lagler, K. F. (1943). Methods of collecting freshwater turtles. *Copeia*, *1943*(1), 21–25.

Lancia, R. A., Nichols, J. D., & Pollock, K. H. (1994). Estimating the number of animals in wildlife populations. *Research and Management Techniques for Wildlife and Habitats.* The Wildlife Society, Bethesda, Maryland, USA, 215–253.

Lancia, R. A., Kendall, W. L., Pollock, K. H., & Nichols, J. D. (2005). Estimating the number of animals in wildlife populations. *Techniques for Wildlife Investigations and Management.* The Wildlife Society, Bethesda, Maryland, *USA*, 106–153.

Legal Information Institute. (2020). *50 CFR § 27.34 – Aircraft*. Cornell Law School. Retrieved on March 2, 2020, https://www.law.cornell.edu/cfr/text/50/27.34.

Letter, A. W., Waldon, K. J., Pollock, D. A., & 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*(3), 204–208.

Linchant, J., Lisein, J., Semeki, J., Lejeune, P., & Vermeulen, C. (2015). Are unmanned aircraft systems (UAS s) the future of wildlife monitoring? A review of accomplishments and challenges. *Mammal Review*, *45*(4), 239–252.

Lindeman, P. V. (2015). Surveys of basking turtles in the rivers of northeastern Oklahoma, with emphasis on *Graptemys geographica* (Common Map Turtle). In *Proceedings of the Oklahoma Academy of Science* (Vol. 94).

Lovich, J. E., & Ennen, J. R. (2013). A quantitative analysis of the state of knowledge of turtles of the United States and Canada. *Amphibia-Reptilia*, *34*(1), 11–23.

Mali, I., Brown, D. J., Jones, M. C., & Forstner, M. R. (2012). Switching bait as a method to improve freshwater turtle capture and recapture success with hoop net traps. *Southeastern Naturalist*, *11*(2), 311–318.

Mali, I., Brown, D. J., Jones, M. C., & Forstner, M. R. (2013). Hoop net escapes and influence of traps containing turtles on Texas spiny softshell (*Apalone spinifera emoryi*) captures. *Herpetological Review*, *44*(1), 40–42.

Mali, I., Brown, D. J., Ferrato, J. R., & Forstner, M. R. (2014). Sampling freshwater turtle populations using hoop nets: testing potential biases. *Wildlife Society Bulletin*, *38*(3), 580–585.

Mali, I. & Forstner, M. R. J. (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, A. W., & Suriyamongkol, T. (2018a). 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.

Mali, I., Duarte, A., & Forstner, M. R. (2018b). Comparison of hoop-net trapping and visual surveys to monitor abundance of the Rio Grande cooter (*Pseudemys gorzugi*). *PeerJ*, *6*, e4677.

McEvoy, J. F., Hall, G. P., & McDonald, P. G. (2016). Evaluation of unmanned aerial vehicle shape, flight path and camera type for waterfowl surveys: disturbance effects and species recognition. *PeerJ*, *4*, e1831.

Mirabal, J., Letter, A. W., Waldon, K. J., & Mali, I. (2018). *Pseudemys gorzugi* (Rio Grande cooter). Attractions to trap baits. *Herpetological Review*, *49*(2), 323–324.

Moon, Mariella. (2017). *DJI Mavic Pro Platinum Edition Boasts Longer Battery Life*. Engadget. Verizon Media. Retrieved on March 1, 2020, https://www.engadget.com/2017/08/31/dji-launches-mavic-pro-platinum-edition/.

Morrison, M. L., Block, W. M., Strickland, M. D., Collier, B. A., & Peterson, M. J. (2008). *Wildlife Study Design*. Springer Science & Business Media, 386 pp.

Mulero-Pázmány, M., Stolper, R., Van Essen, L. D., Negro, J. J., & Sassen, T. (2014). Remotely piloted aircraft systems as a rhinoceros anti-poaching tool in Africa. *PLOS ONE*, *9*(1), e83873.

New Mexico Department of Game and Fish [NMDGF]. 2006. The comprehensive wildlife conservation strategy for New Mexico. Santa Fe: New Mexico Department of Game and Fish.

Pierce, L. J. S., Stuart, J. N., Ward, J. P., & Painter, C. W. (2016). *Pseudemys gorzugi* Ward 1984-Rio Grande Cooter, Western River Cooter, Tortuga de Oreja Amarilla, Jicotéa del Río Bravo. In: Rhodin, A. G. J., Iverson, J. B., van Dijk, P. P., Saumure, R. A., Buhlmann, K. A., Pritchard, P. C. H., Mittermeier R. A., 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: Chelonian Research Foundation*, 1–12.

Plummer, M. V. (1977). Activity, habitat and population structure in the turtle, *Trionyx muticus*. *Copeia*, *2*(4), 431–440.

Ratcliffe, N., Guihen, D., Robst, J., Crofts, S., Stanworth, A., & Enderlein, P. (2015). A protocol for the aerial survey of penguin colonies using UAVs. *Journal of Unmanned Vehicle Systems*, *3*(3), 95–101.

Ream, C., & Ream, R. (1966). The influence of sampling methods on the estimation of population structure in painted turtles. *American Midland Naturalist*, *75*(2), 325–338.

Rees, A. F., Avens, L., Ballorain, K., Bevan, E., Broderick, A. C., Carthy, R. R., ... & Mangel, J. C. (2018). The potential of unmanned aerial systems for sea turtle research and conservation: a review and future directions. *Endangered Species Research*, *35*, 81–100.

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

Sharath, B. K., & Hegde, S. N. (2003). Two new traps for sampling the black pond turtle (*Melanochelys trijuga*) in the tropical rainforest of the Western Ghats (India). *Herpetological Review*, *34*(1), 33.

Sterrett, S. C., Smith, L. L., Schweitzer, S. H., & Maerz, J. C. (2010). An assessment of two methods for sampling river turtle assemblages. *Herpetological Conservation and Biology*, *5*(3), 490–497.

Su, T. C. (2015). Multispectral sensors carried on unmanned aerial vehicle (UAV) for trophic state mapping of the small reservoir in Kinmen, Taiwan. In *2015 IEEE International Geoscience and Remote Sensing Symposium (IGARSS)* (pp. 5348–5351).

Suriyamongkol, T., & Mali, I. (2019). Aspects of the Reproductive Biology of the Rio Grande Cooter (*Pseudemys gorzugi*) on the Black River, New Mexico. *Chelonian Conservation and Biology*, *18*(2), 187–194.

Tesche, M. R., & Hodges, K. E. (2015). Unreliable population inferences from common trapping practices for freshwater turtles. *Global Ecology and Conservation*, *3*, 802–813.

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

Thomas, R. B., Vogrin, N., & Altig, R. (1999). Sexual and seasonal differences in behavior of *Trachemys scripta* (Testudines: Emydidae). *Journal of Herpetology*, *33*(3), 511–515.

Twidwell, D., Allen, C. R., Detweiler, C., Higgins, J., Laney, C., & Elbaum, S. (2016). Smokey comes of age: unmanned aerial systems for fire management. *Frontiers in Ecology and the Environment*, *14*(6), 333–339.

USFWS [United States Fish and Wildlife Service]. (2015). Endangered and threatened wildlife and plants; 90-day findings on 31 species. *Federal Register, 80,* 37568–37579.

Valdeón, A., Crespo-Diaz, A., Egaña-Callejo, A., & Gosá, A. (2010). Update of the Pond Slider *Trachemys scripta* (Schoepff, 1792) records in Navarre (Northern Spain), and presentation of the Aranzadi Turtle Trap for its population control. *Aquatic Invasions*, *5*(3), 297–302.

van Gemert, J. C., Veenman, C. J., & Geusebroek, J. M. (2009). Episode-constrained cross-validation in video concept retrieval. *IEEE Transactions on Multimedia*, *11*(4), 780–785.

van Gemert, J. C., Verschoor, C. R., Mettes, P., Epema, K., Koh, L. P., & Wich, S. (2014). Nature conservation drones for automatic localization and counting of animals. In: Agapito, L., Bronstein, M. M., & Rother, C., eds. *European Conference on Computer Vision. Springer, Cham*., pp. 255–270.

Vermeulen, C., Lejeune, P., Lisein, J., Sawadogo, P., & Bouché, P. (2013). Unmanned aerial survey of elephants. *PLOS ONE*, *8*(2), e54700.

Weber, A. S., & Layzer, J. B. (2011). A comparison of turtle sampling methods in a small lake in Standing Stone State Park, Overton County, Tennessee. *Tennessee Academy of Science. Journal of the Tennessee Academy of Science*, *86*(2), 45.

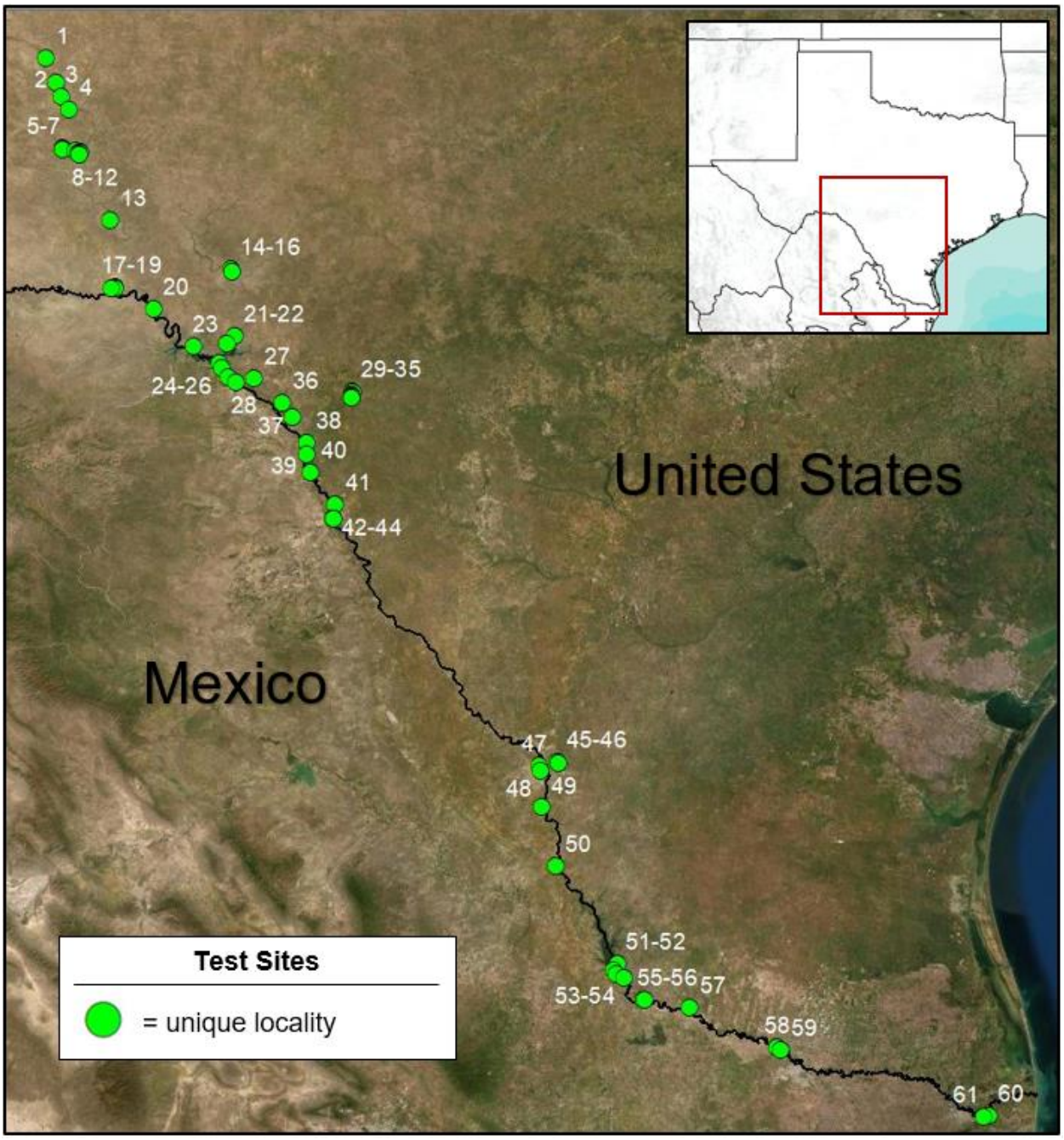

Figure 3. Map illustrating 61 unique localities visited from November 2018–October 2019 as part of our project to survey for *Pseudemys gorzugi* through southwestern Texas. Not all localities underwent full sampling as some were opportunistic site additions. Site numbers correspond to the numbers used in Table 1.

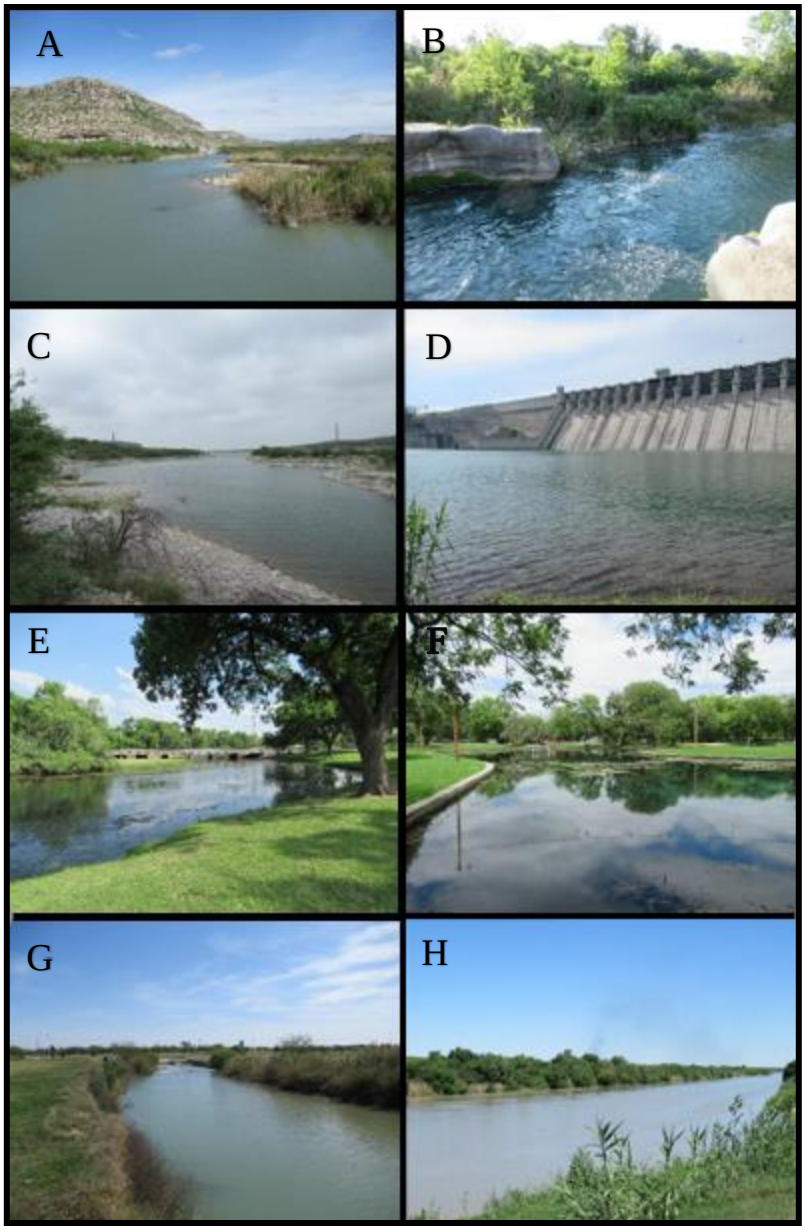

Figure 4. Representative sites depicting the habitat variation at sampling localities. (A) Pecos River, at Pandale crossing, Val Verde County (Site 13); (B) TNC Dolan Falls Preserve, Devils River, Dolan Falls, Val Verde County (Site 16); (C) Lake Amistad, Rough Canyon, Val Verde County (Site 21); (D) 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); and (H) Rio Grande, Laredo, near water treatment center, Webb County (Site 47). All photos by Amy P. Bogolin.

Figure 5. Drone and equipment used to conduct drone surveys for aquatic turtles. (A) DJI Matrice 600 Pro unmanned aerial vehicle with additional survey equipment attached; and (B) Gremsy T-3 gimbal with the SONY digital camera and MAIA multispectral camera attached.

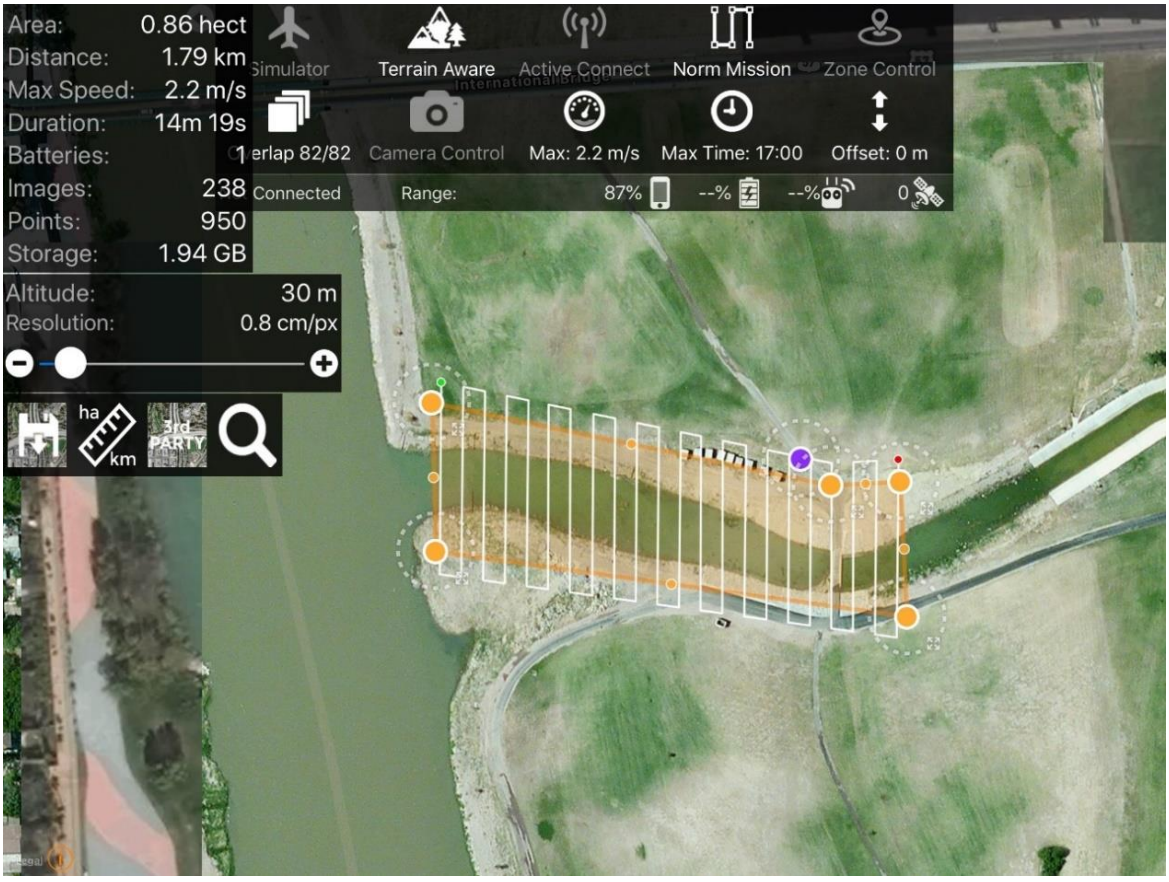

Figure 6. A screenshot from Maps Made Easy, the app used to conduct the majority of drone flights during this project. The projected flight path and flight parameters are depicted for a flight conducted at Eagle Pass Golf Course, spillway into Rio Grande, Maverick County (Site 42).

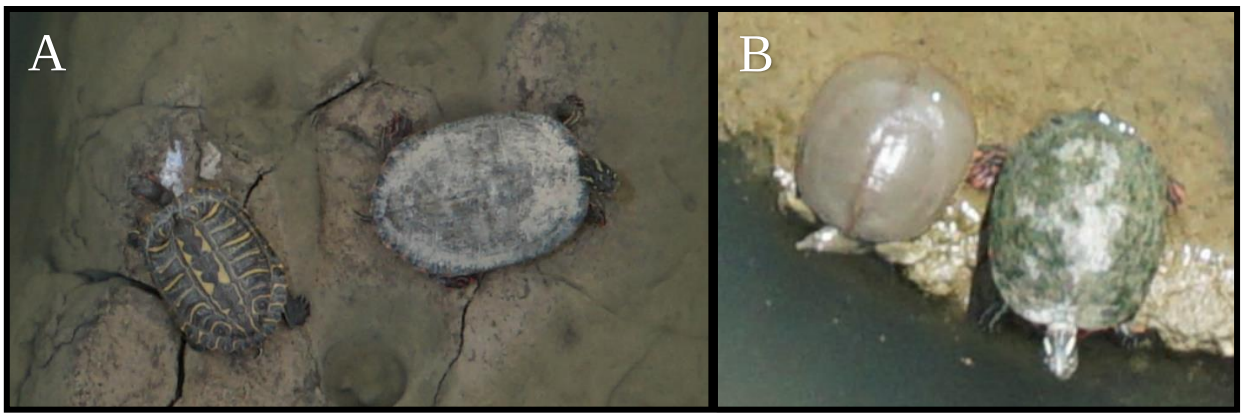

Figure 7. Magnified drone images depicting the species of turtles identified throughout this study. (A) *Trachemys scripta elegans* on left and *Pseudemys gorzugi* on right basking in the Rio Grande, near Salineño, Starr County (Site 54); and (B) *Apalone spinifera* on left and *Pseudemys gorzugi* on right basking at Eagle Pass Golf Course, spillway into Rio Grande, Maverick County (Site 42).

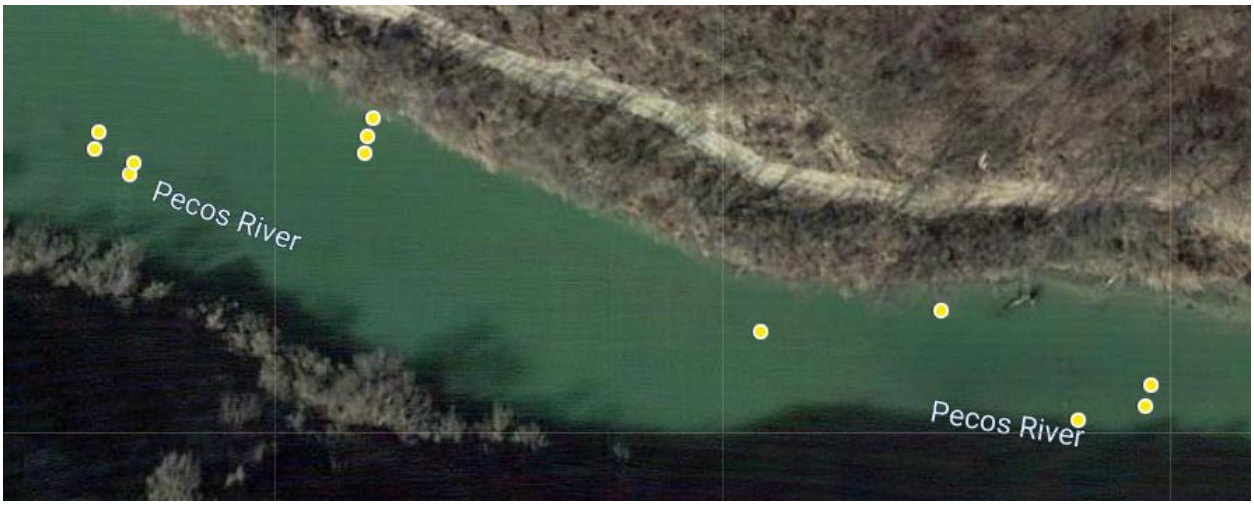

Figure 8. Map created on Google Drive showing locations of turtle detections during a drone survey. By importing the GPS locations of photographs containing turtles the relative location of the detections could be determined to assist in turtle quantification. The yellow dots mark the GPS locations of photos where turtles were detected from a flight on 10 August 2019 at Pecos River, 0.8 river km upstream of confluence with Independence Creek, Crockett County (Site 9). Out of the 12 photos containing turtles, six unique turtles were identified.

Figure 9. Floating trap deployed in sampling area. On the right, the trap, PVC piping (white), pool noodles (blue), and bait can are visible. On the bottom left, the anchor is visible under water. These traps were successful at trapping turtles in locations where habitat characteristics prevented the securement of baited hoop-net traps to shore or where water levels fluctuated due to variable releases from upstream dams.

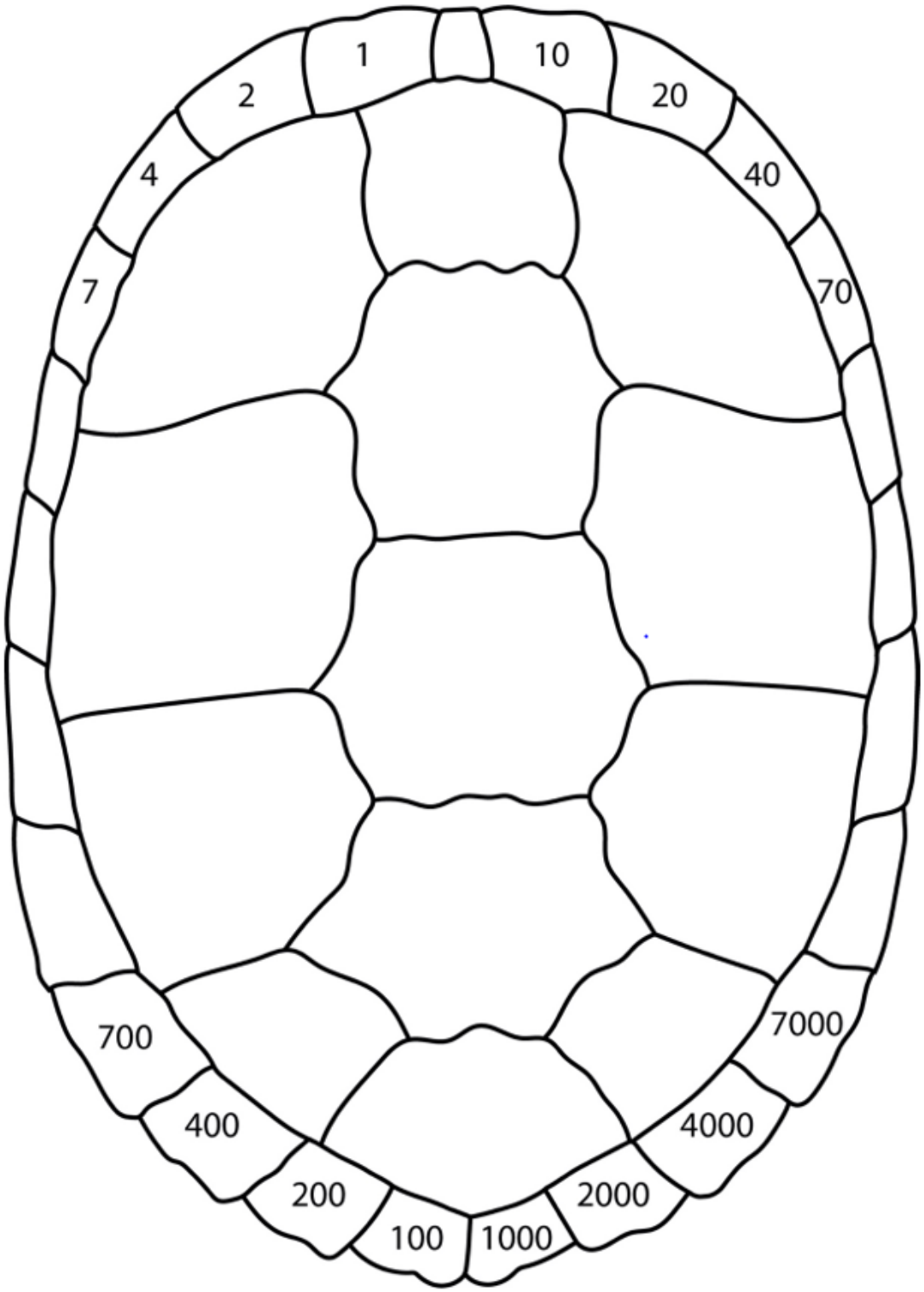

Figure 10. Diagram of the notching scheme used to mark turtles for this study. Notches are made in marginals with the corresponding numbers summing to the turtle ID number. This follows a modified version of the marking scheme by Ernst et al. (1974). Figure drawn by Drew R. Davis from a preserved specimen (TNHC 114463 [DRD 5628]).

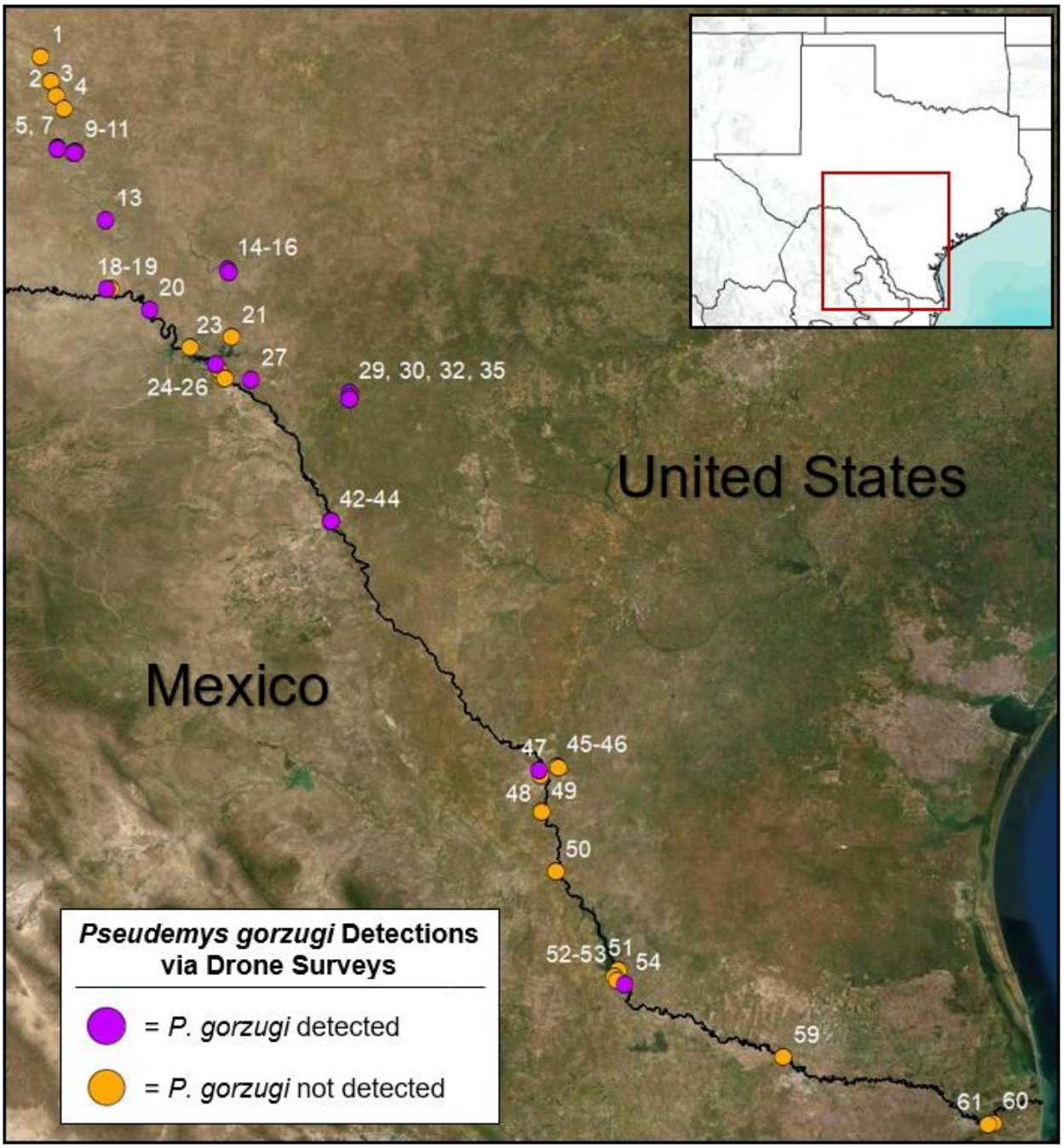

Figure 11. Map of 42 unique localities where drone surveys occurred for *Pseudemys gorzugi* through southwestern Texas. Purple dots indicate positive detections of *P. gorzugi*. Orange dots indicate sites where *P. gorzugi* was not detected. Site numbers correspond to the numbers used in Table 2.

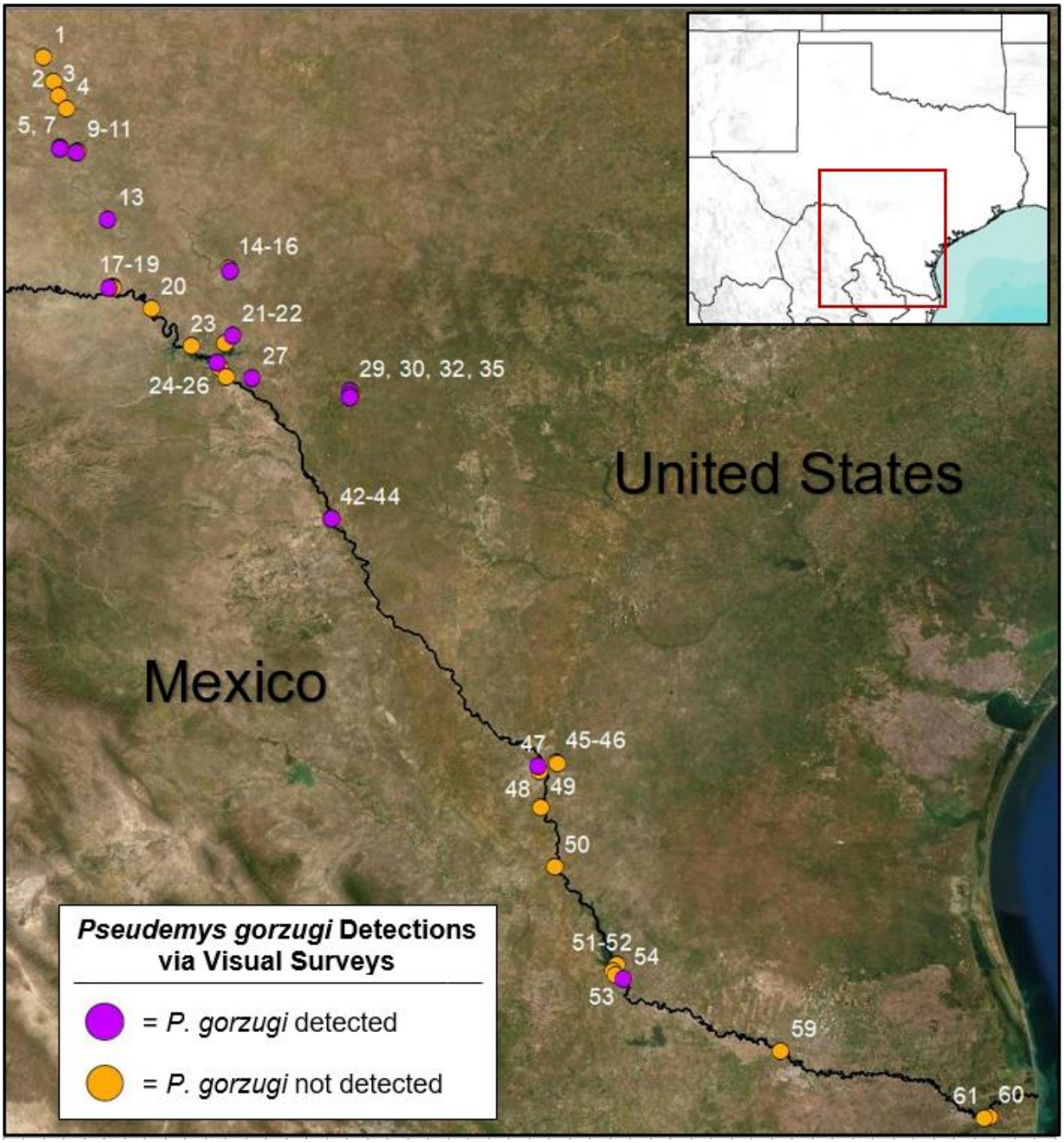

Figure 12. Map of 44 unique localities where visual surveys occurred for *Pseudemys gorzugi* through southwestern Texas. Purple dots indicate positive detections of *P. gorzugi*. Orange dots indicate sites where *P. gorzugi* was not detected. Site numbers correspond to the numbers used in Table 3.

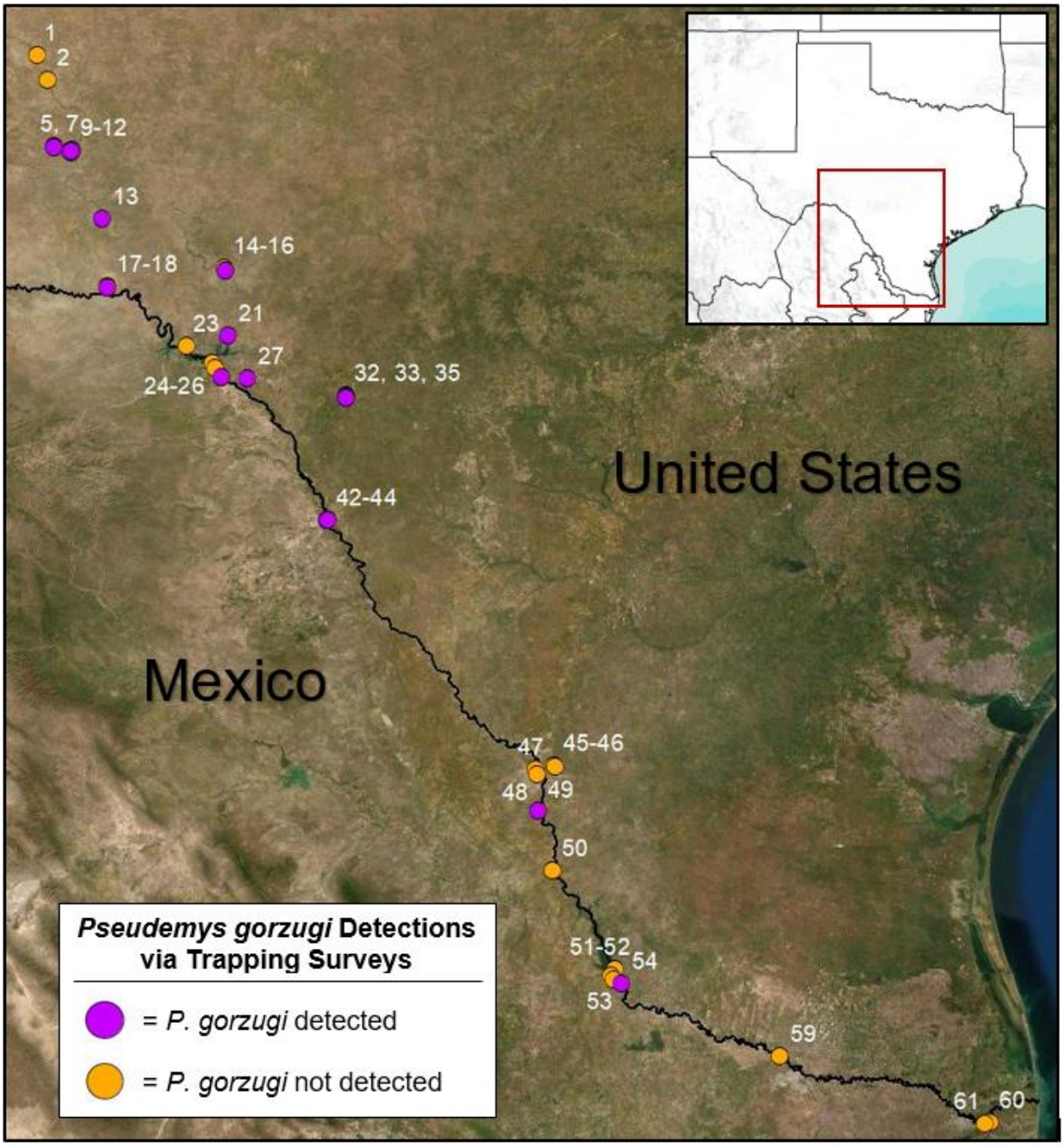

Figure 13. Map of 39 unique localities where trapping surveys occurred for *Pseudemys gorzugi* through southwestern Texas. Purple dots indicate positive detections of *P. gorzugi*. Orange dots indicate sites where *P. gorzugi* was not detected. Site numbers correspond to the numbers used in Table 4.

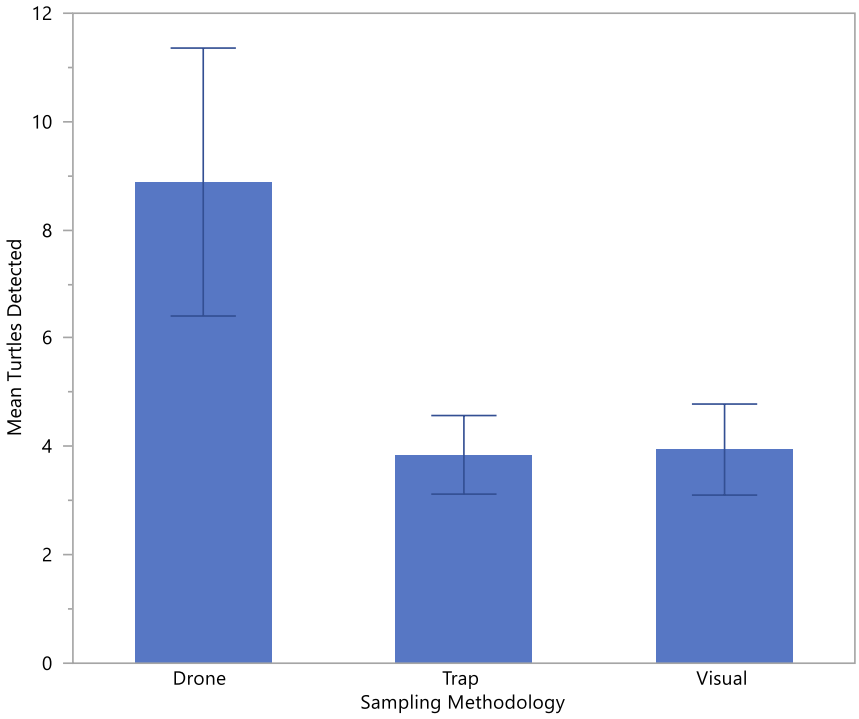

Figure 14. Mean (± 1 SE) detections of turtles for drone, trap, and visual sampling methodologies. Wilcoxon multiple comparisons tests failed to produce a significant difference.

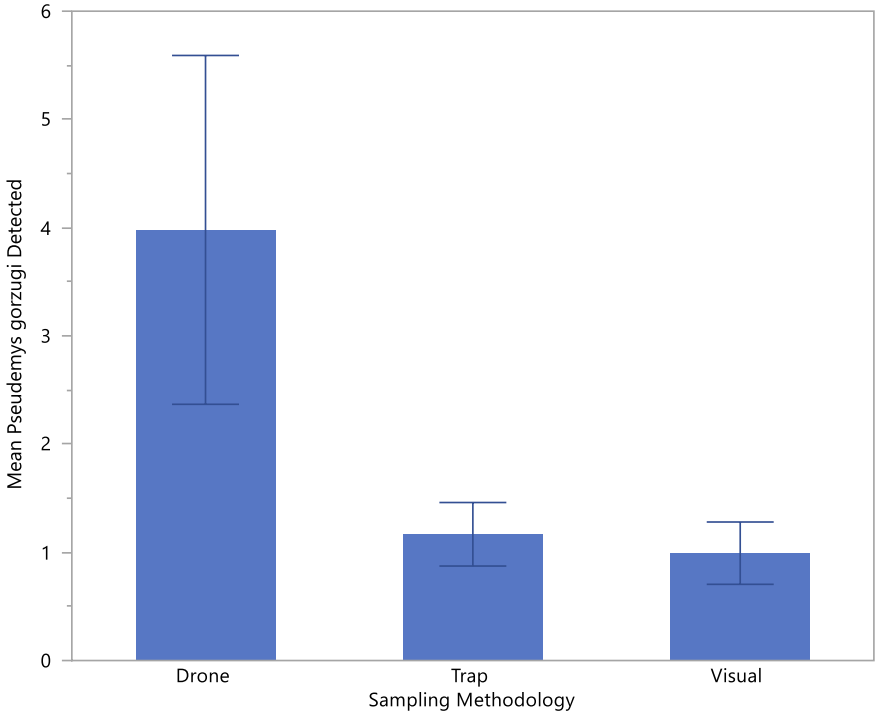

Figure 15. Mean (± 1 SE) *Pseudemys gorzugi* detections for drone, trap, and visual sampling methodologies. Wilcoxon multiple comparisons tests failed to produce a significant difference.

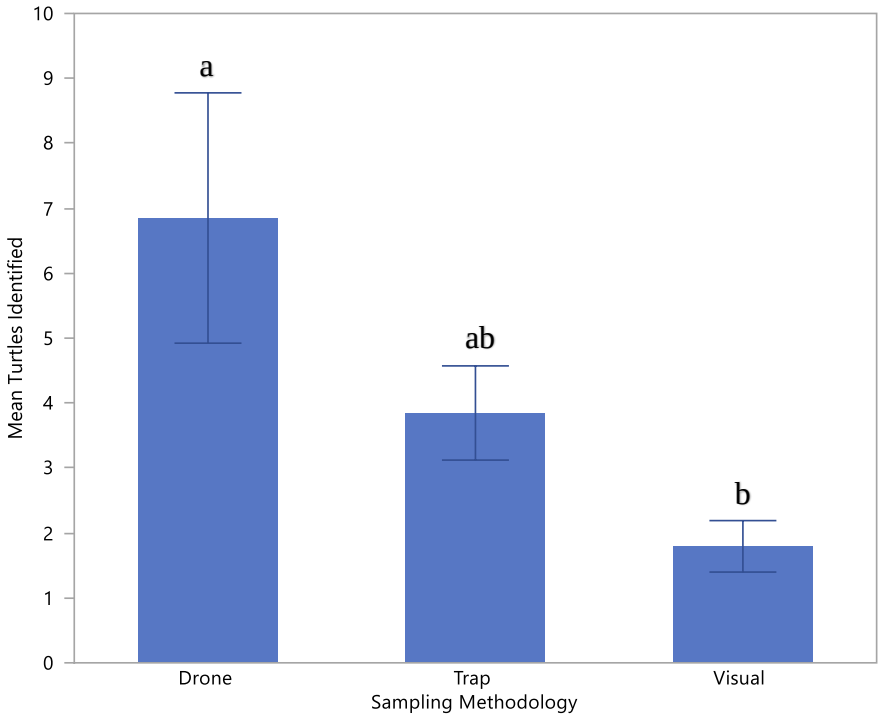

Figure 16. Mean (± 1 SE) identifications of turtles for drone, trap, and visual sampling methodologies. Letters indicate groupings from Wilcoxon multiple comparison tests (α = 0.05).

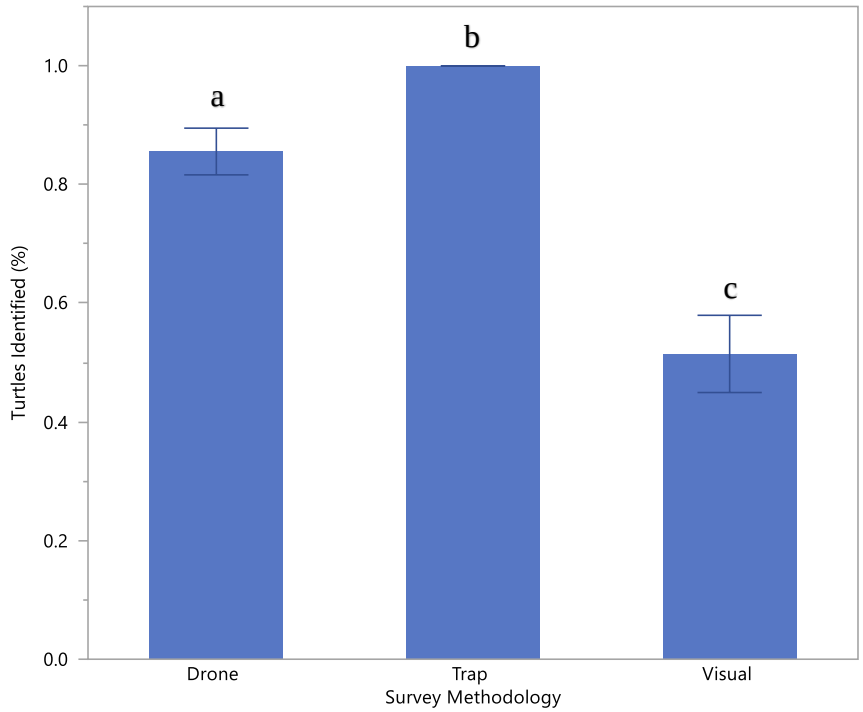

Figure 17. Mean (± 1 SE) turtle identification percentage for drone, trap, and visual sampling methodologies. Letters indicate groupings from Wilcoxon multiple comparison tests (α = 0.05).

Figure 18. Images from drone surveys depicting turtles (*Pseudemys gorzugi* in yellow, unknown turtle in red) that were not visible from shore demonstrating the unique aerial viewpoint of drone imagery. (A) Four turtles are seen in this photo at the Rio Grande, Laredo, near water treatment center, Webb County (Site 47) providing our first documentation of *P. gorzugi* at this site; and (B) One *P. gorzugi* underwater at Fort Clark Springs, Las Moras Creek, Buzzard Roost, Kinney County (Site 35).

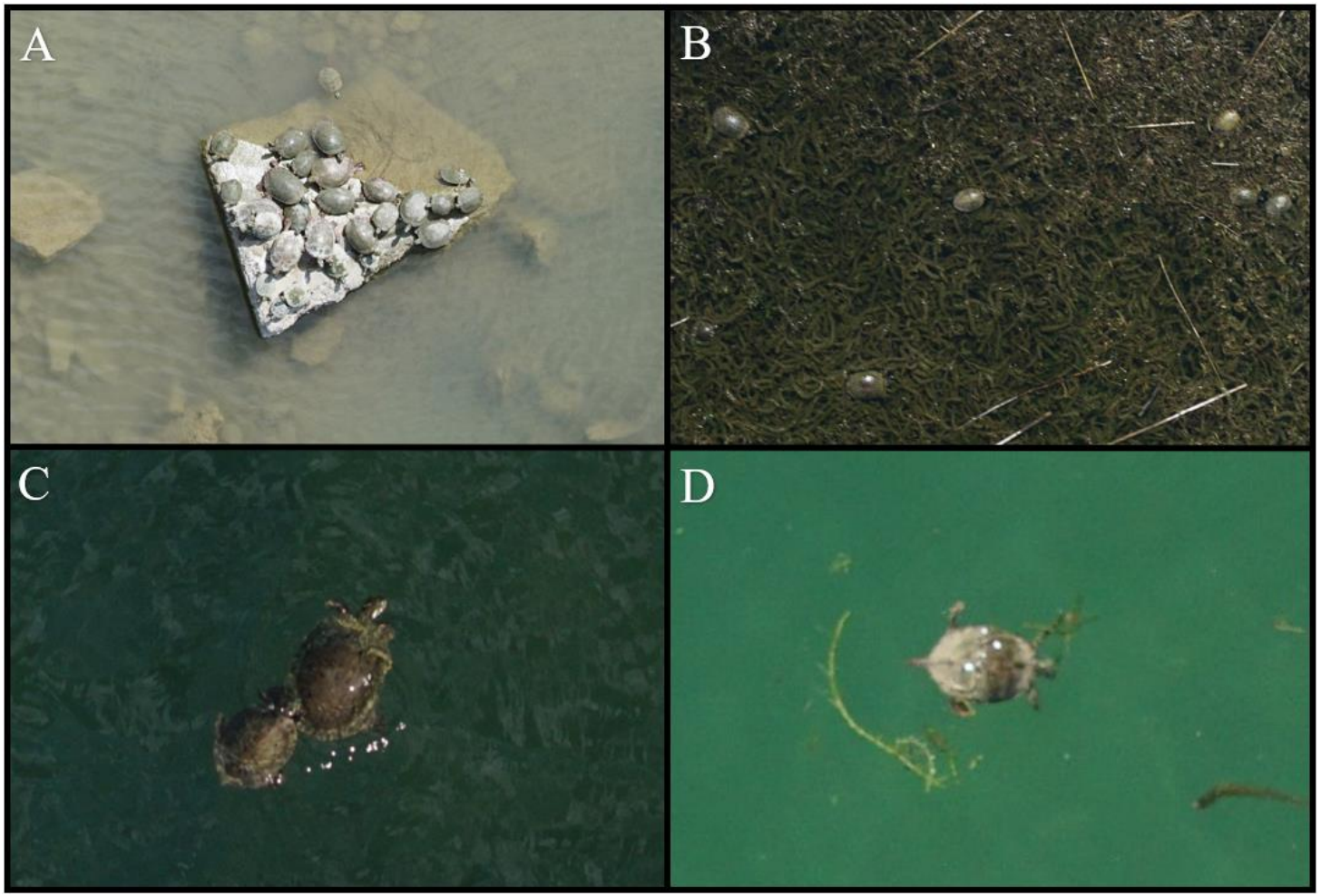

Figure 19. Magnified drone imagery depicting examples of *Pseudemys gorzugi* behavior documented during drone surveys. (A) 26 *P. gorzugi* basking on one rock at Eagle Pass Golf Course, spillway into Rio Grande, Maverick County (Site 42). An additional *P. gorzugi* is seen swimming towards the rock for 27 *P. gorzugi* total in this image; (B) Subaerial basking of *P. gorzugi* on aquatic vegetation at Del Rio, San Felipe Springs Golf Course, San Felipe Creek, Val Verde County (Site 27); (C) Two *P. gorzugi* exhibiting courting behaviors in the Rio Grande, spillway below Amistad Dam, Val Verde County (Site 24); (D) *Pseudemys gorzugi* seen foraging on a piece of aquatic vegetation in TNC Dolan Falls Preserve, Devils River, Dolan Falls, Val Verde County (Site 16).

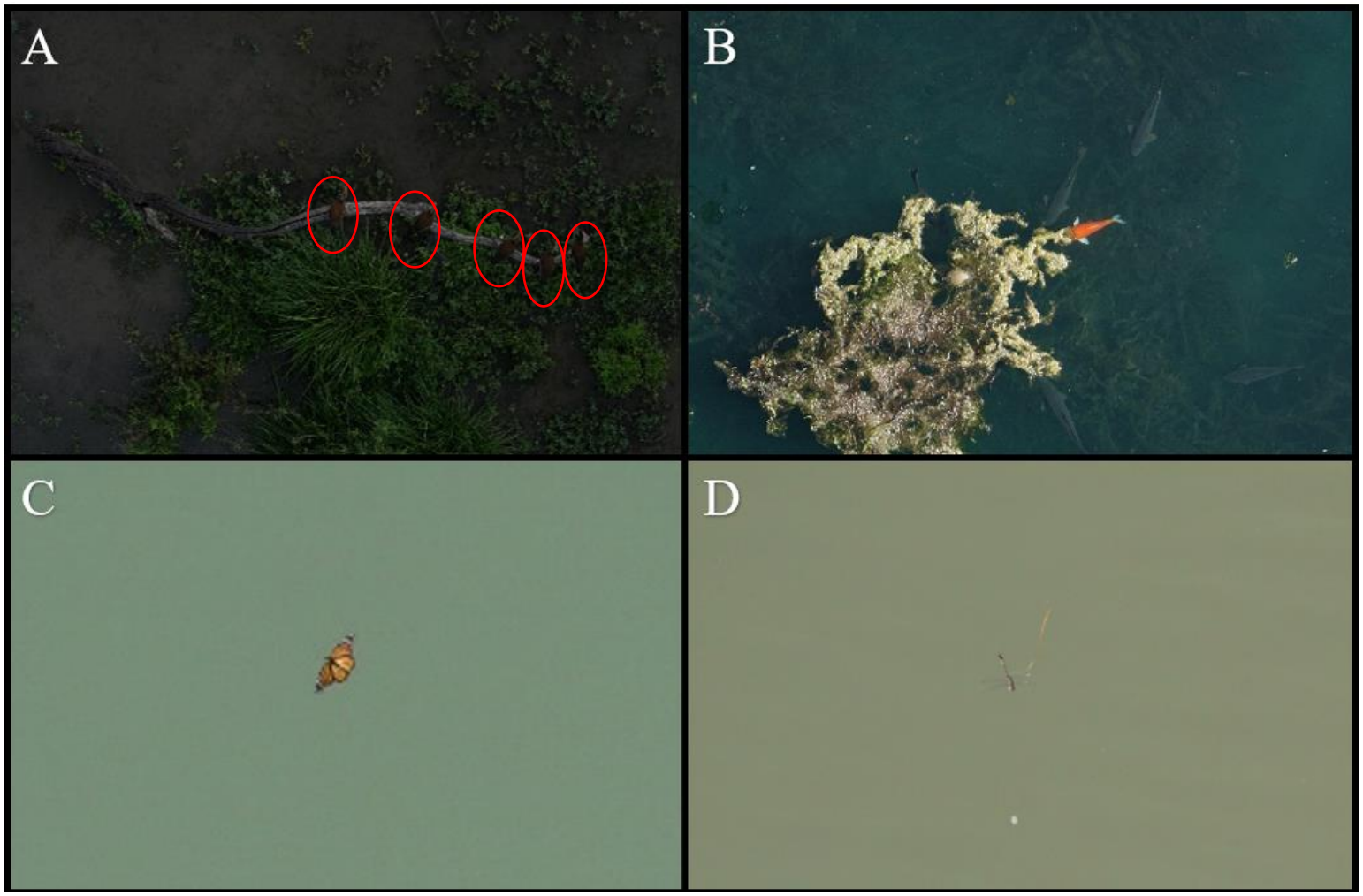

Figure 20. Magnified drone imagery of non-target species photographed during surveys. All species seemed unbothered by the drone flights. (A) Five Black-bellied Whistling Ducks (*Dendrocygna autumnalis*) perched on a log at Fort Clark Springs, Las Moras Creek, Buzzard Roost, Kinney County (Site 35); (B) Native and introduced fish (Cypriniformes) swimming at Fort Clark Springs, Headwater Pond, Kinney County (Site 29); (C) Monarch Butterfly (*Danaus plexippus*) flying over the Pecos River, 0.3 km upstream of confluence with Independence Creek, Crockett County (Site 11); and (D) Dragonfly (Odonata) flying above the Pecos River, at Pandale Crossing (Site 13).

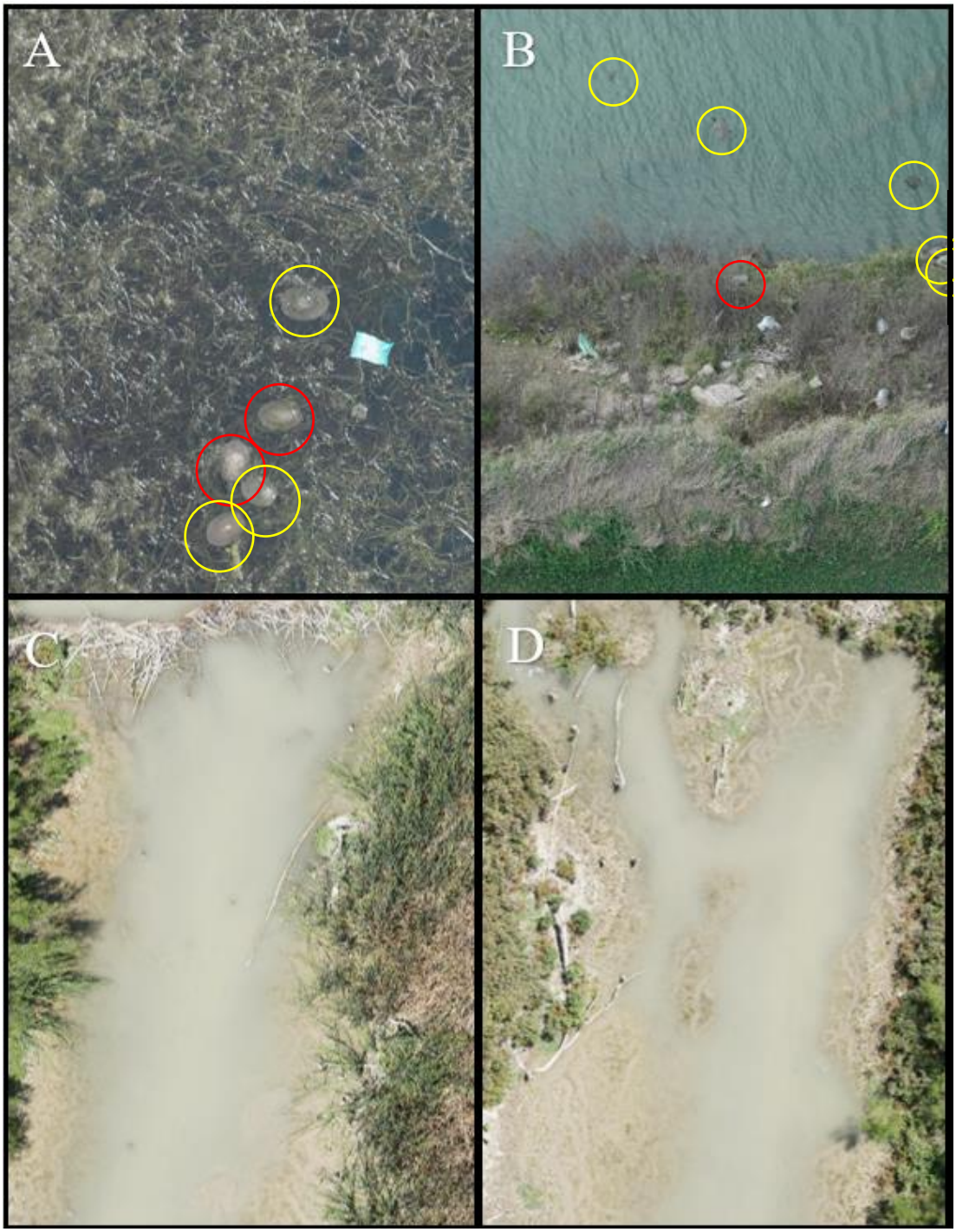

Figure 21. Habitat characteristics depicted in imagery obtained during drone surveys for *Pseudemys gorzugi*. *Pseudemys gorzugi* are indicated in yellow and unknown turtles in red (A) Three *P. gorzugi* and two unidentifiable turtles basking next to trash in Fort Clark Springs, Headwater Pond, Kinney County (Site 29); (B) Five *P. gorzugi* and one unidentified turtle basking and swimming next to trash at Eagle Pass Golf Course, spillway into Rio Grande, Maverick County (Site 42); (C) Beaver dam at Pump Canyon, Langtry, Val Verde County (Site 19); and (D) Turtle tracks seen in the mud at Pump Canyon, Langtry, Val Verde County (Site 19).

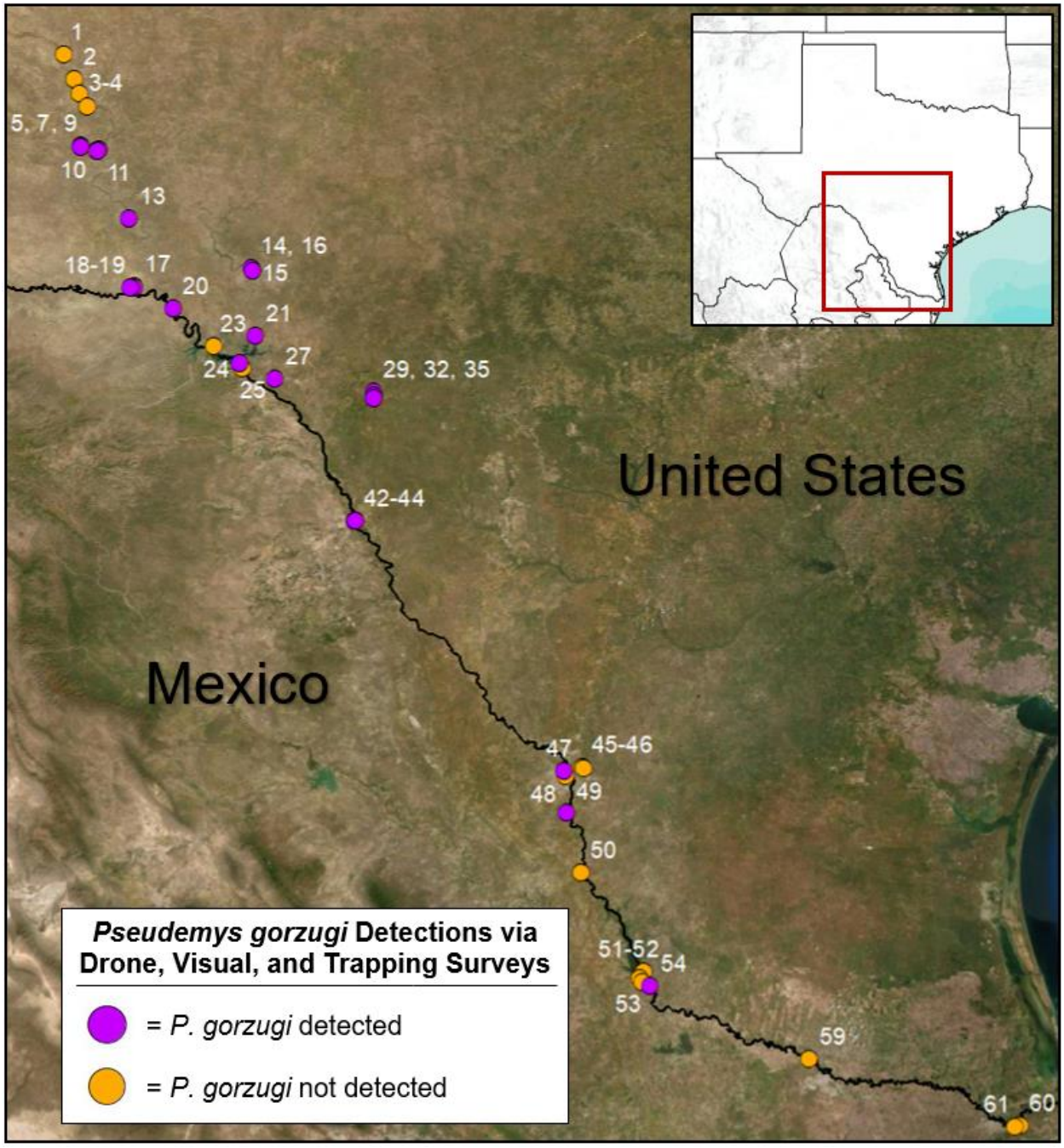

Figure 22. Map of unique localities where *Pseudemys gorzugi* was detected in southwestern Texas through drone, visual, and trapping surveys as a result of a methodology comparison study. Purple dots indicate positive detections of *P. gorzugi*. Orange dots indicate sites where *P. gorzugi* was not detected. Site numbers correspond to the numbers used in Table 1.

Table 1. Site numbers assigned to the 61 unique localities visited during the sampling period with corresponding GPS coordinates. This list includes both sites where sampling methodologies were used as well as opportunistic additions. The number of sampling visits to each site is also included.

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 Parido 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. Average turtle detections per site (± 1 SD) as a result of 15-min drone surveys conducted at sampling sites. Results are broken down by species identified (*Pseudemys gorzugi*, *Trachemys scripta elegans*, and *Apalone spinifera*) with unidentifiable turtles classified as unknown. Average identification percentages per site are displayed as well. Site locality information, *P. gorzugi* (PG) detection, and number of visits are provided, with site numbers corresponding those used in Table 1.

Site #	County	Site	Latitude	Longitude	# of Visits	PG Detected	*Pseudemys gorzugi*	*Trachemys scripta elegans*	*Apalone spinifera*	Unknown	ID %
1	Pecos	Pecos River, at US Hwy 190 crossing	30.90516	-101.88083	2	N	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	N	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	N	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	-101.77022	1	N	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	Y	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	Y	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	Y	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	N	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	Y	1.0 (± 0)	0 (± 0)	2.5 (± 0.7)	0 (± 0)	100.0 (± 0)
13	Val Verde	Pecos River, at Pandale crossing	30.13120	-101.57450	2	Y	3.0 (± 1.4)	0 (± 0)	0 (± 0)	0 (± 0)	-
14	Val Verde	TNC Dolan Falls Preserve, Devils River, upstream of confluence with Dolan Creek	29.89387	-100.99561	2	Y	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 River	29.88591	-100.99292	2	N	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	Y	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	N	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	Y	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	Y	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	N	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	100.0 (± 0)
23	Val Verde	Lake Amistad, Box Canyon	29.52420	-101.17585	2	N	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	Y	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	N	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	N	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	Y	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	Y	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	-100.41745	1	N	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	Y	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	Y	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	Y	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	N	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	Y	1.0 (± N/A)	19.0 (± N/A)	7.0 (± N/A)	0 (± N/A)	-
45	Webb	Lake Casa Blanca International State Park, near El Ranchito pavillion	27.54447	-99.44098	1	N	0 (± N/A)	1.0 (± N/A)	1.0 (± N/A)	0 (± 0)	-
46	Webb	Lake Casa Blanca International State Park, fishing pier	27.53861	-99.43475	1	N	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	Y	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	N	0 (± 0)	0 (± 0)	1.0 (± 1.4)	0 (± N/A)	-
49	Webb	Rio Grande, near El Cenizo	27.33117	-99.51195	2	N	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	100.0 (± N/A)
50	Zapata	Rio Grande, near San Ygancio	27.04330	-99.44496	1	N	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	N	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	N	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	N	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	Y	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	N	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	N	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	N	0 (± 0)	0.5 (± 0.7)	0 (± 0)	0 (± N/A)	100.0 (± N/A)

Table 3. Average turtle detections per site (± 1 SD) as a result of 15-min visual surveys conducted at sampling sites. Results are broken down by species identified (*Pseudemys gorzugi*, *Trachemys scripta elegans*, and *Apalone spinifera*) with unidentifiable turtles classified as unknown. Average identification percentages per site are displayed as well. Site locality information, *P. gorzugi* (PG) detection, and number of visits are provided, with site numbers corresponding to those used in Table 1.

Site #	County	Site	Latitude	Longitude	# of Visits	PG Detected	*Pseudemys gorzugi*	*Trachemys scripta elegans*	*Apalone spinifera*	Unknown	ID %
1	Pecos	Pecos River, at US Hwy 190 crossing	30.90516	-101.88083	3	N	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	N	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	N	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)	-
4	Pecos	Pecos River, at TX Hwy 290 crossing	30.65960	-101.77022	1	N	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	Y	2.0 (± 1.4)	0 (± 0)	0.5 (± 0)	5.0 (± 0)	30.6 (±19.6)
7	Terrell	TNC Independence Creek Preserve, raceway below Upper Lake	30.46736	-101.80181	1	Y	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	N	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	N	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	Y	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	Y	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	N	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	N	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	-

16	Val Verde	TNC Dolan Falls Preserve, Devils River, Dolan Falls	29.88385	-100.99397	3	Y	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	N	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)	-
18	Val Verde	Rio Grande, near Langtry	29.80564	-101.55088	2	N	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	-
19	Val Verde	Pump Canyon, Langtry	29.80343	-101.56750	1	Y	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	N	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	Y	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	N	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)	-
23	Val Verde	Lake Amistad, Box Canyon	29.52420	-101.17585	3	N	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	Y	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	N	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	N	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	Y	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	Y	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	-100.41745	1	N	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	Y	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	Y	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	Y	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	N	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	N	0 (± N/A)	4.0 (± N/A)	0 (± N/A)	15.0 (± N/A)	21.1 (± N/A)
45	Webb	Lake Casa Blanca International State Park, near El Ranchito pavillion	27.54447	-99.44098	2	N	0 (± 0)	1.0 (± 0)	1.5 (± 0.7)	1.5 (± 0.7)	63.3 (± 4.8)
46	Webb	Lake Casa Blanca International State Park, fishing pier	27.53861	-99.43475	2	N	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	Y	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	N	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	-
49	Webb	Rio Grande, near El Cenizo	27.33117	-99.51195	2	N	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	-
50	Zapata	Rio Grande, near San Ygancio	27.04330	-99.44496	1	N	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	N	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	N	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	-
53	Starr	Rio Grande, near Chapeno	26.53233	-99.15546	1	N	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	Y	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	N	0 (± 0)	0 (± 0)	0 (± 0)	3.5 (± 1.7)	0 (± 0)
60	Cameron	Rio Grande, downstream of TNC Southmost Preserve	25.85462	-97.37676	1	N	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	N	0 (± 0)	0.5 (± 0.7)	0 (± 0)	0 (± 0)	100.0 (± N/A)

Table 4. Average turtle detections per site (± 1 SD) as a result of trapping surveys conducted at sampling sites. Results are broken down by species identified (*Pseudemys gorzugi*, *Trachemys scripta elegans*, and *Apalone spinifera*). Site locality information, *P. gorzugi* (PG) detection, and number of visits are provided, with site numbers corresponding to those used in Table 1.

Site #	County	Site	Latitude	Longitude	# of Visits	PG Detected	*Pseudemys gorzugi*	*Trachemys scripta elegans*	*Apalone spinifera*	# of PG/ Trap Hour
1	Pecos	Pecos River, at US Hwy 190 crossing	30.90516	-101.88083	2	N	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	N	0 (± 0)	0.5 (± 0.7)	0 (± 0)	0 (± 0)
5	Terrell	TNC Independence Creek Preserve, Lower Lake	30.46955	-101.80131	2	Y	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	Y	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	Y	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	N	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	Y	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	N	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	Y	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	N	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	N	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	Y	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	N	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	Y	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	Y	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	N	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
24	Val Verde	Rio Grande, spillway below Amistad Dam	29.44737	-101.05667	2	N	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	N	0 (± 0)	1.00 (±1.41)	0 (±0)	0 (±0)
26	Val Verde	Rio Grande, near Lugo property	29.37719	-101.01348	1	Y	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	Y	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	Y	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	Y	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	Y	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	Y	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	Y	3.0 (± N/A)	0 (± N/A)	3.0 (± N/A)	0.02270 (± N/A)
44	Maverick	Eagle Pass Golf Course, settling pond along Rio Grande	28.70146	-100.50979	1	Y	2.0 (± N/A)	16.0 (± N/A)	3.0 (± N/A)	0.0139 (± N/A)
45	Webb	Lake Casa Blanca International State Park, Casa Blanca Lake, near El Ranchito pavillion	27.54447	-99.44098	2	N	0 (± 0)	0 (± 0)	2.0 (± 0)	0 (± 0)
46	Webb	Lake Casa Blanca International State Park, Casa Blanca Lake, fishing pier	27.53861	-99.43475	2	N	0 (± 0)	0.5 (± 0.7)	0 (± 0)	0 (± 0)
47	Webb	Rio Grande, Laredo, near water treatment center	27.52372	-99.52431	2	N	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
48	Webb	Rio Grande, Laredo, near international railroad bridge crossing	27.49835	-99.51674	1	N	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	Y	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	N	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)
51	Starr	Falcon State Park, Falcon Lake	26.58179	-99.15259	2	N	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	N	0 (± 0)	4.0 (± 4.2)	1.0 (± 1.4)	0 (± 0)
53	Starr	Rio Grande, near Chapeno	26.53233	-99.15546	1	N	0 (± N/A)	0 (± N/A)	0 (± N/A)	0 (± N/A)
54	Starr	Rio Grande, near Salineño	26.51429	-99.11662	2	Y	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	N	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	N	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	N	0 (± N/A)	1.0 (± N/A)	0 (± N/A)	0 (± N/A)

Table 5. Average measurements obtained from male (M), female (F), and juvenile (J) *Pseudemys gorzugi* (*P. gorzugi*), *Trachemys scripta elegans* (*T. s. elegans*), and *Apalone spinifera* (*A. spinifera*) throughout the study period from trapping events and opportunistic captures. SCL = straight carapace length, CW = carapace width at widest point, PL = plastron length down midline, PW = plastron width as measured between the junction of the marginal, pectoral, and abdominal scutes, SH = maximum shell height.

Species	Sex	N	SCL	CW	PL	PW	SH	Mass
P. gorzugi	M	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)
P. gorzugi	F	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)
P. gorzugi	J	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)
T. s. elegans	M	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)
T. s. elegans	F	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)
A. spinifera	M	28	168.8 (± 20.2)	136.9 (± 14.3)	122.3 (± 13.7)	N/A	45.2 (± 8.2)	2438.4 (± 1450.7)
A. spinifera	F	26	294.5 (± 76.5)	225.1 (± 54.9)	209.8 (± 55.9)	N/A	75.1 (± 23.3)	516.0 (± 187.6)

Table 6. Comparisons of the unique characteristics of each sampling methodology used throughout the survey period. The information includes level of invasiveness, effort, identification percentage, cost, requirements, advantages, and challenges of each method.

Method	Invasiveness	Effort	ID %	Cost	Requirements	Advantages	Challenges
Drone	low	low–medium	high	high initial, then low–medium	favorable weather, pilot license, launch area	unique aerial viewpoint	technological issues, short flight time
Visual	medium	low	medium	low	shore access	quick and easy assessment	observer bias, lower ID%
Trap	high	high	100%	medium	water access, penetrable substrate	provides population health data	trap theft, sampling bias
eDNA	low	low–medium	high	high initial, then low–medium	water access	detection possible without observation	not quantifiable, delayed results

CHAPTER III

ENVIRONMENTAL DNA ANALYSIS FOR THE RIO GRANDE COOTER (*PSEUDEMYS GORZUGI*)

Introduction

The Rio Grande Cooter, *Pseudemys gorzugi*, is an understudied, elusive freshwater aquatic turtle species only recently described by Ernst (1990), breaking from its previous classification as a subspecies of River Cooter, *P. concinna* (Collins, 1991; Lovich and Ennen, 2013). With a questionable conservation status that is currently undergoing review by the United States Fish and Wildlife Service (USFWS, 2015), *P. gorzugi* is listed as Threatened in New Mexico (New Mexico Department of Game and Fish [NMDGF], 2006) and Mexico (Secretaríade Medio Ambiente y Recursos Naturales, 2010), Near Threatened by the IUCN (Pierce et al., 2016), and a Species of Greatest Conservation Need in Texas (Texas Parks and Wildlife Department, 2012). Observations of *P. gorzugi* have been spotty and infrequent, with few detections in a large portion of their historical range (Pierce et al., 2016; iNaturalist.org, 2020). Traditional sampling methodologies have struggled to detect *P. gorzugi*, particularly amongst the Texas populations, leading to a data deficiency in this area (Degenhardt et al. 1996; Bailey et al., 2014; Bonner and Littrell, 2016). Moreover, traditional sampling methods are often expensive, invasive, and labor intensive, warranting improvements in survey techniques (Beauvais and Buskirk, 1999; Gu and Swihart, 2004; Lancia et al., 2005).

Environmental DNA (eDNA) analysis is a novel sampling methodology that has recently shown great promise in the detection of wildlife, particularly for aquatic species (Goldberg et al.2015; Ficetola et al., 2008; Rees et al., 2014). Organisms continually shed DNA into their surrounding environments from skin cells, urine, feces, etc., and these minute amounts of DNA can be collected and analyzed (Hofreiter et al., 2003; Ficetola et al., 2008; Goldberg et al., 2016). 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; Thomsen et al., 2012; Turner et al., 2014; Takahara et al., 2013; Renshaw et al., 2015). eDNA can then be extracted from the filter, amplified through PCR, purified through a gel, and sequenced to confirm that the DNA came from the species of interest (Rees et al., 2014; Goldberg et al., 2016; Clusa et al., 2017). Primers are developed to ensure species-specificity, which is often confirmed through Sanger sequencing the amplified DNA product (Díaz-Ferguson and Moyer, 2014; de Souza et al., 2016; Baker et al., 2018) and through this procedure the presence of a species in a water body can be detected (Davy et al., 2015; Dougherty et al., 2016; Klymus et al., 2017).

The cytochrome oxidase I sequence has been successfully used to create primers for many turtle species (Reid et al., 2011; Davy et al., 2015; Kundu et al., 2016), from common and invasive species, such as Red-eared Slider, *Trachemys scripta elegans* (Lawson, 2018), to rare and endangered species, such as Wood Turtle, *Glyptemys insculpta* (Akre et al., 2019). While no previous studies have attempted to detect *P. gorzugi* using eDNA, mitochondrial fragments listed on GenBank show enough genetic differences between sympatric turtle species *T. s. elegans* and Spiny Softshell (*Apalone spinifera*) to develop primer sets specific to *P. gorzugi*. With the unique ability to confirm a species presence despite lack of visual or auditory detection (Ficetola et al., 2008; Hoffman et al., 2016), eDNA analysis can provide critical data on *P. gorzugi*

presence, filling in gaps on distribution maps created by shortfalls of traditional sampling methodologies (Davy et al., 2015). Additionally, the ease of use (Roussel et al., 2015) and low cost (Davy et al., 2015) should logistically facilitate its implementation as a survey methodology, allowing for its utilization in the future by managers and additional researchers to document populations.

In this study, we developed and utilized eDNA surveys for the detection of *P. gorzugi* in several water bodies throughout the southwestern Texas portion of its historical range. Specifically, our objectives were to (1) create initial and nested primer sets specific to *P. gorzugi* eDNA, (2) develop and optimize a PCR protocol to amplify *P. gorzugi* eDNA, (3) use the developed primer sets and protocol to conduct a wide-scale survey for the presence of *P. gorzugi* eDNA throughout southwestern Texas, and (4) compare the effectiveness of eDNA surveys against traditional methodologies.

Materials and Methods

Sample Collection

Immediately prior to sample collection, the sample collector put on a fresh set of nitrile gloves and sprayed all sampling equipment with a 50% bleach and distilled (DI) water solution to eliminate existing DNA which could contaminate the sample. This was followed by a rinse with DI water to wash off the bleach which could break down our sample. Afterwards, sampling equipment was sprayed with a 100 g/L sodium thiosulfate solution to neutralize any remaining bleach. After a final DI rinse, a sample blank was obtained by filtering 1 L of DI water though a 47 mm filter cup that was attached to a PVC arm and inserted into a hand-powered automotive fluid evacuator (Figure 23). Water samples were filtered through a Whatman Grade 4 filter (cat.

WHA1004047, GE Healthcare, Little Chalfont, Buckinghamshire, U.K.) with a pore size of ca. 25 microns. The filter was folded and placed in a labelled 2 mL microcentrifuge tube containing 700 µL of DNAzol (cat. # 10503027, Molecular Research Center, Inc., Cincinnati, OH, USA), a DNA buffer/extraction solution, for a minimum of 3 d at room temperature. Three water samples were collected from each survey area (2 m from shore and 1 m below the water surface when possible). This was done by attaching a 2 L plastic pitcher to the end of a large telescoping pole. Different locations or depths were noted if field conditions required a change to the sampling protocol. Up to 2 L of water was filtered through the hand-pump filtering apparatus with a Whatman Grade 4 eDNA filter three separate times. Turbid water samples often resulted in the filter clogging, and prevented the full 2 L from filtering through. When this occurred, the amount of water successfully filtered was noted, and the filter folded and stored as described previously. The microcentrifuge tube containing the samples were stored in a black box which blocked out sunlight to prevent the degradation of DNA while in storage.

Filter Extraction

After a minimum storage time of 3 d at room temperature, samples were extracted following a modified protocol from the DNAzol manual. First, test tubes containing the DNAzol and filters were heated at 55°C for 30 min in a water bath. Test tubes were vortexed to ensure adequate mixing and then centrifuged at 5000 *g* for 1 min. The filter was then removed from the test tube with clean forceps (sterilized with a 50% bleach solution), and the excess DNAzol removed by squeezing to retain all the DNAzol fluid in the microcentrifuge tube. Five hundred µL of chloroform was then added to each test tube and vortexed for 30 s. After standing for 1 min, the samples were centrifuged at 12000 *g* for 2 min. The supernatant was extracted into a clean 1.5 mL microcentrifuge tube with a pipette and 500 µL of 100% ethanol was added for

precipitation of a DNA pellet. Test tubes were inverted until mixed and centrifuged at 16,000 *g* for 10 min. The supernatant was then discarded, and the pellet washed with the addition of 500 µL of 95% ethanol. This was followed by a vortex of 30 s and centrifugation at 5000 *g* for 1 min. The supernatant was discarded, and the procedure repeated with 500 µL of 75% ethanol to ensure it was thoroughly washed. The pellet was left to air dry for at least 30 m and once dry, dissolved in 22 µL of a 30% TE buffer at 55°C. A subset of extracted samples were quantified for total DNA concentration using a Qubit Fluorometer (cat. # Q33238, Thermo Fisher Scientific, Waltham, MA, USA) following the procedure in the manual, and stored at -20°C until analyzed. To prevent contamination, all filter extractions were conducted in a clean lab that was separate from where PCR analysis occurred (Goldberg et al., 2016). Field blanks were extracted separately from field samples, to minimize contamination risk during filter extraction. Additionally, benchtop and micropipettors were sanitized before extractions, and sterile filter pipette tips were used. Nitrile gloves were worn throughout the entire extraction procedure.

Primer Design and eDNA Validation

Forward and reverse oligomer primers were designed in Geneious v11.0.1 using nucleotide sequences based on the mitochondrial genome sequence of the cytochrome oxidase I mitochondrial gene for *P. gorzugi* available in GenBank (GenBank: HQ329656.1; Table 7). Primer design was completed using Primer3 software, to develop primer sets that were specific to *P. gorzugi*, had similar melting temperatures, and had minimal formation of dimers. To increase specificity and sensitivity, a nested primer set was additionally designed (Table 7).

Primers were tested with extracted *P. gorzugi* tissue samples to ensure specificity of our primers for *P. gorzugi* DNA. Tissue samples were obtained from a *P. gorzugi* that was collected in November 2018 from Fort Clark Springs, Las Moras Creek (Site 33; Figure 3; Table 1). The

tissue was stored in a modified Longmire buffer (Longmire et al., 1988; Appendix C) at 4°C until analysis was able to be completed. Tissue samples from the hind foot webbing of sympatric turtle species, *Trachemys scripta elegans* (DRD 6170 [Fort Clark Springs, Las Moras Creek; Site 33; Figure 3; Table 1]) and *Apalone spinifera*, (DRD 6289 [Lake Casa Blanca International State Park, Casa Blanca Lake, near El Ranchito pavilion; Site 45; Figure 3; Table 1]) were obtained and stored in DNAzol at room temperature. These tissue samples were obtained to ensure that the primers did not experience any cross-specificity. The mouse tail DNA extraction procedures from the GenCatch Blood & Tissue Mini-Prep Kit (cat. # 1460050, Epoch Life Science, Sugar Land, TX, USA) were used to extract all the tissues obtained. Polymerase chain reaction (PCR) was conducted according to our protocol outlined below with resulting PCR products run through a 1% agarose gel electrophoresis. The produced gel was examined for visible bands.

Additionally, a positive control eDNA water sample was generated by placing a juvenile *P. gorzugi* (Chandler Ranch, Cement Pond; Site 8; Figure 3; Table 1) in ca. 8 L of water collected from TNC Independence Creek Preserve, Lower Lake (Site 5; Figure 3; Table 1) for ca. 48 h. The sample underwent the filter extraction protocol (described above), followed by a nested PCR procedure, as described below. Afterwards, the nested PCR product was visualized using gel electrophoresis using the procedure described above. The final sequence was sent away for Sanger sequencing at Eurofins Genomics LLC (Louisville, KY, USA) to confirm that PCR amplified out the *P. gorzugi* target DNA sequence. Lastly, this procedure was completed with a filter from a site with known *P. gorzugi* inhabitance, Eagle Pass Golf Course, spillway into Rio Grande (Site 42; Figure 3; Table 1) to ensure that the primers were able to detect DNA in more dilute field conditions. With all eDNA analyses, a no template control and 100 base-pair ladder

were analyzed alongside the samples, to ensure that contamination was not occurring during analysis.

Polymerase Chain Reaction

All eDNA samples were ran through both an initial and nested PCR, which followed an optimized procedure specifically designed for *P. gorzugi* eDNA. Preparation of PCR samples occurred under a PCR hood to prevent contamination. Both rounds of PCR were conducted in 25 μL reactions, consisting of 12.5 μL of GoTaq HotStart Master Mix (cat. # M5122, Promega Corp, Madison, WI, USA), 5.5 μL water, 1 μL 10 μM forward primer, 1 μL 10 μM reverse primer, and 5 μL of sample, which was replaced by 5 μL water for the no template control. The sample for the initial PCR consisted of 5 μL of the filter extract and the sample for the nested PCR consisted of 5 μL of the purified product from the initial PCR. To purify the initial PCR product, Monarch PCR & DNA Cleanup Kits (cat. # T1030S, New England Biolabs, Ipswich, MA, USA) were used following a modified protocol. The optimized protocol for the initial PCR was an initial 3 min denaturation at 94°C, followed by 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 57°C, extension for 30 s at 72°C, and a cooling period for 10 min at 4°C.

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, products were purified again using Monarch PCR & DNA Cleanup Kits and quantified for total DNA concentration through a Qubit Fluorometer following the procedure outlined in the manual. Samples with total DNA concentrations that were unmeasurable by the Quibit Fluorometer were considered eDNA negative samples, and samples with measurable amounts were then prepared and sent for Sanger sequencing at Eurofins Genomics LLC. The resulting sequence data was then compared to the GenBank database using the BLAST query

and aligned with a known *P. gorzugi* sequence (GenBank: KC687314) to confirm sequence identity and designate samples as eDNA positive.

Ethical Statement

All research was conducted under a TPWD Scientific Research Permit (SPR-1018-294), TPWD State Park Scientific Study Permit (2019_R2_RGV_02), 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).

Results

Primer Design and eDNA Validation

The initial primer set, PG_CO1_FW1 and PG_CO1_RV1mod1, successfully amplified a 155-bp sequence of DNA from *P. gorzugi* tissue, producing visual bands after gel electrophoresis. The nested primer set, PG_CO1_FW1_nest and PG_CO1_RV1_nest, successfully amplified a 118-bp sequence of DNA from a *P. gorzugi* positive-control eDNA sample, resulting in a visual band after gel electrophoresis (Figure 24). Sanger sequencing from the nested primer set PCR products confirmed that these PCR products matched the targeted cytochrome oxidase region I of *P. gorzugi*. The nested primer sets successfully amplified eDNA from our initial field-collected eDNA sample, with visible bands observed in the gel, which were confirmed to be *P. gorzugi* DNA through Sanger sequencing. In all analyses, the no template controls produced no visible band (Figure 24).

Sampling Results

In total, 42 unique localities were chosen to validate the *P. gorzugi* eDNA assay (Table 8). Sites were chosen to encompass the full range of *P. gorzugi* abundance, from localities where *P. gorzugi* has not been recently documented, to localities with known, large populations. Additionally, sites were chosen to encompass the full geographical range of our survey area (Figure 3), ensuring that the outer reaches of our survey area were covered, including areas outside of recent *P. gorzugi* observations. Varying turbidity levels led to a range of average filtered water volumes from 250 to 2000 mL, with a mean volume of water (± 1 SD) of 1650 ± 690 mL (Table 8).

Of these 42 sites, eDNA analysis resulted in positive detections at 22 sites, and failed to detect *P. gorzugi* eDNA at 20 sites (Figure 25; Table 8). Thirty-four of these sites were part of a methodology comparison study, comparing the effectiveness of drone, visual, and trapping surveys, and sites where detection of *P. gorzugi* occurred through at least one of these methodologies were deemed *P. gorzugi* positive (Table 8). Out of the 34 methodology comparison sites, 21 were previously considered *P. gorzugi* positive, and eDNA analysis failed to detect *P. gorzugi* eDNA at nine of these sites (Table 8). Five of these detection failures occurred amongst the nine spring-fed sites (55%), and four detection failures occurred amongst the 25 sites that were not spring-fed (16%; Table 8).

Thirteen of the method comparison sites were previously considered *P. gorzugi* negative as drone, visual, and trapping surveys failed to result in any detections, however, eDNA analysis resulted in positive detections at six of these sites (Table 8). Included in this grouping were Pecos River at US Hwy 190 crossing, Pecos County (Site 1; Figure 25; Table 8) the northernmost detection of *P. gorzugi* resulting from this study, and the Rio Grande, near the

National Butterfly Center, Hidalgo County (Site 59; Figure 25; Table 8) the southernmost detection of *P. gorzugi* resulting from this study (Figure 25; Table 8). Amongst these unique detections, one occurred out of the nine spring-fed sites (11%) and five occurred amongst the 25 sites that were not spring-fed (20%; Table 8).

Discussion

Environmental DNA (eDNA) analysis successfully detected *Pseudemys gorzugi* DNA in several different types of aquatic systems, showing the potential of eDNA analysis to survey for this species. While eDNA studies have been conducted on other turtle species (Davy et al., 2015; de Souza et al., 2016; Raemy and Ursenbacher, 2018), this was the first implementation of eDNA analysis on *P. gorzugi*, adding *P. gorzugi* to the growing list of species that have undergone this survey methodology (Rees et al., 2014).

Several advantages of eDNA surveys became apparent throughout the course of this study. eDNA surveys proved to be fairly low effort and easy to implement in the field, and thus samples were collected at almost every site we sampled. There was one instance at Pump Canyon, Langtry (Site 19; Figure 3; Table 1) in which a water sample was not able to be acquired as steep canyon walls prevented water access from the shore. However, with the exception of this site, the telescoping pole was always sufficient for sample collection and despite water access, few other requirements existed. Additionally, these surveys appeared to be minimally invasive, with few observed changes to *P. gorzugi* behavior. In one instance, a *P. gorzugi* was observed swimming in water ca. 2 m from the collection site while the eDNA water sample was being collected. With high specificity of the primers, misidentification is unlikely to occur, and detection rates can be superior to other methodologies, as visual observation is not

required (Ficetola et al., 2008; Hoffman et al., 2016). Due to these numerous strengths, eDNA appears to be a simple and cost-effective (Davy et al., 2015) method to survey for *P. gorzugi*.

Despite its strengths, one of the shortfalls of eDNA analysis is that abundance estimates are not possible, and thus only presence at a site can be determined. While studies have found correlations between eDNA concentrations and biomass using quantitative PCR (qPCR) (Klymus et al., 2015; Sassoubre et al., 2016; Lacoursière-Roussel et al., 2016), numerous factors affect DNA shedding rates of organisms, including water temperature (Lacoursière-Roussel et al., 2016) and diet (Klymus et al., 2015). Therefore, quantification cannot be accurately incorporated into field studies at this time. With eDNA analysis, absence cannot be definitively stated, as there is always a chance that a species is present, but not detected (Moyer et al., 2014). Previous studies have produced variable detection rates (Moyer et al., 2014; Biggs et al., 2015; Takahara et al., 2015) with detection probability increasing with number of replicates (Ficetola et al., 2014).

Throughout the survey period of March–October 2019, 42 sites were analyzed for *P. gorzugi* eDNA and at some of these sites, *P. gorzugi* was detected through other methods, but was not detected through eDNA analysis (Figure 25; Table 8). While repeat sampling occurred at most sites, due to time constraints these additional samples were not analyzed. Analysis of these remaining samples may show whether eDNA consistently fails to detect *P. gorzugi* at these sites, or if it is an occasional occurrence. Literature suggests that increased replicates may result in more detections and minimize the occurrence of false negatives (Ficetola et al., 2014; Piggott, 2016). eDNA detections have at times been seasonal in nature, especially when the target organism has activity levels that are seasonally dependent (de Souza et al., 2016). While *P. gorzugi* maintain some level of activity year-round, they may be more active in certain seasons,

and environmental factors that vary seasonally could influence detection results as well. Additionally, organic compounds, such as tannic and humic acids are known to inhibit PCR amplification and can lead to false negatives (Hunter et al., 2019). Methodological enhancements can reduce PCR inhibition (Hunter et al., 2019) and should be further explored to see if PCR inhibitors were responsible for any missed detections in this study.

Numerous factors can impact eDNA degradation such as temperature, pH, and UV rays (Strickler et al., 2015). The bulk of sampling occurred during the summer months in southwestern Texas, when temperatures and UV levels were high. It is likely that eDNA would degrade faster in these systems during this time period, and more detections may occur in cooler months when eDNA persists for a longer time period. Estimates on the persistence of eDNA in aquatic systems range from less than 1 d to over 2 wk (Barnes et al., 2014), and under our sampling conditions it would be expected to be on the shorter end of that time frame.

Additionally, detection failures could be prevented by filtering greater amounts of water through the filtering apparatus to capture larger amounts of eDNA. Schultz and Lance (2015) found a sample volume increase to be the most effective way to enhance the sensitivity of eDNA surveys. While some sites had turbid water or high amounts of algae which resulted in only small volumes of water successfully filtered, the 2 L sample was easily filtered at most of the sites. While 2 L was used for consistency, 6 L of water was successfully filtered at Fort Clark Springs, Headwater Pond (Site 29; Figure 25; Table 8), and it is likely similar, if not larger, amounts of water could have been filtered, particularly at spring-fed sites. Spring-fed sites had a greater likelihood of missed detections than our non-spring-fed sites. These sites were often near, or close to, the headwaters, and generally had high flow. High flowing systems are known to result in decreased eDNA counts (Jane et al., 2014) as eDNA is quickly transported downstream

preventing buildup of eDNA in these systems. Greater volumes of water filtered may thus result in more detections at these sites, and this should be further explored.

While most of our results correlated with what we expected, positive detections in known localities, and no detection in areas where *P. gorzugi* has not been observed, there were also a few instances in which we had positive detections in areas where *P. gorzugi* had not been recently documented. This includes Rio Grande, near National Butterfly Center (Site 59; Figure 25; Table 8) a southeastern expansion of their range and Pecos River, at US Hwy 190 crossing (Site 1; Figure 25; Table 8) a northwestern expansion. The distance eDNA can travel in lotic systems before it becomes too degraded for analysis varies between studies from 239.5 m (Jane et al., 2014) to 12.3 km (Deiner and Altermatt, 2014). Several variables such as environmental conditions and biomass determine this difference (Kessler et al., 2020). Given these estimates, it seems likely that undocumented populations of *P. gorzugi* may exist outside of their recognized current range. The next documented population of *P. gorzugi* upstream from Pecos River, at US Hwy 190 crossing (Site 1; Figure 25; Table 8) is 160 km from that site in the Delaware River in Texas along the New Mexico border (Bonner and Littrell, 2016). It appears unlikely that eDNA would persist in that river system for such a great distance, which suggests *P. gorzugi* populations exist nearby. Contamination is also unlikely as field blanks and no template controls failed to amplify *P. gorzugi* eDNA throughout the study. While other survey methods failed to detect *P. gorzugi* during the three sampling visits to this site, it is recommended that both this area, and further upstream, undergo more thorough analysis to confirm this detection.

Our site at Rio Grande, near National Butterfly Center (Site 59; Figure 25; Table 8) lies outside of areas where *P. gorzugi* has been directly overserved, 92 river-km downriver from the next known locality, Rio Grande, near Rio Grande City (Site 57; Figure 25; Table 8), which was

only recently discovered (iNaturalist 35887108, 35887109). Both of these sites appear very similar in appearance with no apparent distinguishing characteristics that should prevent *P. gorzugi* from occurring at the location further downstream. With Rio Grande, near Rio Grande City (Site 57; Figure 25; Table 8) being a recent extension to the current range, it seems likely that *P. gorzugi* may exist further downstream than thought. Historical range maps (Figure 1) encompass the Rio Grande to Brownsville, Texas, and though the locality information on records in that area are dated and vague, it is reasonable to think that *P. gorzugi* could occur even further downriver than Rio Grande, near National Butterfly Center (Site 59; Figure 25; Table 8). It is recommended that this area between Rio Grande, near Rio Grande City (Site 57; Figure 25; Table 8) and Rio Grande, near National Butterfly Center (Site 59; Figure 25; Table 8) undergo more extensive surveys to see if a visual detection can be confirmed. It is important to note that false positives in eDNA analysis likely result from contamination or lack of primer specificity (Schultz and Lance, 2015), which can be determined through sample controls and sequencing PCR products. If these are accounted for, false positives are often rare (Moyer et al., 2014; Biggs et al., 2015) which increases the validity of these detections. Future studies should continue to explore the distance that eDNA can travel downstream before it becomes too degraded for analysis in different aquatic systems to determine the likely proximity of a species to its detection point.

By combining the eDNA detection data with the detections that resulted from drone, visual, and trapping surveys (Chapter II), we were able to create a current range map for *P. gorzugi* in southwestern Texas (Figure 26). This is the most comprehensive map that we were able to produce as a result of this study, and greatly adds to the knowledge of *P. gorzugi* current distribution. This map illustrates the benefits that arise from combining multiple methodologies,

as there are far more detections than what arose from the individual detection maps of each method (Figure 11–13, 25). Our conclusion after implementing drone, visual, trapping, and eDNA surveys, is that each method has its different advantages which lead to unique detections. Whenever possible, all of these methods should be applied in survey efforts to ensure a more thorough analysis.

REFERENCES

Akre, T. S., Parker, L. D., Ruther, E., Maldonado, J. E., Lemmon, L., & McInerney, N. R. (2019). Concurrent visual encounter sampling validates eDNA selectivity and sensitivity for the endangered wood turtle (*Glyptemys insculpta*). *PLOS ONE*, *14*(4), e0215586.

Bailey, L. A., Forstner, M. R., Dixon, J. R., & Hudson, R. (2014). Contemporary status of the Rio Grande cooter (Testudines: Emydidae: *Pseudemys gorzugi*) in Texas: phylogenetic, ecological and conservation consideration. In *Proceedings of the Sixth Symposium on the Natural Resources of the Chihuahuan Desert Region. Texas: Chihuahuan Desert Research Institute* (pp. 320–334).

Baker, C. S., Steel, D., Nieukirk, S., & Klinck, H. (2018). Environmental DNA (eDNA) from the wake of the whales: droplet digital PCR for detection and species identification. *Frontiers in Marine Science*, *5*, 133.

Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., & Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science & Technology*, *48*(3), 1819–1827.

Beauvais, G. P., & Buskirk, S. W. (1999). Modifying estimates of sampling effort to account for sprung traps. *Wildlife Society Bulletin*, *27*(1), 39–43.

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R. A., ... & Williams, P. (2015). Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biological Conservation*, *183*, 19–28.

Bonner, T. H. & Littrell, B. M. (2016). Aquatic surveys of Delaware River of Texas. Final report to Texas Comptroller of Public Accounts.

Clusa, L., Ardura, A., Fernández, S., Roca, A. A., & García-Vázquez, E. (2017). An extremely sensitive nested PCR-RFLP mitochondrial marker for detection and identification of salmonids in eDNA from water samples. *PeerJ*, *5*(2), e3045.

Collins, J. T. (1991). Viewpoint: a new taxonomic arrangement for some North American amphibians and reptiles. *Herpetological Review*, *22*(2), 42–43.

Davy, C. M., Kidd, A. G., & Wilson, C. C. (2015). Development and validation of environmental DNA (eDNA) markers for detection of freshwater turtles. *PLOS ONE*, *10*(7), e0130965.

Degenhardt, W. G., Painter, C. W., & Price, A. H. (1996). *Amphibians and reptiles of New Mexico*. University of New Mexico Press, Albuquerque, New Mexico. 431 pp., 123 plates.

de Souza, L. S., Godwin, J. C., Renshaw, M. A., & Larson, E. (2016). Environmental DNA (eDNA) detection probability is influenced by seasonal activity of organisms. *PLOS ONE*, *11*(10), e0165273.

Deiner, K., & Altermatt, F. (2014). Transport distance of invertebrate environmental DNA in a natural river. *PLOS ONE*, *9*(2), e88786.

Díaz-Ferguson, E. E., & Moyer, G. R. (2014). History, applications, methodological issues and perspectives for the use environmental DNA (eDNA) in marine and freshwater environments. *Revista de Biologia Tropical*, *62*(4), 1273–1284.

Dougherty, M. M., Larson, E. R., Renshaw, M. A., Gantz, C. A., Egan, S. P., Erickson, D. M., & Lodge, D. M. (2016). Environmental DNA (eDNA) detects the invasive rusty crayfish *Orconectes rusticus* at low abundances. *Journal of Applied Ecology*, *53*(3), 722–732.

Ernst, C. H. (1990). *Pseudemys gorzugi*. *Catalogue of American Amphibians and Reptiles (CAAR)*, *461*, 1–2.

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

Ficetola, G. F., Pansu, J., Bonin, A., Coissac, E., Giguet-Covex, C., De Barba, M., ... & Rayé, G. (2014). Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Molecular Ecology Resources*, *15*(3), 543–556.

Goldberg, C. S., Pilliod, D. S., Arkle, R. S., & Waits, L. P. (2011). Molecular detection of vertebrates in stream water: a demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. *PLOS ONE*, *6*(7), e22746.

Goldberg, C. S., Strickler, K. M., & Pilliod, D. S. (2015). Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. *Biological Conservation*, *183*, 1–3.

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., ... & Laramie, M. B. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, *7*(11), 1299–1307.

Gu, W., & Swihart, R. K. (2004). Absent or undetected? Effects of non-detection of species occurrence on wildlife–habitat models. *Biological Conservation*, *116*(2), 195–203.

Hoffmann, C., Schubert, G., & Calvignac-Spencer, S. (2016). Aquatic biodiversity assessment for the lazy. *Molecular Ecology*, *25*(4), 846–848.

Hofreiter, M., Mead, J. I., Martin, P., & Poinar, H. N. (2003). Molecular caving. *Current Biology*, *13*(18), R693–R695.

Hunter, M. E., Ferrante, J. A., Meigs-Friend, G., & Ulmer, A. (2019). Improving eDNA yield and inhibitor reduction through increased water volumes and multi-filter isolation techniques. *Scientific Reports*, *9*(1), 1–9.

iNaturalist. (2020). *Observations of Rio Grande Cooter*. Retrieved February 4, 2020, https://www.inaturalist.org/observations?taxon_id=39823.

Jane, S. F., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., ... & Whiteley, A. R. (2015). Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. *Molecular Ecology Resources*, *15*(1), 216–227.

Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters*, *4*(2), 150–157.

Kessler, E. J., Ash, K. T., Barratt, S. N., Larson, E. R., & Davis, M. A. (2020). Radiotelemetry reveals effects of upstream biomass and UV exposure on environmental DNA occupancy and detection for a large freshwater turtle. *Environmental DNA*, *2*(1), 13–23.

Klymus, K. E., Richter, C. A., Chapman, D. C., & Paukert, C. (2015). Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*. *Biological Conservation*, *183*, 77–84.

Klymus, K. E., Marshall, N. T., & Stepien, C. A. (2017). Environmental DNA (eDNA) metabarcoding assays to detect invasive invertebrate species in the Great Lakes. *PLOS ONE*, *12*(5), e0177643.

Kundu, S., Kumar, V., Laskar, B. A., Chandra, K., & Tyagi, K. (2016). Mitochondrial DNA effectively detects non-native Testudines: Invisible wildlife trade in northeast India. *Gene Reports*, *4*, 10–15.

Lacoursière-Roussel, A., Rosabal, M., & Bernatchez, L. (2016). Estimating fish abundance and biomass from eDNA concentrations: variability among capture methods and environmental conditions. *Molecular Ecology Resources*, *16*(6), 1401–1414.

Lancia, R. A., Kendall, W. L., Pollock, K. H., & Nichols, J. D. (2005). Estimating the number of animals in wildlife populations. *Techniques for wildlife investigations and management.* The Wildlife Society, Bethesda, Maryland, USA, 106–153.

Lawson, G. (2018). Development of eDNA techniques for the detection of *Trachemys scripta* in aquatic samples and detection of prey items (*Danio rerio*) in *Trachemys scripta* fecal samples. Biological Sciences Theses.

Lovich, J. E., & Ennen, J. R. (2013). A quantitative analysis of the state of knowledge of turtles of the United States and Canada. *Amphibia-Reptilia*, *34*(1), 11–23.

Longmire, J. L., Lewis, A. K., Brown, N. C., Buckingham, J. M., Clark, L. M., Jones, M. D., ... & Wagner, R. P. (1988). Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. *Genomics*, *2*(1), 14–24.

Moyer, G. R., Diaz-Ferguson, E., Hill, J. E., & Shea, C. (2014). Assessing environmental DNA detection in controlled lentic systems. *PLOS ONE*, *9*(7), e103767.

New Mexico Department of Game and Fish [NMDGF]. 2006. The comprehensive wildlife conservation strategy for New Mexico. Santa Fe: New Mexico Department of Game and Fish.

Pierce, L. J. S., Stuart, J. N., Ward, J. P., & Painter, C. W. (2016). *Pseudemys gorzugi* Ward 1984-Rio Grande Cooter, Western River Cooter, Tortuga de Oreja Amarilla, Jicotéa del Río Bravo. In: Rhodin, A. G. J., Iverson, J. B., van Dijk, P. P., Saumure, R. A., Buhlmann, K. A., Pritchard, P. C. H., Mittermeier R. A., 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: Chelonian Research Foundation*, 1–12.

Piggott, M. P. (2016). Evaluating the effects of laboratory protocols on eDNA detection probability for an endangered freshwater fish. *Ecology and Evolution*, *6*(9), 2739–2750.

Raemy, M., & Ursenbacher, S. (2018). Detection of the European pond turtle (*Emys orbicularis*) by environmental DNA: is eDNA adequate for reptiles? *Amphibia-Reptilia*, *39*(2), 135–143.

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R., & Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA–a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, *51*(5), 1450–1459.

Reid, B. N., Le, M., McCord, W. P., Iverson, J. B., Georges, A., Bergmann, T., ... & NARO-MACIEL, E. (2011). Comparing and combining distance-based and character-based approaches for barcoding turtles. *Molecular Ecology Resources*, *11*(6), 956–967.

Renshaw, M. A., Olds, B. P., Jerde, C. L., McVeigh, M. M., & Lodge, D. M. (2015). The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol–chloroform–isoamyl alcohol DNA extraction. *Molecular Ecology Resources*, *15*(1), 168–176.

Roussel, J. M., Paillisson, J. M., Treguier, A., & Petit, E. (2015). The downside of eDNA as a survey tool in water bodies. *Journal of Applied Ecology*, *52*(4), 823–826.

Sassoubre, L. M., Yamahara, K. M., Gardner, L. D., Block, B. A., & Boehm, A. B. (2016). Quantification of environmental DNA (eDNA) shedding and decay rates for three marine fish. *Environmental Science & Technology*, *50*(19), 10456–10464.

Schultz, M. T., & Lance, R. F. (2015). Modeling the sensitivity of field surveys for detection of environmental DNA (eDNA). *PLOS ONE*, *10*(10), e0141503.

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

Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, *183*, 85–92.

Takahara, T., Minamoto, T., & Doi, H. (2013). Using environmental DNA to estimate the distribution of an invasive fish species in ponds. *PLOS ONE*, *8*(2), e56584.

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

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. (2012). Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLOS ONE*, *7*(8), e41732.

Turner, C. R., Barnes, M. A., Xu, C. C., Jones, S. E., Jerde, C. L., & Lodge, D. M. (2014). Particle size distribution and optimal capture of aqueous macrobial eDNA. *Methods in Ecology and Evolution*, *5*(7), 676–684.

USFWS [United States Fish and Wildlife Service]. (2015). Endangered and threatened wildlife and plants; 90-day findings on 31 species. *Federal Register, 80,* 37568–37579.

Figure 23. Equipment used to acquire environmental DNA (eDNA) samples. This includes the plastic pitcher, telescoping sampling pole, 47-mm filter cup, and hand-powered fluid excavator.

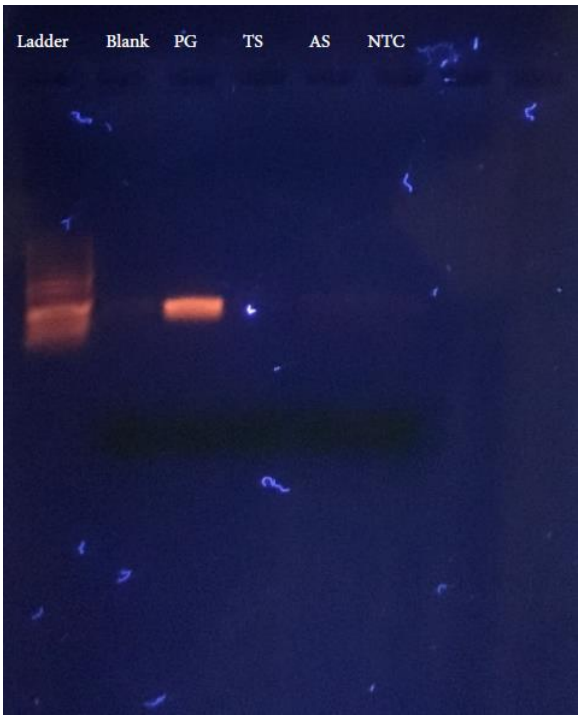

Figure 24. Amplification of *Pseudemys gorzugi* DNA using nested primer set PG_CO1_FW1_nest and PG_CO1_RV1_nest produced a band of expected size from the *P. gorzugi* (PG) tissue sample after electrophoresis through agarose gel. Tissue samples from *Trachemys scripta elegans* (TS) and *Apalone spinifera* (AS), the no template control (NTC), and sample blank (Blank) failed to produce any visible bands.

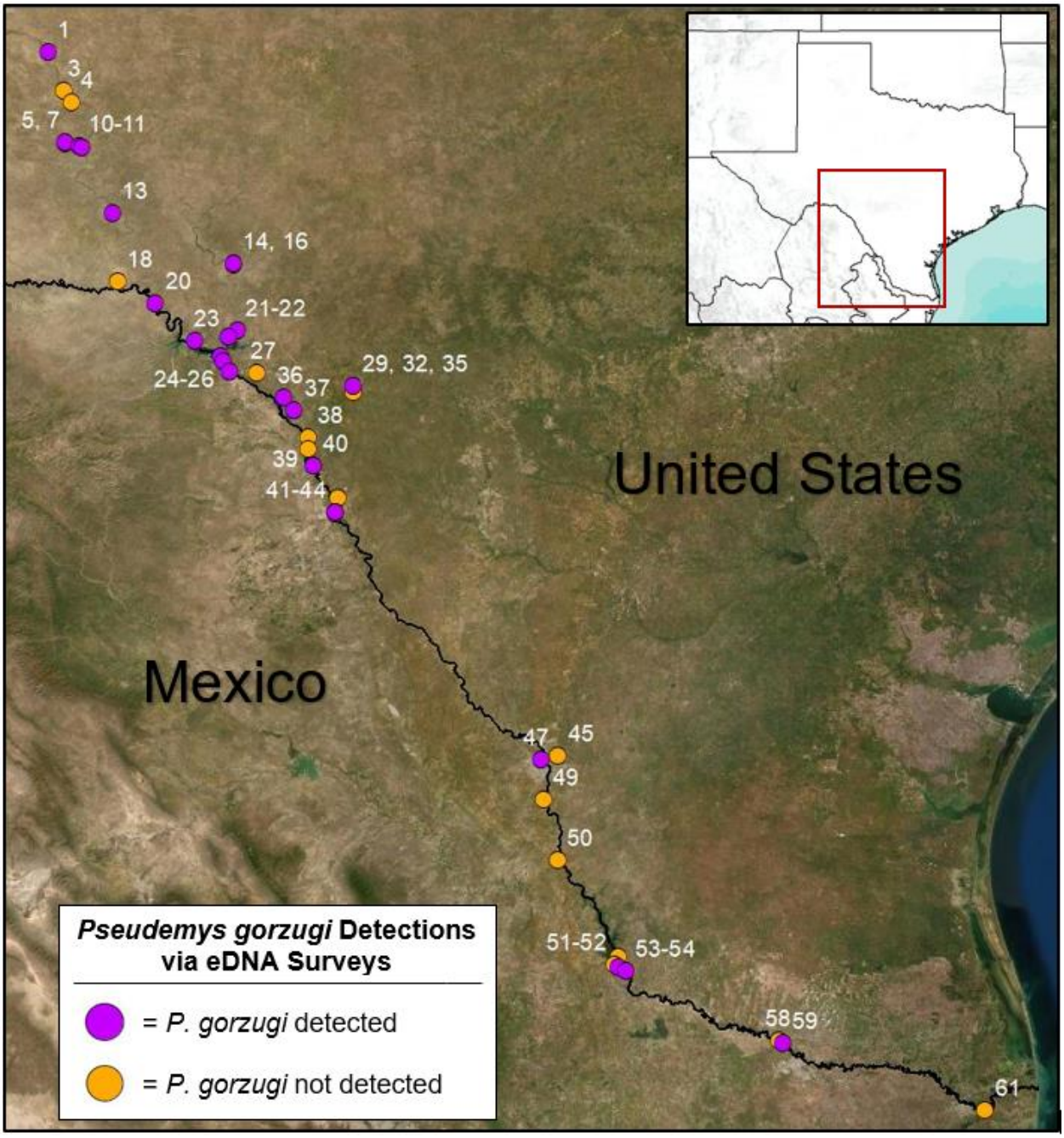

Figure 25. Map of 42 unique localities where eDNA analysis occurred for *Pseudemys gorzugi* through southwestern Texas. Purple dots indicate positive detections of *P. gorzugi*. Orange dots indicate sites where *P. gorzugi* was not detected. Site numbers correspond to the numbers used in Table 8.

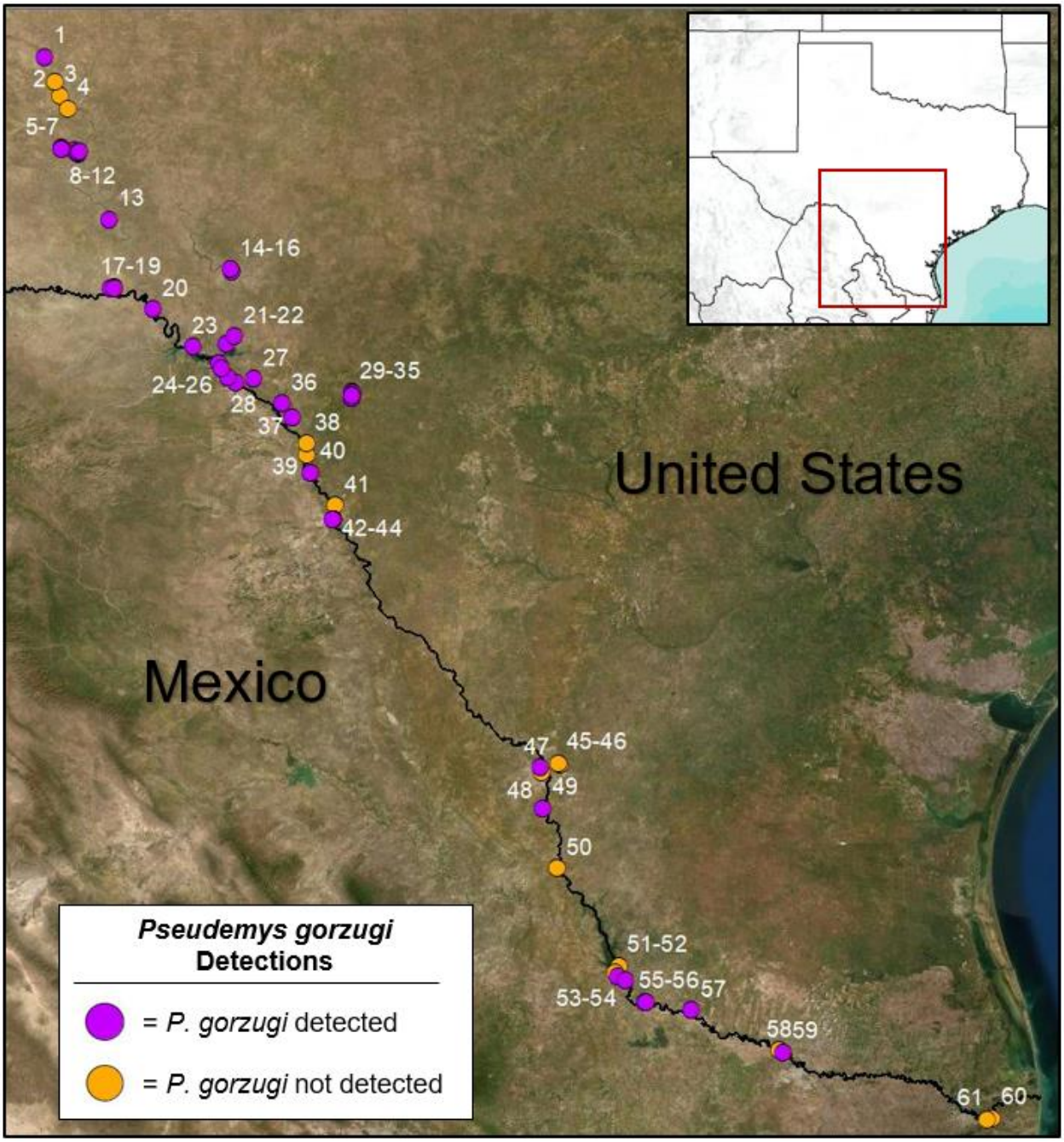

Figure 26. Map of unique localities where *Pseudemys gorzugi* was detected in southwestern Texas. Purple dots indicate positive detections of *P. gorzugi*. Orange dots indicate sites where *P. gorzugi* was not detected. Site numbers correspond to the numbers used in Table 1.

Table 7. Initial and nested primer sets that were designed and used for environmental DNA (eDNA) analysis for the detection of *Pseudemys gorzugi*. Primer characteristics including annealing temperature (°C) and product size (bp) are included.

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

Table 8. The 42 sites that were analyzed for *Pseudemys gorzugi* environmental (eDNA). Sites that were a part of the methodology comparison study as well as those with the spring-fed classification are indicated. Average volume of water filtered (± 1 SD) through each filter (mL), concentrations of each of the three eDNA samples (ng/µL), and final outcomes regarding whether *P. gorzugi* (PG) eDNA was detected are indicated. Boldfaced letters denote locations where eDNA resulted in unique detections, and underlined letters denote locations where eDNA analysis failed to detect *P. gorzugi* eDNA despite a known presence. For eDNA detections to be considered positive, two of the three samples analyzed had to be successfully sequenced as *P. gorzugi* (indicated in bold). Site numbers correspond to Table 1. Information regarding sampling dates can be found in Appendix D.

Site #	County	Site	Latitude	Longitude	Methods Site	Spring-fed	Average Volume Filtered	Sample 1	Sample 2	Sample 3	PG eDNA detected
1	Pecos	Pecos River, at US Hwy 190 crossing	30.90516	-101.88083	Y	N	1600.0 (± 173.2)	**2.4**	**3.02**	**1.78**	**Y**
3	Pecos	Pecos River, at I-10 crossing	30.71808	-101.80954	Y	N	833.3 (± 144.3)	too low	too low	too low	N
4	Pecos	Pecos River, at TX Hwy 290 crossing	30.65960	-101.77022	Y	N	1000.0 (± 0)	0.108	**0.388**	too low	N
5	Terrell	TNC Independence Creek Preserve, Lower Lake	30.46955	-101.80131	Y	Y	2000.0 (± 0)	**13.4**	**5.12**	**9.66**	Y
7	Terrell	TNC Independence Creek Preserve, raceway below Upper Lake	30.46736	-101.80181	Y	Y	2000.0 (± 0)	0.204	too low	too low	N
10	Terrell	Independence Creek, at County Road crossing	30.45026	-101.73124	Y	Y	2000.0 (± 0)	**5.44**	0.89	**1.07**	**Y**
11	Crockett	Pecos River, 0.3 river km upstream of confluence with Independence Creek	30.44767	-101.72119	Y	N	1916.7 (± 144.3)	**0.506**	**1.12**	**0.578**	Y
13	Val Verde	Pecos River, at Pandale crossing	30.13120	-101.57450	Y	N	900.0 (± 91.7)	**0.446**	**0.222**	0.242	Y
14	Val Verde	TNC Dolan Falls Preserve, Devils River, upstream of confluence with Dolan Creek	29.89387	-100.99561	Y	Y	2000.0 (± 0)	too low	**0.144**	**0.378**	Y
16	Val Verde	TNC Dolan Falls Preserve, Devils River, Dolan Falls	29.88385	-100.99397	Y	Y	2000.0 (± 0)	too low	too low	too low	N
18	Val Verde	Rio Grande, near Langtry	29.80564	-101.55088	Y	N	250.0 (± 0)	too low	1.04	2	N

20	Val Verde	Pecos River, near confluence with Rio Grande	29.70431	-101.36667	Y	N	1333.3 (± 577.4)	**0.446**	too low	**0.88**	Y
21	Val Verde	Lake Amistad, Rough Canyon	29.57490	-100.97809	Y	N	2000.0 (± 0)	**2.22**	**0.426**	**1.03**	Y
22	Val Verde	Lake Amistad, along Spur 406	29.54023	-101.01623	N	N	1916.7 (± 144.3)	**0.412**	**4.04**	**0.348**	**Y**
23	Val Verde	Lake Amistad, Box Canyon	29.52420	-101.17585	Y	N	2000.0 (± 0)	**0.698**	**0.416**	**0.49**	**Y**
24	Val Verde	Rio Grande, spillway below Amistad Dam	29.44737	-101.05667	Y	N	2000.0 (± 0)	**0.202**	**0.15**	**0.354**	Y
25	Val Verde	Rio Grande, weir below Amistad Dam	29.42455	-101.04118	Y	N	2000.0 (± 0)	**0.242**	**0.104**	**0.2**	**Y**
26	Val Verde	Rio Grande, near Lugo property	29.37719	-101.01348	Y	N	2000.0 (± 0)	**0.884**	too low	**0.552**	Y
27	Val Verde	Del Rio, San Felipe Springs Golf Course, San Felipe Creek	29.37029	-100.88526	Y	Y	1000.0 (± 0)	**0.452**	0.108	too low	N
29	Kinney	Fort Clark Springs, Headwater Pond	29.30944	-100.42125	Y	Y	2000.0 (± 0)	17.6	**2.28**	**0.202**	Y
32	Kinney	Fort Clark Springs, Las Moras Creek, upstream of golf pro shop	29.29043	-100.42386	Y	Y	1916.7 (± 144.3)	too low	too low	too low	N
35	Kinney	Fort Clark Springs, Las Moras Creek, Buzzard Roost	29.28034	-100.42076	Y	Y	2000.0 (± 0)	too low	too low	too low	N
36	Val Verde	Sycamore Creek, at US Hwy 277 crossing	29.25473	-100.75216	N	Y	2000.0 (± 0)	**2.9**	**5.2**	**10.4**	Y
37	Kinney	Pinto Creek, at US Hwy 277 crossing	29.18898	-100.70340	N	Y	2000.0 (± 0)	**1.57**	**0.97**	**0.636**	Y
38	Maverick	Tequesquite Creek, at US Hwy 277 crossing	29.06453	-100.63899	N	Y	2000.0 (± 0)	too low	0.488	too low	N
39	Maverick	Irrigation canal along US Hwy 277, near Las Moras Creek	29.00785	-100.63817	N	N	1416.7 (± 381.9)	too low	too low	0.136	N
40	Maverick	Quemado Creek, along US Hwy 277	28.92578	-100.61490	N	Y	666.7 (± 144.3)	**0.186**	**0.156**	too low	Y
41	Maverick	Elm Creek, near US Hwy 277	28.77016	-100.49828	N	Y	2000.0 (± 0)	too low	too low	too low	N

42	Maverick	Eagle Pass Golf Course, spillway into Rio Grande	28.70416	-100.51046	Y	N	1916.7 (± 144.3)	too low	too low	too low	N
43	Maverick	Rio Grande, along Eagle Pass Golf Course	28.70294	-100.51089	Y	N	1883.3 (± 202.1)	**0.304**	**0.182**	**0.136**	Y
44	Maverick	Eagle Pass Golf Course, settling pond along Rio Grande	28.70146	-100.50979	Y	N	600.0 (± 173.2)	0.408	0.406	**0.498**	N
45	Webb	Lake Casa Blanca International State Park, near El Ranchito pavillion	27.54447	-99.44098	Y	N	1750.0 (± 250.0)	0.156	0.184	0.102	N
47	Webb	Rio Grande, Laredo, near water treatment center	27.52372	-99.52431	Y	N	333.3 (± 144.3)	**1.52**	too low	**0.15**	Y
49	Webb	Rio Grande, near El Cenizo	27.33117	-99.51195	Y	N	583.3 (± 144.3)	**0.986**	0.442	0.89	N
50	Zapata	Rio Grande, near San Ygancio	27.04330	-99.44496	Y	N	443.3 (± 268.6)	0.14	too low	0.25	N
51	Starr	Falcon State Park, Falcon Lake	26.58179	-99.15259	Y	N	666.7 (± 144.3)	0.1	3.18	0.108	N
52	Starr	Rio Grande, spillway below Falcon Dam	26.54608	-99.17093	Y	N	1633.3 (± 321.5)	0.27	**0.388**	0.24	N
53	Starr	Rio Grande, near Chapeno	26.53233	-99.15546	Y	N	2000.0 (± 0)	**2.68**	**0.524**	**0.112**	**Y**
54	Starr	Rio Grande, near Salineño	26.51429	-99.11662	Y	N	2000.0 (± 0)	0.51	**0.38**	**0.812**	Y
58	Hidalgo	Bentsen-Rio Grande Valley State Park, La Parido Banco	26.17906	-98.38716	N	N	1500.0 (± 500.0)	**1.24**	too low	0.378	N
59	Hidalgo	Rio Grande, near National Butterfly Center	26.16934	-98.36742	Y	N	2000.0 (± 0)	**2.12**	1.58	**2.18**	**Y**
61	Cameron	Rio Grande, near TNC Southmost Preserve Office	25.85008	-97.39865	Y	N	1666.7 (± 577.4)	too low	too low	too low	N

CHAPTER IV

ASSESSMENT AND CHARACTERIZATION OF RIO GRANDE COOTER (*PSEUDEMYS GORZUGI*) HABITAT

Introduction

The Rio Grande Cooter (*Pseudemys gorzugi*) is a freshwater, aquatic turtle species, with a small range throughout the Rio Grande, Pecos, and Devils river systems in southwestern Texas, southeastern New Mexico, and northeastern Mexico, including the Mexican states of Tamaulipas, Nuevo León, and Coahuila (Iverson, 1992; Degenhardt et al., 1996; Dixon, 2013). Research on *P. gorzugi* is scarce, but concerns over habitat degradation, collection for pet trade, and intentional destruction have led to a designation of Threatened in New Mexico (New Mexico Department of Game and Fish [NMDGF], 2006) and Mexico (Secretaríade Medio Ambiente y Recursos Naturales, 2010), Near Threatened by the IUCN (Pierce et al., 2016), and a Species of Greatest Conservation Need in Texas (Texas Parks and Wildlife Department [TPWD], 2012). This has also made it a candidate for a federal listing as an endangered species, with a decision to be made in 2021 (USFWS, 2015).

Despite habitat degradation being cited as a major reason for its conservation status review, little is known about *P. gorzugi* habitat requirements. Its current range encompasses substantial habitat variation covering many different ecoregions, including the South Texas Plains, Edwards Plateau, and Trans-Pecos ecoregions in Texas (Texas Parks and Wildlife, 2020),

the Chihuahuan Desert ecoregion in New Mexico (Griffith et al., 2006), as well as several ecoregions in Mexico (Mexican Biodiversity, 2020). However, the small range and patchy distribution of *P. gorzugi* suggests that specific habitat parameters may be required. An elevational limit has been suggested at 1082 m with no *P. gorzugi* yet detected above this elevation (Degenhardt and Christiansen, 1974) and with salinity being a known limiting factor of freshwater aquatic turtles, one study has suggested that their conductivity threshold is above 2264–2593 μS/cm (Bonner and Littrell, 2016). Besides these two factors, data regarding *P. gorzugi* habitat is sparse. With *P. gorzugi* having a questionable conservation status it is imperative to understand the habitat parameters for this species to ensure that conservation measures are undertaken for appropriate management.

Habitat characterization can be accomplished through various methods, one of which is multispectral imaging. Multispectral imagers record light reflectance from various bands of the electromagnetic spectrum and create images from these reflectance values (Dickson et al., 2001). Bands such as near-infrared (NIR) go beyond the visible light portion of the spectrum and allow valuable data to be obtained that is indiscernible by human sight, which has been used for a wide variety of applications from detecting fecal contamination on apples (Kim et al., 2002) to determining depths of burns (Eisenbeiß et al., 1999). Multispectral imagers can be attached to drones, which has expanded the availability of this technology (Bendig et al., 2012). Farmers have recently taken advantage of multispectral imagery in crop field surveys, 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; Ouédraogo et al., 2014; Candiago et al., 2015). Imagery obtained from multispectral imagers can generate information regarding vegetation species, presence,

abundance, biomass, distribution, and structural attributes (Smith et al., 1990; Berni et al., 2009; Goncalves et al., 2015). This information can be used in habitat characterization, which has been successfully demonstrated in riverine habitat (Whited et al., 2002) and for wildlife habitat distribution (Viña et al., 2008).

Pseudemys gorzugi have demonstrated preferences for different habitat parameters including the presence of algal mats (see Water Quality section below). The characteristics of these algal mats that are important for habitat selection are unknown. While several sites with algal mats had *P. gorzugi* detections (n = 12), there were other sites that had algal mats, but no detections (n = 3). Algal mats are composed of several different types of aquatic vegetation (Tison et al., 1981; Zedler et al., 1982; Wharton et al., 1983) and it is likely that species composition differs between sites. If differences occur, this could suggest that a species-specific preference may exist for *P. gorzugi* and explain why *P. gorzugi* were not found at some sites where algal mats were present. Multispectral analysis of algal mats has been conducted previously, however, primarily in marine environments (Richardson, 1996; Al-AbdulKader et al., 2002; Hajjdiah et al., 2017). Unique spectral reflectance signatures for different species of algae composing marine algal mats has been determined previously (Richardson, 1996; Aberle et al., 2006; Thorhaug et al., 2007). By using the spectral reflectance data from a multispectral imager, unique classes containing algae should be able to be created for sites.

The National Land Cover Database (NLCD) is another method that can be used to characterize habitat. This publicly available database was created by the Multi-Resolution Land Characteristics Consortium (MRLC), a partnership of Federal agencies, using the Landsat Thematic Mapper (Multi-Resolution Land Characteristics Consortium, 2020). The MRLC has produced a map of land cover classifications which covers the United States and Puerto Rico by

assigning a land cover class to each 30-m pixel (Homer et al., 2015). This dataset can be used in several different analyses such as determining land cover change over time (Radeloff et al., 2005), the effects of climate change (Wylie et al., 2014) and habitat associations of target species (Collins et al., 2010). Several studies have used NLCD data for habitat analysis of various turtle species including Snapping Turtles (*Chelydra serpentina*; Patrick and Gibbs, 2010), Painted Turtles (*Chrysemys picta*; Patrick and Gibbs, 2010), Spotted Turtles (*Clemmys guttata*; Dailey, 2017), and Wood Turtles (*Glyptemys insculpta*; Brown et al., 2016). To the best of our knowledge, no formal analysis of *P. gorzugi* habitat has yet occurred, and by using NLCD data to compare the percent of land cover classes between sites with *P. gorzugi* detections and sites that did not result in *P. gorzugi* detections, we can start to look for associations between land cover class and *P. gorzugi* presence to better characterize *P. gorzugi* habitat.

Aquatic turtle habitat selection is often determined by water quality parameters with many species choosing habitats in minimally impacted areas (Gibbons, 1990). Aquatic turtles' susceptibility to water pollution has led to their frequent classification as biological indicators (Gibbons, 1990; Hinton and Scott, 1990; Herbert et al., 1993; Bonin et al., 1995; Golet and Haines, 2001). *Pseudemys gorzugi* is presumed to choose habitats with higher water quality as well, 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 in Texas and New Mexico has even been thought to be attributed to water pollution from oil and natural gas well runoff (Ward, 1984) as both the Rio Grande and Pecos River are subject to pollution from sewage inflow, as well as agricultural and mining runoff, leading to variable water quality amongst the rivers and their tributaries (Bailey et al., 2014). Additionally, factors such as salinity (Dunson and Seidel, 1986),

pH (Doupe et al., 2009), dissolved oxygen (Rasmussen and Litzgus, 2010), and water temperature (Storey et al., 2008) can impact habitat selection.

The Rio Grande is already considered an endangered river system with significantly degraded water quality (American Rivers, 2003; USDOI, 1998), and the surrounding habitats are degraded (Levings, 1998) and becoming worse (Brown et al., 2012). The Pecos River is in a similar state, with anthropogenic factors causing issues in water quality and habitat degradation (Robertson, 1997). It is essential to evaluate the state of these habitats and determine *P. gorzugi* habitat requirements to ensure that habitat is available and maintained for the survival of this species. In order to accomplish this, we have collected and analyzed multispectral imagery and water quality data from our sampling sites throughout southwestern Texas. Specifically, our objectives were to (1) collect and analyze multispectral imagery to characterize *P. gorzugi* habitat and (2) collect and analyze water quality data for differences between positive and negative *P. gorzugi* detection sites.

Materials and Methods

Multispectral Imaging

The MAIA, an eight spectral band multispectrometer (cat. # MAIA-WV, SAL Engineering, Russi, RA, Italy) was used for multispectral imaging and attached to a DJI Matrice 600 Pro unmanned aerial vehicle (cat. # CP.SB.000308, SZ DJI Tehnology Co., Ltd, Shenzhen, Guangdong, China) with a Gremsy T-3 gimbal (cat. # Gremsy T3V3, Gremsy.com, Ho Chi Minh City, Vietnam). Flights were programmed using the Maps Made Easy App (Drones Made Easy, San Diego, CA, USA) 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. This overlap was calculated off of pre-set settings within this app and are not accurate for the camera that we used. The overlap between transects

was less than the app calculated, but sufficiently covered the area surveyed. Flights were conducted in linear transects and perpendicular to the direction of flow in lotic systems to assist in photo-stitching. The entire study area was surveyed when possible, amounting to ca. 1.2 ha with a 10 m border around the water body. This area was determined as it 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 frontal 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. All drone flights were conducted by Amy P. Bogolin and under a Federal Aviation Administration remote pilot license (license # 4189203).

The MAIA has eight bands which range 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. Multilayer tiffs were created of three study sites using MAIA MultiCam Stitcher Pro Software (cat. # MAIA-WV, SAL Engineering, Russi, RA, Italy and 3DOM, Yokohama, Kanagawa, Japan), and the resulting tiff files were stitched together using Agisoft Metashape photogrammetry software (Agisoft LLC, St. Petersburg, Russia) to create photomosaics of the study sites. Three study sites were chosen for analysis, TNC Independence Creek Preserve, Lower Lake (Site 5; Figure 3; Table 1), Del Rio, San Felipe Springs Golf Course, San Felipe Creek (Site 27; Figure 3; Table 1), and Fort Clark

Springs, Headwater Pond (Site 29; Figure 3; Table 1), as each exhibited high *P. gorzugi* density and algal mat presence.

Multispectral imagery underwent a series of unsupervised classifications using Erdas Imagine 2020 (Hexagon AB, Stockholm, Sweden). The optimal classification occurred using seven classes, a convergence of 0.950, and 20 maximum iterations, and thus the thematic image for each study site with these parameters was used for analysis. Each class was masked out of the resulting image and the mean and mode spectral values were determined using the metadata function. The mean spectral values for each band were then input into Excel to create a graph for each class at each study site. As unsupervised classification results in the generation of randomly numbered classes, spectral signatures in the graphs were compared across the study sites to correlate these classes. Once these new corresponding classes were identified, they were verified by comparing the original unclassified images to see if object type (e.g. grass, water, trees, etc.) matched. Additionally, algal mats were further examined to see what classes composed each mat. The spectral values present in the algal mats were compared between the three sites to look for similarities and differences in the aquatic vegetation.

Photomosaics of the multispectral imagery additionally underwent supervised classification. The unsupervised classification image for Fort Clark Springs, Headwater Pond was used to create a signature file by inputting the pixel data from areas of interest located within each class. This signature file was used to run a supervised classification on each of the three study sites.

Land Class Characterization

The NLCD 2016 Land Cover (CONUS) data was downloaded from the MRLC website and the resulting raster data was uploaded into ArcGIS Desktop 10.6.1 (Esri, Redlands, CA,

USA). Buffers were created around each unique locality (Figure 3; Table 1) at several distances- 100, 250, 500, and 1000 m (Figure 27). These distances were chosen as they fall within the linear movements of the closely related *P. concinna,* with Buhlmann and Vaughn (1991) reporting a maximum observed distance range of 39–777 m and Dreslik et al. (2003) reporting a mean daily movement (± 1 SD) of 122.1 m (± 76.9) and a maximum daily movement mean of 336.6 m (± 200.9) for this species. Little is known about linear movements of *P. gorzugi*, however, one study found adults to travel an average of 61 m (± 14) for short-term movements with a maximum distance of 114 m (Mali and Forstner, 2017). Additionally, seasonal movements have been suggested to be 300 m (Degenhardt, et al., 1996). Another study noted the maximum downstream movement was 1.2 km for the individuals they studied (MacLaren et al., 2017). Considering these aforementioned movement distances for *P. gorzugi* and *P. concinna,* we feel that our buffers accurately represent the extent of the surrounding landscape that *P. gorzugi* is likely to interact with, representing both more and less conservative estimates of their movement distances.

Areas of the NLCD land cover classes were calculated within each buffer using the Tabulate Area 2 function in ArcGIS, which accommodates for overlap. While this does create an overrepresentation of certain land class types within our dataset, this allows for categorical analysis between study sites which would not be possible utilizing the merge function. Only two instances of overlap occurred at the 100 m buffer size, but more instances occurred as buffer size increased since many of our study sites are clustered. Therefore, the effect that overlap may have on the output would be increased in our larger scale analyses. Additionally, the NLCD land cover classification does not include Mexico, but labels these pixels as unclassified. The unclassified pixels were subtracted from the total buffer area before calculating percent land

covers, thus only representing the United States side of the habitat. It is likely that the Mexican habitat is similar to that on the United States side of the river due to the close proximity, but it is important to acknowledge that differences may exist that were unaccounted for.

Similar land cover class types were grouped to create nine classes: open water, developed, forest, shrub/scrub, grassland/herbaceous, wetlands, barren land, cultivated crops, and pasture/hay. The land cover area of each class was summed to determine a total land area for each buffer. These values varied slightly due to the number of pixels encompassed in each buffer. As the pixel size is relatively large at 30 m in comparison with some of the buffer sizes, especially at the 100 m buffer, the location of the GPS point within the pixel changed how many pixels were included in the analysis. Next, unclassified pixels were subtracted if present to determine an adjusted area, and then the percent area of each class was determined using this adjusted value. The mean percent of each land cover class type was compared between sites which had resulted in *P. gorzugi* detections throughout the study (Figure 26; Table 1) and sites where *P. gorzugi* were never detected at each buffer size.

For each site and at each buffer size, the percentage of each land class cover type was binned into 20% intervals (0–20%, 21–40%, 41–60%, 61–80%, and 81–100%) and tallied. This was performed separately for sites with *P. gorzugi* detections (n = 43) and sites without *P. gorzugi* detections (n = 18) to determine whether differences existed between these sites. Additionally, sites were grouped into different descriptive habitat categories: mainstem, tributary, Rio, Pecos, or spring-fed, and some study sites met conditions for multiple categories. This categorization was to group similar habitat sites together to see whether differences occurred in land class cover between sites with and without *P. gorzugi* detections, while minimizing the substantial habitat variability exhibited throughout *P. gorzugi* range. Within each

specific habitat category, differences in land cover class percentages were analyzed between sites with and without *P. gorzugi* detections at each buffer size.

All NLCD data was non-normally distributed and had unequal variances as determined by Shapiro-Wilk and Welch's t-tests. Therefore, non-parametric Mann Whitney tests were used for analyses. Means for all analyses are reported as mean (± 1 SD). All statistical analyses were conducted in JMP v14 statistical software (SAS Institute, Cary, NC, USA).

Water Quality

Water quality data was gathered at each location alongside eDNA water sampling throughout the survey period of November–October 2019. A Hach HQ40D Portable Multi Meter water quality sonde (cat. # HQ40D53000000, Hach Company, Loveland, CO, USA) was placed in the water ca. 1 m from the shoreline and nearby the eDNA sampling location. The water quality sonde obtained measurements of water temperature (°C), pH, dissolved oxygen (mg/L), conductivity (µS/cm), and oxidation-reduction potential (mV). Hach AquaChek water quality strips (cat. # 2745425; 2755325; 2744850; 5745250, Hach Company, Loveland, CO, USA) were also utilized to measure nitrate (ppm), nitrite (ppm), ammonia (ppm), alkalinity (ppm), and hardness (ppm). Visual estimates on turbidity, depth, flow, connectivity, as well as evidence of dredging, surface films, algal mats, and permanence of water body were noted as well. The habitat was additionally characterized noting the percentages of substrate and floating, submerged, and emergent vegetation cover. Absence or presence of woody debris and trees was noted, as well as fish, amphibian, or reptile species observed. Any anthropogenic disturbance of the surrounding habitat was also listed as well as visual detection of *P. gorzugi*.

Quantitative water quality data was analyzed to determine whether there were significant differences in the mean, maximum, and minimum values of air temperature, water temperature,

pH, dissolved oxygen, conductivity, oxidation reduction potential, nitrate, nitrite, ammonia, alkalinity, and hardness between sites where *P. gorzugi* was detected via any survey methodology, and sites where it was never detected. Additionally, qualitative data from the categorical analyses (Appendix A) was analyzed. The average maximum amount of *P. gorzugi* detections per site was determined by averaging the highest number of detections per site visit across drone, visual, and trapping methodologies. This was to avoid pseudoreplication in our dataset as different methods likely detected some of the same individuals. The average maximum of *P. gorzugi* detections was analyzed to determine whether *P. gorzugi* exhibited a preference for the following categories: spring-fed, turbidity, flow, connectivity, algal mat presence, woody debris presence, and tree presence. Additionally, the average maximum amount of *P. gorzugi* detections was used to see if *P. gorzugi* exhibited a preference for mainstem, tributary, or reservoir water systems.

All water quality data was non-normally distributed and had unequal variances as determined by Shapiro-Wilk and Welch's t-tests, so Mann Whitney and Wilcoxon multiple comparison tests were used for analyses. Means for all analyses are reported as mean (± 1 SD). All statistical analyses were conducted in JMP v14 statistical software (SAS Institute, Cary, NC, USA).

Results

Multispectral Imaging

The unsupervised classification resulted in similar classes at each study site (Figure 28). All but two of the spectral signature graphs appeared to have matches in other study sites. Four distinct differences appeared between open water, grass, mixed vegetation, and rock/soil. Class 1

was composed of open water; Class 2 was also composed of open water, but in this class dark green aquatic vegetation appeared on the substrate of the water body (Figure 29). Two classes from the classified TNC Independence Creek Preserve, Lower Lake (Site 5; Figure 3; Table 1) image also comprised open water, but did not appear to have a corresponding class at the other sites (Figure 29). Class 3 was composed of various types of vegetation, including algae, shoreline herbaceous vegetation, and trees. This class was not found at TNC Independence Creek Preserve, Lower Lake (Site 5; Figure 3; Table 1), but was present at the other two sites. Class 4 was similar to Class 3, composed of algae, vegetation, and trees, but was lusher than Class 3, which contained some brown, potentially dead vegetation (Figure 30). Class 4 was also present at all three sites. Class 5 and Class 6 both contained grass, but Class 6 composed paler, potentially dead grass, whereas Class 5 was lusher (Figure 31). Class 7 was composed of rock, soil, and cement, but also contained some algae (Figure 32). This class was also not present at TNC Independence Creek Preserve, Lower Lake.

Algal mats were found to contain several different spectral classes at each site. The algal mat at TNC Independence Creek Preserve, Lower Lake had the fewest classes and contained primarily Class 4 as well as some of Class 5 (Figure 33). Algal mats at Del Rio, San Felipe Springs Golf Course, San Felipe Creek (Site 27; Figure 3; Table 1) were the most diverse and contained primarily Class 3 and Class 4, but also had some of Class 5, Class 7, and Class 6, in order of abundance (Figure 33). The algal mats at Fort Clark Springs, Headwater Pond (Site 29; Figure 3; Table 1) contained primarily Class 7 and Class 3, but also contained Class 6 (Figure 33). Spectral values were similar between sites for each band within these classes, however there was some greater variance in spectral values in Class 5 and Class 7, with TNC Independence

Creek Preserve, Lower Lake have higher values for Class 5 and Del Rio, San Felipe Springs Golf Course having higher values for Class 7.

The supervised classifications were difficult to compare due to variances in spectral values between the sites. The classification returned a similar visual output for TNC Independence Creek Preserve, Lower Lake and Del Rio, San Felipe Springs Golf Course, San Felipe Creek, but the spectral values differed tremendously. Fort Clark Springs, Headwater Pond had similar spectral values for each class as Del Rio, San Felipe Springs Golf Course, San Felipe Creek, but a much different make up of these classes. Due to these differences, comparisons were not made with supervised classifications, and the unsupervised classifications were used instead.

Land Class Characterization

The number of sites with each land class broken into 20% intervals is presented in Table 9–16. This data is also broken down by sites with and without *P. gorzugi* detection at each buffer size (Table 9–16). In the land cover class comparisons between sites with *P. gorzugi* detections and sites where they were never detected, two significant differences resulted in the mean percent area. At the 250 m buffer a significant difference resulted in the cultivated crop class ($H = 4.33$, $df = 1$, $p = 0.04$) with sites that had *P. gorzugi* detections having lower percent of cultivated crop cover (mean = 2%) than sites where *P. gorzugi* were not detected (mean = 10%; Figure 34). Additionally, a significant difference in the cultivated crop class resulted in the 500 m buffer ($H= 6.39$, $df = 1$, $p = 0.01$) with sites with *P. gorzugi* detections having a lower percent of cultivated crop cover (mean = 2%) than sites where *P. gorzugi* were never detected (mean = 10%). Sites with high percentages of cultivated crop cover include the Rio Grande, downstream of TNC Southmost Preserve (Site 60; Figure 3; Table 1), the Rio Grande, downstream of TNC

Southmost Preserve (Site 61; Figure 3; Table 1), and the Rio Grande, near National Butterfly Center (Site 59; Figure 3; Table 1). At the 100 m buffer the percent cultivated crop cover for these sites is 77%, 67%, and 46%, respectively. No significant differences resulted in any other analyses.

In the categorical analyses a few significant differences resulted at different buffer sizes. In the 100 m buffer a significant difference was observed in the grassland/herbaceous class for all sites that were categorized as mainstem ($H = 3.89$, $df = 1$, $p < 0.05$) with sites where *P. gorzugi* was detected having a higher percentage of this land class (mean = 10%) than sites where *P. gorzugi* was never detected (mean = 1%; Figure 35). A significant difference resulted in the cultivated crop class for both tributaries ($H = 4.58$, $df = 1$, $p = 0.03$) and spring-fed systems ($H = 6.00$, $df = 1$, $p = 0.01$) at the 500 m buffer level. For tributaries, a lower percent cover of cultivated crops was found at sites where *P. gorzugi* was detected (mean = 3%) than sites where it was not detected (mean = 5%). For spring-fed systems, a lower percent cover of cultivated crops was also found at sites where *P. gorzugi* was detected (mean = 0%) than sites where it was never detected (mean = 1%). At the 1000 m buffer scale for mainstem study sites, two significant differences resulted. A significant difference was found between percent land cover of shrub/scrub ($H = 4.69$, $df = 1$, $p = 0.03$) with sites where *P. gorzugi* was detected having a greater percent of this land cover (mean = 56%) than sites where *P. gorzugi* was never detected (mean = 21%; Figure 36). A significant difference was also found in the open water class ($H = 4.69$, $df = 1$, $p = 0.03$) with sites where *P. gorzugi* was detected having a lower percent of this land cover class (mean = 8%) than sites where it was never detected (mean = 9%; Figure 37). All other analyses failed to result in any significant differences.

Water Quality

Water quality parameters were measured at 52 unique localities throughout the sampling period of November 2018–October 2019 (Table 17 and 18). There were instances when some parameters were unable to be obtained due to issues with the sampling equipment, and at one site, Pump Canyon, Langtry (Site 19; Figure 3; Table 1) steep canyon walls prevented water access and data collection. The majority of unique localities were sampled, however, and most sampling events produced a complete dataset. Additionally, upon review of the water quality data some points (n = 4) with unreasonable values were discarded to ensure data integrity. Our finalized dataset covered a wide range of values with differences noted between and amongst sites. Sites that resulted in *P. gorzugi* detections encompassed most of this range. *Pseudemys gorzugi* was found in water bodies with average water temperatures ranging from 17.5–33.6°C, average pH from 7.75–9.76, average dissolved oxygen from 2.49–9.64 mg/L, average conductivity from 433–23,197 µS/cm, and an average oxidation-reduction potential of 12.2–205.2 mV (Table 17). Additionally, we detected *P. gorzugi* in water bodies with an average nitrate of 0–1 ppm, average nitrite of 0–0.15 ppm, average ammonia of 0–0.25 ppm, average alkalinity of 63–240 ppm, and an average hardness of 250–425 ppm (Table 18). Maximum, minimum, and average values for each water quality parameter can be found in Table 17 and 18.

Some of our quantitative water quality parameters were found to be significantly different between sites where *P. gorzugi* was detected and sites where it was never detected. A significant difference was observed in average conductivity ($H = 4.85$, $df = 1$, $p = 0.03$) with sites where *P. gorzugi* was detected reporting a significantly lower average conductivity (mean = 2103.4 µS/cm) than sites where *P. gorzugi* was not detected (mean = 3940.2 µS/cm; Figure 38). Minimum conductivity was found to be significantly lower ($H = 5.84$, $df = 1$, $p = 0.02$) at sites

where *P. gorzugi* was detected (mean = 1961.9 µS/cm) than sites where it was not detected (mean = 3906.8 µS/cm; Figure 38). A significant difference was observed in minimum pH (H = 5.43, df = 1, p = 0.02) with minimum pH significantly lower at sites with *P. gorzugi* detections (mean = 8.07), than sites where it was never detected (mean = 8.22; Figure 39). All other analyses failed to result in a significant difference.

Categorical water quality analysis resulted in several statistical differences. A statistical difference was observed in the spring-fed analysis (H = 8.57, df = 1, p = 0.003) with higher average maximum *P. gorzugi* detections resulting in spring-fed environments (mean = 7.01) than sites that were not spring-fed (mean = 2.39; Figure 40). Additionally, a statistical difference was noted in the presence of algal mats (H = 4.01, df = 1, p = 0.045) with sites with algal mats having higher average maximum *P. gorzugi* detections (mean = 6.61) than sites without algal mats (mean = 2.09; Figure 41). The presence of woody debris resulted in a statistical difference in average maximum *P. gorzugi* detections (H = 5.86, df = 1, p = 0.016), with sites with woody debris resulting in more average maximum *P. gorzugi* detections (mean = 6.18) than sites without woody debris (mean = 2.01; Figure 42).

Lastly, a significant difference was noted between the highest average maximum *P. gorzugi* detections between the type of water body, with pairwise comparisons resulting in higher average maximum *P. gorzugi* detections in tributaries (mean = 7.02) than mainstem systems (mean = 2.79, p = 0.009) as well as reservoirs (mean = 0.27, p = 0.015; Figure 43). No significant differences were observed in the turbidity, flow, connectivity, or tree presence categories.

Discussion

Multispectral Imaging

From the unsupervised classification we were able to return four groups of classes with spectral signatures similar to what was expected for the object type. Open water had low reflectance across all bands, vegetation and grass classes had high reflectance of the green and NIR bands, and the rock/soil category was similar to the vegetation class, but had higher reflectance in the red band. Additionally, when looking at the classes within the groups, Class 4 had higher reflectance of NIR than Class 3, which is characteristic of the lusher vegetation found within this class. In a similar manner, Class 6 reflected more of the red band than Class 5, which explains why this vegetation appears browner. While each of these spectral signatures was characteristic of the object types they were identified as, there were some variances between sites which could be the result of actual spectral differences, or differences in the classifications between sites. While classes were confirmed by visually analyzing the original photomosaic, ground-truthing would further enhance this process and could explain some of these deviations, as noted in Govender et al. (2009) when ground-truthing was used to complement multispectral imagery to analyze plant water stress.

Furthermore, some spectral differences are likely explained by the diversity between sites. TNC Independence Creek Preserve, Lower Lake (Site 5; Figure 3; Table 1) did not have trees or manmade structures in the photomosaic, which resulted in the remaining areas having a more detailed classification. This can be seen with the four open water classes that were formed at this site, in comparison to the two open water classes found at the other two sites. To minimize the effects of diversity in object type on the classifications, photomosaics could be trimmed, as demonstrated in Rossiter and Hengl (2001), to include only the water body before a classification

is run. This may help to result in classes that have more similar spectral values between locations.

One of the disadvantages to unsupervised classifications is that each image is classified separately so while classes will be similar if reflectance values are similar between locations, the classes will not be identical. While similar spectral values can be matched, without dictating the parameters of each class there will likely be some variation and overlap in class values between sites. Additionally, depending on the range and distribution of spectral values in the original photomosaic, some bands appear to have less variance in values than others. Histograms depicting the spectral values for each pixel in Class 3 at Del Rio, San Felipe Springs Golf Course, San Felipe Creek (Site 27; Figure 3; Table 1) show the variance of pixel values in the bands composing this class. Ideally, a sharp peak is desired, relaying that the pixel values are closely related. Upon examination of the histograms from these classifications, however, this was often not the case. Figure 44 is characteristic of the classes found throughout each study site. One solution to this is to perform supervised classifications, however, the supervised classification ran on these study sites failed to adequately classify the sites due to the variation in spectral values between sites. Additionally, thresholding algorithms have been developed to minimize intra-class variance (Arifin and Asano, 2006) which may be beneficial to further explore.

Between the study sites, five different classes were found in algal mats, but none of these classes were present across all three sites illustrating the diversity of algal populations as previously noted in Steven et al. (2012). All the classes from TNC Independence Creek Preserve, Lower Lake were found at Del Rio, San Felipe Springs Golf Course, San Felipe Creek, but none of these classes were present at Fort Clark Springs, Headwater Pond (Site 29; Figure 3; Table 1).

Additionally, all classes from Fort Clark Springs, Headwater Pond were found at Del Rio, San Felipe Springs Golf Course, San Felipe Creek, but none were present at TNC Independence Creek Preserve, Lower Lake. No classes were unique to Del Rio, San Felipe Springs Golf Course, San Felipe Creek, but instead this site contained all the classes present at the other two sites. This suggests that there is no specific multispectral class that is required for *P. gorzugi* inhabitance; however, the overlap in classes between sites suggest that certain classes may be beneficial for *P. gorzugi*. Additionally, the relative composition of the classes of the algal mats differed greatly between sites, which means that the classes analyzed may not be an important factor for *P. gorzugi* occurrence. Similarly, Walton (2006) failed to identify a unique spectral signature for wetlands in Bog Turtle (*Glyptemys muhlenbergii*) habitats, but added that seasonality and image quality may have prevented this identification. It is important to note that reflectance values of vegetation can change seasonally (Brown et al., 1999) and our imagery was taken over a month and a half time span. Algal mats could have changed their spectral values within this time span, for example if hot temperatures led to algal death, or an increase in nutrients led to additional growth.

Several recommendations can be made going forward. We suggest that a more robust analysis including both sites with and without *P. gorzugi* detections be conducted. By examining algal mats in water systems where *P. gorzugi* were not found, similarities or differences that are not apparent now may become noticeable. A more temporal analysis is additionally recommended to discern whether algal mats have similarities that were not apparent in this analysis. Ground truthing would be advantageous to see if any visual differences or similarities from the ground correspond to similarities and differences in these classes, and whether classes compose different species or just different stages of algae. A different outcome may arise from

different classifications as well, and the number of classes could be adjusted. Lastly, if a supervised classification is fine-tuned, the added user input may produce a more optimal classification. While this study provided the foundation for multispectral analysis of *P. gorzugi* habitats, additional analyses will need to be conducted before class associations can be determined.

Land Class Characterization

In total, nine land cover classes were detected around study sites encompassing a great diversity which matched our field observations of habitat diversity throughout this study (Figure 4). We did notice that the shrub/scrub category had more occurrences as the higher percentage intervals than other land class categories, particularly at the larger buffer sizes, which is understandable given that this is the dominant land class type throughout most of our survey areas. Additionally, developed areas presented some high percentage of land class cover which is also expected, given that many sampling areas were located near development as this is where river access occurred through the use of boat launches and public access points. These trends were seen in both sites with and without *P. gorzugi* detections, suggesting that these trends are applicable to all our study sites.

Significant differences appeared in several analyses of different buffer sizes and categories for the cultivated crops land cover class. Sites with high percentages of the cultivated crop class were typically located in south Texas, where cotton, sorghum, corn, and sugarcane are commonly grown (United States Department of Agriculture, 2019). In each significant difference that resulted for the cultivated crops class, lower percentages were seen in sites where *P. gorzugi* was detected. This suggests that *P. gorzugi* is unlikely to select habitats in agricultural areas, a trend that has been seen in other turtle species (Bodie and Semlitsch, 2000). Agriculture can

reduce growth and recruitment (Saumure and Bider, 1998), decrease hatchling success (Thompson et al., 2018), and degrade water quality (Ribaudo et al., 2006), which could explain the avoidance of areas with cultivated crops. Both sites with and without *P. gorzugi* detections had relatively low percentages of cultivated crops, however, and thus further analysis should be conducted on a larger scale to explore whether a relationship between *P. gorzugi* habitat selection and agriculture truly exists.

In the mainstem category, three significant differences resulted at different buffer sizes. Mainstem sites included those along the Rio Grande and Pecos River. As these habitats are similar, this eliminates some of the variability that occurs amongst our entire dataset of study sites. At the 100 m buffer a significant difference resulted in the grassland/herbaceous class, with sites where *P. gorzugi* was detected, having a higher percentage of this land cover type than sites that did not have *P. gorzugi* detections. The grassland/herbaceous category is broad, encompassing multiple habitat types, and the term grassland is ambiguous in itself, with several terms used interchangeably including desert, desertification, and rangeland (Weddell, 1996). In one study with finer resolution data, aquatic turtles were found to have lower occupancy rates in grassland, but higher occupancy in areas with herbaceous vegetation (Rizkalla and Swihart, 2005), suggesting that it would be beneficial to increase the resolution of the class types for this analysis to better understand our contradictory results.

A significant difference also resulted in the mainstem category at the 1000 m buffer size for the open water class with sites where *P. gorzugi* was detected having a lower amount of open water than sites where they were not detected. While this may seem counterintuitive for an aquatic turtle species, it could show a preference for smaller water bodies, which would support our findings that *P. gorzugi* demonstrated a preference for tributaries over mainstem and

reservoir sites, as tributaries are typically smaller than these latter two systems (see Water Quality section). Several aquatic turtle species have demonstrated different preferences for water body size and depth (Rizkalla and Swihart, 2006) and while *P. gorzugi* have been found in a diversity of water bodies, an apparent preference for smaller tributaries has been noted in previous observations (Pierce et al., 2016). Additionally, it is important to note that the difference between these means was only 1% and thus additional analysis is highly recommended before this relationship is definitively stated.

At the 1000 m buffer size for mainstem sites, a significant difference emerged in the shrub/scrub class with a higher percent cover of this land class in sites where *P. gorzugi* was detected. Literature on aquatic turtle use of this habitat class is sparse, and one study found that painted turtles experienced higher abundances in habitats with less shrub-scrub vegetation (Winchell and Gibbs, 2016). As this significant difference only resulted in one category at the largest buffer size, there is a chance that the shrub/scrub class may be overrepresented in this instance, which led to this result.

Several potential trends emerged as a result of these analyses, however, further analysis of these habitat classes would be beneficial to determine whether our findings were biologically meaningful. Additionally, increasing the resolution of the analysis by examining factors that differ between the habitat classes (e.g. soil type, vegetation type, vegetation structure) would be advantageous to determine what attributes of each land class may be responsible for the increased prevalence of *P. gorzugi* at sites with certain land cover classes. With little literature on *P. gorzugi* habitat selection, the knowledge base to which we can compare our results to is meager, but these analyses may provide a foundation for further exploration into *P. gorzugi* habitat selection, which is essential for the conservation of this species.

Water Quality

From the quantitative data we collected, it appears that *P. gorzugi* can encompass a range of water quality values, suggesting that they may trend towards a habitat generalist. When values were sorted from largest to smallest, sites with *P. gorzugi* detections were generally distributed evenly throughout the sorted list and often encompassed both the highest and lowest values. While we were unable to establish thresholds in any of the water quality parameters in this study, noting the ranges of values where *P. gorzugi* was detected is a start to this process. While a previous study suggested the conductivity threshold was above 2264–2593 µS/cm (Bonner and Littrell, 2016) we detected *P. gorzugi* through eDNA analysis at Pecos River, at US Hwy 190 crossing (Site 1; Figure 3; Table 1) which had an average conductivity of 23,197 µS/cm and trapped individuals at Pecos River, 0.3 river km upstream of confluence with Independence Creek (Site 11; Figure 3; Table 1) which had an average conductivity of 11,440 µS/cm, with both conductivity values much higher than the previous findings. These detections suggest that the conductivity threshold is even beyond these new, highly elevated values. While *P. gorzugi* may be able to tolerate a wide range of water quality parameters, it is still likely that they have certain preferences.

Two significant findings in conductivity suggests that there may be a relationship between conductivity and *P. gorzugi* habitat selection. With an average conductivity significantly lower at sites where *P. gorzugi* was detected, and a significantly lower minimum conductivity at sites where we were able to detect this species, it seems that *P. gorzugi* may prefer sites with lower conductivity. Freshwater turtle species generally struggle to osmoregulate in hyperosmotic environmental conditions (Bower et al., 2016) and salinity is a known limiting factor to freshwater turtle distribution (Dunson and Mazzotti, 1989). While some freshwater

turtle species have been shown to behaviorally osmoregulate (Dunson, 1981; Dunson, 1986) inhabiting higher saline locations for limited time periods, tolerance of these environments is short-term, and a lower saline environment is needed for extended survival (Agha et al., 2018). Detections at the higher end of the range of our conductivity values shows that *P. gorzugi* is able to tolerate water levels with high conductivity, at least temporarily. The specific conductivity threshold for this species is still unknown, and it is possible that they may tolerate even higher values. Often tradeoffs factor into habitat selection (Futuyma and Moreno, 1988) and it is possible that *P. gorzugi* will inhabit sites with higher conductivity values if other benefits may result, for example a robust food source.

Literature on the effects of environmental pH on turtles is sparse, but pH is a known indicator of water quality (Higgins, 2003) and certain thresholds have been established for aquatic organisms (Deming et al., 1992; Lacoul et al., 2011). In this study, a significantly lower minimum pH was observed at sites with *P. gorzugi* detections. However, the difference between the two means is small, 8.07 and 8.22, and it is unknown whether this difference has any implications for habitat selection. Furthermore, the average and maximum pH analyses failed to result in significant differences, suggesting that the lower minimum pH observed at sites with *P. gorzugi* detections could be a coincidental occurrence.

We were surprised to see no significant difference result in any of our water temperature analyses. *Pseudemys gorzugi* appear to exhibit a preference toward spring-fed environments, which often experience cool water temperatures with little temperature fluctuation. Aquatic ectotherms experience greater rates of heat transfer in water than on land and studies have shown that water temperatures influence habitat selection in other aquatic turtle species (Nebeker and Bury, 2000; Fitzgerald and Nelson, 2011). The aquatic nature of *P. gorzugi* suggests that water

temperature would be a likely factor for habitat selection for this species. As water temperature varies seasonally, however, true differences in temperature between sites were difficult to determine as our results were heavily influenced by which season(s) sampling occurred at each site, and therefore, prevented an equal comparison. Future studies should attempt to analyze the relationship between water temperature and *P. gorzugi* inhabitance in a more concurrent manner in order to minimize the effects of seasonal trends.

Nitrogenous compounds, including nitrate, nitrite, and ammonia can be toxic to aquatic turtles (Ip et al., 2008) and inhibit reproduction (de Solla and Martin, 2009) in high concentrations. Water bodies with high levels of nitrogenous compounds often have ample algal growth, which depletes dissolved oxygen, but algae largely constitutes *P. gorzugi* diet (Letter et al., 2019). With mostly negative, but some potentially advantageous effects, we suspected that nitrogenous compounds may play a role in *P. gorzugi* habitat selection; however, no statistical difference resulted from our data.

Categorical water quality analyses resulted in several statistically significant differences in average maximum *P. gorzugi* detections, suggesting that *P. gorzugi* prefers certain habitat features. Pierce et al. (2016) noted that few *P. gorzugi* records were from the mainstem of the Rio Grande and that most were from tributaries instead, which our analysis supports. It remains unknown as to what element(s) of spring-fed environments they are selecting for, but our data suggests that spring-fed sites within *P. gorzugi* range are likely viable habitat for this species and have a high likelihood of resulting in *P. gorzugi* detection. Only one spring-fed site, TNC Dolan Falls Preserve, Dolan Creek, near confluence with Devils River failed to result in any *P. gorzugi* detections in this study (Site 15; Figure 3; Table 1). Detections were noted, however, in TNC Dolan Falls Preserve, Devils River, upstream of confluence with Dolan Creek (Site 14; Figure 3;

Table 1) and TNC Dolan Falls Preserve, Devils River, Dolan Falls (Site 16; Figure 3; Table 1), which are 0.92 and 0.28 river km, respectively, from this sampling site. This suggests that they may be present, but undetected in this system, or are at least utilizing nearby habitat.

Pseudemys gorzugi seem to exhibit a preference towards environments with algal mats, with higher average maximum *P. gorzugi* detections noted at these sites. Detections may be more likely in these environments as *P. gorzugi* subaerially basking in algal mats may be easier to detect than those swimming, but the large difference observed between these means suggests that there is a greater explanation for this difference. Basking is an essential behavior of turtle species required for thermoregulation, and subaerial basking has been observed as the preferred basking behavior for turtles in warm habitats (Bury et al., 2012). Algal mats provide an upper thermal layer where turtles can reach suitable body temperatures in areas where aerial basking may be too hot, while additionally providing cover (Bury et al., 2012). The majority of *P. gorzugi* detected throughout this site were subaerially basking, with only a few seen aerially basking, primarily in the cooler spring and fall months. It is likely that algal mats offer a substantial advantage for individuals for thermoregulation and reduction in predation resulting in *P. gorzugi* selecting for these environments. Additionally, algae is a known food source of *P. gorzugi*, with adult female fecal composition 66.6% filamentous algae (Letter et al., 2019). The presence of this food source offers an additional and likely explanation for their preference of habitats with algal mats.

The presence of woody debris at a study site appeared to have a favorable impact on *P. gorzugi* habitat selection, with higher average maximum *P. gorzugi* detections occurring at sites with woody debris. While subaerially basking was the predominant form of basking in this study, *P. gorzugi* were still observed aerially basking in cooler times of the day and year. Woody debris

functions as basking substrate (Chaney and Smith, 1950), provides cover (Riedle et al., 2006), regulates the thermal profile of aquatic habitats (Welty et al., 2002), and provides foraging sites (Moll, 1976), and has been known to determine the distribution of emydid turtles (Lovich, 1988; Lindeman, 1999). It is likely a combination of these factors that has led *P. gorzugi* to select for sites with woody debris.

Turbidity, flow, connectivity, and tree presence seemed to have no influence *on P. gorzugi* habitat selection. However, it is important to note that few study sites were not flowing (n = 6), had no connectivity (n = 1), and lacked trees (n = 6), and as a result these analyses are not very robust. It is suggested that further studies be conducted at sites with these characteristics to determine if there truly is no relationship between these habitat categories and *P. gorzugi* presence. Our turbidity analysis, however, was more robust yet failed to result in a significant difference. This leads us to conclude that turbidity is not an important factor in *P. gorzugi* habitat selection.

Pseudemys gorzugi have been documented in diverse water bodies, from lentic and lotic systems, to large reservoirs and rivers, and smaller tributaries (Degenhardt et al., 1996; Pierce et al., 2016). While they seem capable of inhabiting a wide range of habitats, a preference did arise for tributaries over both mainstem rivers and reservoir systems. These habitats have several factors that differ from one another and it is unknown which of these factors causes this trend. All but two of our tributary sites were spring-fed, but all of our reservoir or mainstem sites were not, so this association could be related to that. Additionally, tributaries are generally smaller bodies of water than the mainstem rivers they feed, and it is possible that *P. gorzugi* prefer sites with less open water. Several aquatic turtle species have been found to exhibit unique preferences for different types of water bodies (Cagle, 1942), and past literature has suggested

that *P. gorzugi* are more abundant in tributaries (Pierce et al., 2016), which our findings corroborate.

We highly recommend that future studies continue to explore the relationships identified in these analyses. In particular, further examination of conductivity could seek to establish a conductivity threshold for this species. Additionally, as the water quality test strips were not very precise and required a subjective interpretation of the displayed result to determine the value, we encourage future studies to evaluate these parameters with greater precision to assess whether a relationship exists. Additional studies on water quality may further assist in characterizing *P. gorzugi* habitat which can identify areas of suitable habitat for this species.

REFERENCES

Aberle, N., Beutler, M., Moldaenke, C., & Wiltshire, K. H. (2006). 'Spectral fingerprinting' for specific algal groups on sediments in situ: a new sensor. *Archiv für Hydrobiologie*, *167*(1-2), 575–592.

Agha, M., Ennen, J. R., Bower, D. S., Nowakowski, A. J., Sweat, S. C., & Todd, B. D. (2018). Salinity tolerances and use of saline environments by freshwater turtles: implications of sea level rise. *Biological Reviews*, *93*(3), 1634–1648.

Al-AbdulKader, K. A., Farrand, W. H., & Blundell, J. S. (2002). Marine Habitat Mapping Using High Spatial Resolution Multispectral Satellite Data. In: *SPE International Conference on Health, Safety and Environment in Oil and Gas Exploration and Production*. Society of Petroleum Engineers.

American Rivers. (2003). *America's most endangered rivers of 2003*. Retrieved March 16, 2020, from https://s3.amazonaws.com/american-rivers-website/wp-content/uploads/2016/02/24220916/2003-mer-report.pdf.

Arifin, A. Z., & Asano, A. (2006). Image segmentation by histogram thresholding using hierarchical cluster analysis. *Pattern Recognition Letters*, *27*(13), 1515-1521.

Bailey, L. A., Forstner, M. R., Dixon, J. R., & Hudson, R. (2014). Contemporary status of the Rio Grande cooter (Testudines: Emydidae: *Pseudemys gorzugi*) in Texas: phylogenetic, ecological and conservation consideration. In: *Proceedings of the Sixth Symposium on the Natural Resources of the Chihuahuan Desert Region. Texas: Chihuahuan Desert Research Institute* (pp. 320–334).

Bendig, J., Bolten, A., & Bareth, G. (2012). Introducing a low-cost mini-UAV for thermal-and multispectral-imaging. *Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci*, *39*, 345–349.

Berni, J., Zarco-Tejada, P., Suárez, L., González-Dugo, V., & Fereres, E. (2009). Remote sensing of vegetation from UAV platforms using lightweight multispectral and thermal imaging sensors. *The International Archives of the Photogrammetry Remote Sensing and Spatial Information Sciences*, *38*(6), 6.

Bodie, J. R., & Semlitsch, R. D. (2000). Spatial and temporal use of floodplain habitats by lentic and lotic species of aquatic turtles. *Oecologia*, *122*(1), 138–146.

Bonin, J., DesGranges, J. L., Bishop, C. A., Rodrigue, J., Gendron, A., & Elliott, J. E. (1995). Comparative study of contaminants in the mudpuppy (*Amphibia*) and the common snapping turtle (*Reptilia*), St. Lawrence River, Canada. *Archives of Environmental Contamination and Toxicology*, *28*(2), 184–194.

Bonner, T. H. & Littrell, B. M. (2016). Aquatic surveys of Delaware River of Texas. Final report to Texas Comptroller of Public Accounts.

Bower, D. S., Scheltinga, D. M., Clulow, S., Clulow, J., Franklin, C. E., & Georges, A. (2016). Salinity tolerances of two Australian freshwater turtles, *Chelodina expansa* and *Emydura macquarii* (Testudinata: Chelidae). *Conservation Physiology*, *4*(1), cow042.

Brown, B. E., Dunne, R. P., Ambarsari, I., Le Tissier, M. D. A., & Satapoomin, U. (1999). Seasonal fluctuations in environmental factors and variations in symbiotic algae and chlorophyll pigments in four Indo-Pacific coral species. *Marine Ecology Progress Series*, *191*, 53–69.

Brown, D. J., Schultz, A. D., Dixon, J. R., Dickerson, B. E., & Forstner, M. R. (2012). Decline of red-eared sliders (*Trachemys scripta elegans*) and Texas spiny softshells (*Apalone spinifera emoryi*) in the Lower Rio Grande Valley of Texas. *Chelonian Conservation and Biology*, *11*(1), 138–143.

Brown, D. J., Nelson, M. D., Rugg, D. J., Buech, R. R., & Donner, D. M. (2016). Spatial and temporal habitat-use patterns of Wood Turtles at the western edge of their distribution. *Journal of Herpetology*, *50*(3), 347–356.

Buhlmann, K. A., & Vaughan, M. R. (1991). Ecology of the turtle *Pseudemys concinna* in the New River, West Virginia. *Journal of Herpetology*, *25*(1), 72–78.

Bury, R. B., Ashton, D. T., Welsh Jr, H. H., Reese, D. A., & Germano, D. J. (2012). Synopsis of biology. *Western pond turtle− biology, sampling techniques, inventory and monitoring, conservation and management: northwest fauna. Olympia: The Society for Northwestern Vertebrate Biology*, 9–19.

Cagle, F. R. (1942). Turtle populations in southern Illinois. *Copeia*, *1942*(3), 155-162.

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*(4), 4026–4047.

Chaney, A., & Smith, C. L. (1950). Methods for collecting map turtles. *Copeia*, 1950(4), 323–324.

Collins, M. L., Small, M. F., Veech, J. A., Baccus, J. T., & Benn, S. J. (2010). Dove habitat association based on remotely sensed land cover types in South Texas. *The Journal of Wildlife Management*, *74*(7), 1568–1574.

Dailey, C. (2017). Revising a Habitat Suitability Model for Spotted Turtles (*Clemmys guttata*) in Upstate New York. Masters Theses.

de Solla, S. R., & Martin, P. A. (2007). Toxicity of nitrogenous fertilizers to eggs of snapping turtles (*Chelydra serpentina*) in field and laboratory exposures. *Environmental Toxicology and Chemistry: An International Journal*, *26*(9), 1890–1895.

Degenhardt, W. G., & Christiansen, J. L. (1974). Distribution and habitats of turtles in New Mexico. *The Southwestern Naturalist*, *19*(1), 21–46.

Degenhardt, W. G., Painter, C. W., & Price, A. H. (1996). *Amphibians and Reptiles of New Mexico*. University of New Mexico Press, Albuquerque, New Mexico. 431 pp., 123 plates.

Deming, W., Yicheng, X., Dehui, Z., Baoyuan, L., Xinfu, L., Qijun, K., & Shida, W. (1992). A study of effects of water acidification on aquatic organisms of different trophic levels [J]. *Acta Scientiae Circumstantiae*, *1*.

Dickson, M. A., Hendrickson, L. L., & Reid, J. F. (2001). U.S. Patent Application No. 09/411,414.

Dixon, J. R. (2013). *Amphibians and Reptiles of Texas: With Keys, Taxonomic Synopses, Bibliography, and Distribution Maps.* Third Edition. College Station, Texas: Texas A&M University Press, 447 pp.

Doupe, R. G., Schaffer, J., Knott, M. J., & Dicky, P. W. (2009). A description of freshwater turtle habitat destruction by feral pigs in tropical north eastern Australia. *Herpetological Conservation and Biology*, *4*(3), 331–339.

Dreslik, M. J., Kuhns, A. R., Phillips, C. A., & Jellen, B. C. (2003). Summer movements and home range of the cooter turtle, *Pseudemys concinna*, in Illinois. *Chelonian Conservation and Biology*, *4*(3), 706–710.

Dunson, W. A. (1981). Behavioral osmoregulation in the key mud turtle, *Kinosternon b. baurii*. *Journal of Herpetology*, *15*(2), 163–173.

Dunson, W. A. (1986). Estuarine populations of the snapping turtle (*Chelydra*) as a model for the evolution of marine adaptations in reptiles. *Copeia*, *(3)*, 741–756.

Dunson, W. A., & Mazzotti, F. J. (1989). Salinity as a limiting factor in the distribution of reptiles in Florida Bay: a theory for the estuarine origin of marine snakes and turtles. *Bulletin of Marine Science*, *44*(1), 229–244.

Dunson, W. A., & Seidel, M. E. (1986). Salinity tolerance of estuarine and insular emydid turtles (*Pseudemys nelsoni* and *Trachemys decussata*). *Journal of Herpetology*, *93*(1), 237–245.

Eisenbeiß, W., Marotz, J., & Schrade, J. P. (1999). Reflection-optical multispectral imaging method for objective determination of burn depth. *Burns*, *25*(8), 697–704.

Fitzgerald, L. A., & Nelson, R. E. (2011). Thermal biology and temperature-based habitat selection in a large aquatic ectotherm, the alligator snapping turtle, *Macroclemys temminckii*. *Journal of Thermal Biology*, *36*(3), 160–166.

Futuyma, D. J., & Moreno, G. (1988). The evolution of ecological specialization. *Annual Review of Ecology and Systematics*, *19*(1), 207–233.

Gibbons, J. W. Lovich. JE (1990). Sexual dimorphism in turtles with emphasis on the slider turtle (*Trachemys scripta*). *Herpetological Monographs*, *4*, 1–29.

Global Scan Technologies LLC. (2019). *WorldView-2 Satellite Imagery and Sensor Specifications*. Retrieved May 15, 2019, from http://www.gstdubai.com/satelliteimagery/worldview-2.html.

Golet, W. J., & Haines, T. A. (2001). Snapping turtles (*Chelydra serpentina*) as monitors for mercury contamination of aquatic environments. *Environmental Monitoring and Assessment*, *71*(3), 211–220.

Gonçalves, J., Henriques, R., Alves, P., Sousa-Silva, R., Monteiro, A. T., Lomba, Â., ... & 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*(1), 132–146.

Govender, M., Govender, P. J., Weiersbye, I. M., Witkowski, E. T. F., & Ahmed, F. (2009). Review of commonly used remote sensing and ground-based technologies to measure plant water stress. *Water SA*, *35*(5), 741–752.

Grenzdörffer, G. J., 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*, *31*(B3), 1207–1214.

Griffith, G. E., Omernik, J. M., McGraw, M. M., Jacobi, G. Z., Canavan, C. M., Schrader, T. S., ... & Moran, B. C. (2006). Ecoregions of New Mexico (color poster with map, descriptive text, summary tables, and photographs): Reston, Virginia. *US Geological Survey (map scale 1: 1,400,000)*.

Hajjdiab, H., Farok, H., & Howari, F. M. (2017, February). The Application of Narrow-Band NDVI as a Tool for Mapping Mangrove Environment in Abu Dhabi. In: *Proceedings of the 9th International Conference on Machine Learning and Computing* (pp. 472–475).

Hebert, C. E., Glooschenko, V., Haffner, G. D., & Lazar, R. (1993). Organic contaminants in snapping turtle (*Chelydra serpentina*) populations from southern Ontario, Canada. *Archives of Environmental Contamination and Toxicology*, *24*(1), 35–43.

Higgins, B. M. (2003). Sea turtle husbandry. *The Biology of Sea Turtles, 2*, 411–440.

Hinton, T. G., & Scott, D. E. (1990). Radioecological techniques for herpetology, with an emphasis on freshwater turtles. In: J. Whitfield Gibbons (ed.), *.Life History and Ecology of the Slider Turtle.* Smithsonian Institution Press, Washington, DC, pp. 267–287.

Homer, C., Dewitz, J., Yang, L., Jin, S., Danielson, P., Xian, G., ... & Megown, K. (2015). Completion of the 2011 National Land Cover Database for the conterminous United States–representing a decade of land cover change information. *Photogrammetric Engineering & Remote Sensing*, *81*(5), 345–354.

Iverson, J. B. (1992). *A Revised Checklist with Distribution Maps of the Turtles of the World*. Privately printed, Richmond, Indiana. 363pp.

Ip, Y. K., Lee, S. M. L., Wong, W. P., & Chew, S. F. (2008). Mechanisms of and defense against acute ammonia toxicity in the aquatic Chinese soft-shelled turtle, *Pelodiscus sinensis*. *Aquatic Toxicology*, *86*(2), 185–196.

Kim, M. S., Lefcourt, A. M., Chao, K., Chen, Y. R., Kim, I., & Chan, D. E. (2002). Multispectral detection of fecal contamination on apples based on hyperspectral imagery: Part I Application of visible and near–infrared reflectance imaging. *Transactions of the ASAE*, *45*(6), 2027.

Lacoul, P., Freedman, B., & Clair, T. (2011). Effects of acidification on aquatic biota in Atlantic Canada. *Environmental Reviews*, *19*(NA), 429–460.

Letter, A. W., Waldon, K. J., Pollock, D. A., & 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*(3), 204–208.

Levings, G. W. (1998). *Water Quality in the Rio Grande Valley, Colorado, New Mexico, and Texas, 1992–95* (Vol. 1162). US Geological Survey, Water Resources Division.

Lindeman, P. V. (1999). Surveys of basking map turtles *Graptemys* spp. in three river drainages and the importance of deadwood abundance. *Biological Conservation*, *88*(1), 33–42.

Lovich, J. (1988). Aggressive basking behavior in eastern painted turtles (*Chrysemys picta picta*). *Herpetologica*, *44*(2), 197–202.

MacLaren, A. R., Foley, D., Sirsi, S., & Forstner, M. R. (2017). Updating methods of satellite transmitter attachment for long-term monitoring of the Rio Grande Cooter (*Pseudemys gorzugi*). *Herpetological Review*, *48*(1), 48–52.

Mexican Biodiversity. (2020). *Terrestrial ecoregions*. Retrieved on February 5, 2020, from https://www.biodiversidad.gob.mx/v_ingles/region/terrestrialEco.html.

Moll, D. (1976). Food and feeding strategies of the Ouachita map turtle (*Graptemys pseudogeographica ouachitensis*). *American Midland Naturalist, 96*(2), 478–482.

Multi-Resolution Land Characteristics Consortium. (2020). *Multi-Resolution Land Characteristics (MRLC) Consortium.* Retrieved on April 10, 2020, from https://www.mrlc.gov/.

Nebeker, A. V., & Bury, R. B. (2000). Temperature selection by hatchling and yearling Florida red-bellied turtles (*Pseudemys nelsoni*) in thermal gradients. *Journal of Herpetology*, *34*(3), 465–469.

New Mexico Department of Game and Fish [NMDGF]. 2006. The comprehensive wildlife conservation strategy for New Mexico. Santa Fe: New Mexico Department of Game and Fish.

Ouédraogo, M. M., 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.

Patrick, D. A., & Gibbs, J. P. (2010). Population structure and movements of freshwater turtles across a road-density gradient. *Landscape Ecology*, *25*(5), 791–801.

Pierce, L. J. S., Stuart, J. N., Ward, J. P., & Painter, C. W. (2016). *Pseudemys gorzugi* Ward 1984-Rio Grande Cooter, Western River Cooter, Tortuga de Oreja Amarilla, Jicotéa del Río Bravo. In: Rhodin, A. G. J., Iverson, J. B., van Dijk, P. P., Saumure, R. A., Buhlmann, K. A., Pritchard, P. C. H., Mittermeier R. A., 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: Chelonian Research Foundation,* 1–12.

Radeloff, V. C., Hammer, R. B., Stewart, S. I., Fried, J. S., Holcomb, S. S., & McKeefry, J. F. (2005). The wildland–urban interface in the United States. *Ecological Applications*, *15*(3), 799–805.

Rasmussen, M. L., & Litzgus, J. D. (2010). Habitat selection and movement patterns of spotted turtles (*Clemmys guttata*): effects of spatial and temporal scales of analyses. *Copeia*, *1*(1), 86–96.

Ribaudo, M., & Johansson, R. (2006). Water quality impacts of agriculture. In: Wiebe, K. & Gollehon, N. (eds.), *Agricultural Resources and Environmental Indicators*. Nova Science Publishers, Inc., New York City, New York, pp. 39–47.

Richardson, L. L. (1996). Remote sensing of algal bloom dynamics. *BioScience*, *46*(7), 492–501.

Riedle, J. D., Shipman, P. A., Fox, S. F., & Leslie Jr, D. M. (2006). Microhabitat use, home range, and movements of the alligator snapping turtle, *Macrochelys temminckii*, in Oklahoma. *The Southwestern Naturalist, 51*(1) 35–40.

Rizkalla, C. E., & Swihart, R. K. (2006). Community structure and differential responses of aquatic turtles to agriculturally induced habitat fragmentation. *Landscape Ecology*, *21*(8), 1361–1375.

Robertson, L. (1997). Water operations on the Pecos river, New Mexico and the Pecos Bluntnose Shiner, a federally-listed minnow. *Competing Interests in Water Resources-Searching for Consensus*, 407.

Rossiter, D. G., & Hengl, T. (2001). Creating geometrically-correct photo-interpretations, photomosaics, and base maps for a project GIS. *International Institute for Geoinformation Science and Earth Observation (ITC), C–24*(11), 1113–1119.

Saumure, R. A., & Bider, J. R. (1998). Impact of agricultural development on a population of wood turtles (*Clemmys insculpta*) in southern Quebec, Canada. *Chelonian Conservation and Biology*, *3*, 37–45.

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

Smith, M. O., Ustin, S. L., Adams, J. B., & Gillespie, A. R. (1990). Vegetation in deserts: I. A regional measure of abundance from multispectral images. *Remote Sensing of Environment*, *31*(1), 1–26.

Steven, B., McCann, S., & Ward, N. L. (2012). Pyrosequencing of plastid 23S rRNA genes reveals diverse and dynamic cyanobacterial and algal populations in two eutrophic lakes. *FEMS microbiology ecology*, *82*(3), 607–615.

Storey, E. M., Kayes, S. M., De Vries, I., & Franklin, C. E. (2008). Effect of water depth, velocity and temperature on the surfacing frequency of the bimodally respiring turtle *Elseya albagula*. *Functional Ecology*, *22*(5), 840–846.

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

Texas Parks and Wildlife Department. (2020). *Texas Ecoregions*. Retrieved February 5, 2020, from https://tpwd.texas.gov/education/hunter-education/online-course/wildlife-conservation/texas-ecoregions-ecoregions.

Thompson, M., Coe, B. H., Andrews, R. M., Cristol, D. A., Crossley, D. A., & Hopkins, W. A. (2018). Agricultural land use creates evolutionary traps for nesting turtles and is exacerbated by mercury pollution. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *329*(4–5), 230–243.

Thorhaug, A., Richardson, A. D., & Berlyn, G. P. (2007). Spectral reflectance of the seagrasses: *Thalassia testudinum, Halodule wrightii, Syringodium filiforme* and five marine algae. *International Journal of Remote Sensing*, *28*(7), 1487–1501.

Tison, D. L., Wilde, E. W., Pope, D. H., & Fliermans, C. B. (1981). Productivity and species composition of algal mat communities exposed to a fluctuating thermal regime. *Microbial Ecology*, *7*(2), 151–165.

United States Department of Agriculture. (2019). *USDA National Agricultural Statistics Service*. United States Department of Agriculture. Retrieved April 30, 2020, from https://www.nass.usda.gov/Statistics_by_State/Texas/Publications/County_Estimates/ce_pdf/index.php.

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.

USFWS [United States Fish and Wildlife Service]. (2015). Endangered and threatened wildlife and plants; 90-day findings on 31 species. *Federal Register, 80,* 37568–37579.

Viña, A., Bearer, S., Zhang, H., Ouyang, Z., & Liu, J. (2008). Evaluating MODIS data for mapping wildlife habitat distribution. *Remote Sensing of Environment*, *112*(5), 2160–2169.

Walton, E. M. (2006). Using remote sensing and geographical information science to predict and delineate critical habitat for the bog turtle, *Glyptemys muhlenbergii*. Masters Thesis.

Ward, J. P. (1984). Relationships of Chrysemyd Turtles of North America (Testudines: Emydidae). *Special Publications, the Museum Texas Technical University* 21:1–50.

Weddell, B. J. (1996). Geographic overview: Climate, phenology, and disturbance regimes in steppe and desert communities. *United States Department of Agriculture Forest Service General Technical Report RM*, 3–12.

Welty, J. J., Beechie, T., Sullivan, K., Hyink, D. M., Bilby, R. E., Andrus, C., & Pess, G. (2002). Riparian aquatic interaction simulator (RAIS): a model of riparian forest dynamics for the generation of large woody debris and shade. *Forest Ecology and Management*, *162*(2-3), 299–318.

Westoby, M. J., Brasington, J., Glasser, N. F., Hambrey, M. J., & Reynolds, J. M. (2012). 'Structure-from-Motion'photogrammetry: A low-cost, effective tool for geoscience applications. *Geomorphology*, *179*, 300–314.

Wharton Jr, R. A., Parker, B. C., & Simmons Jr, G. M. (1983). Distribution, species composition and morphology of algal mats in Antarctic dry valley lakes. *Phycologia*, *22*(4), 355–365.

Whited, D., Stanford, J. A., & Kimball, J. S. (2002). Application of airborne multispectral digital imagery to quantify riverine habitats at different base flows. *River Research and Applications*, *18*(6), 583–594.

Winchell, K. M., & Gibbs, J. P. (2016). Golf courses as habitat for aquatic turtles in urbanized landscapes. *Landscape and Urban Planning*, *147*, 595–70.

Wylie, B., Rigge, M., Brisco, B., Murnaghan, K., Rover, J., & Long, J. (2014). Effects of disturbance and climate change on ecosystem performance in the Yukon River Basin boreal forest. *Remote Sensing*, *6*(10), 9145–9169.

Zedler, J. B. (1982). Salt marsh algal mat composition: spatial and temporal comparisons. *Bulletin of the Southern California Academy of Sciences*, *81*(1), 41–50.

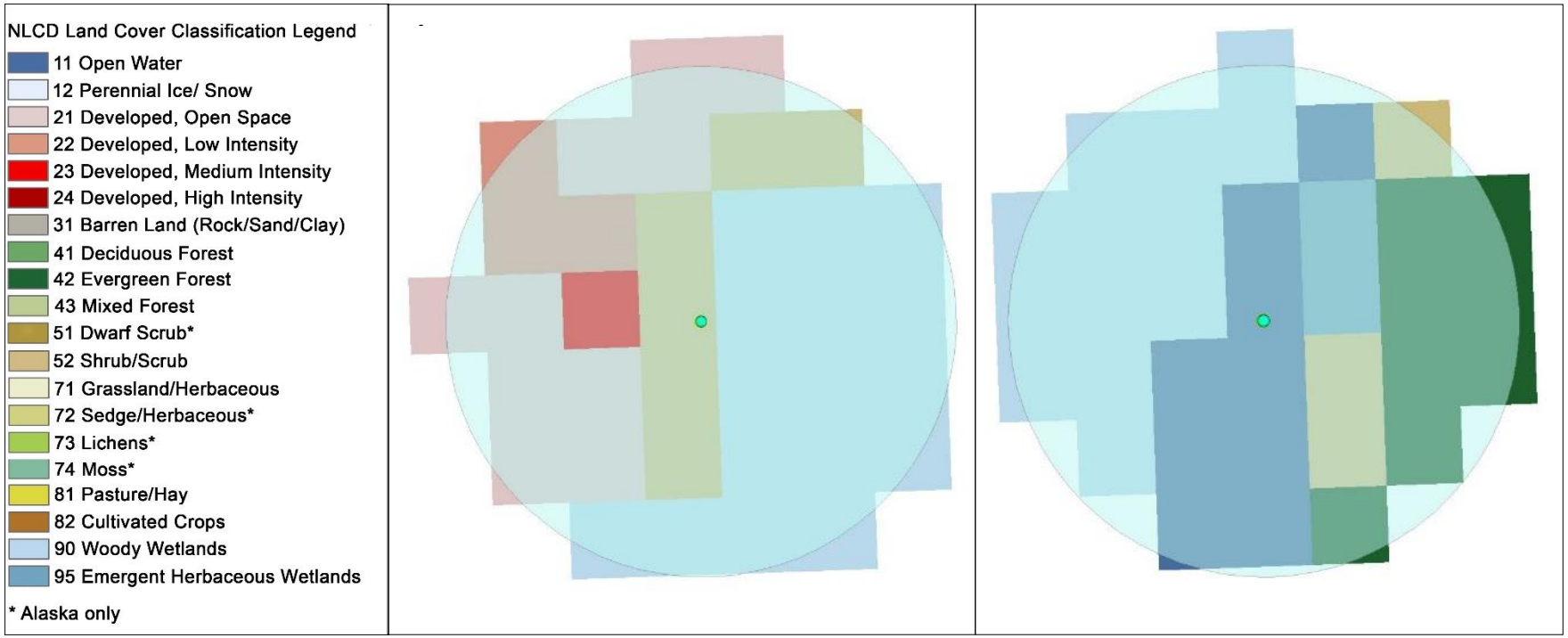

Figure 27. Two examples of the National Land Cover Database raster data used for land classification analysis at the 100 m buffer scale for (A) Fort Clark Springs, Headwater Pond, Kinney County (Site 29) and (B) Pecos River, 0.3 river km upstream of confluence with Independence Creek (Site 11). The light blue circle indicates the 100 m buffer around the GPS point used to mark each locality, which is indicated by a bright green circle.

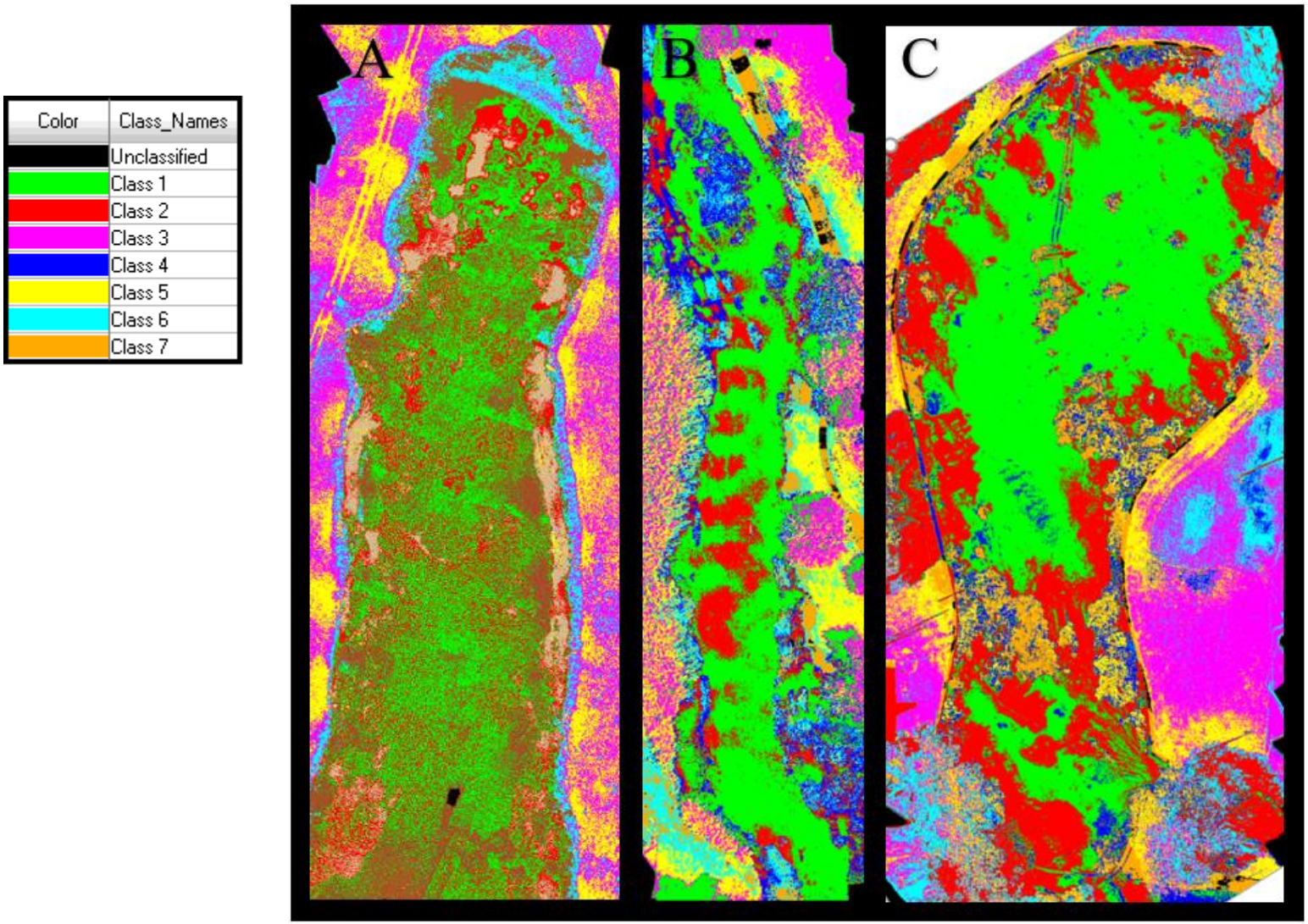

Figure 28. The thematic images that resulted from the unsupervised classification for (A) TNC Independence Creek Preserve, Lower Lake, Terrell County (Site 5); (B) Del Rio, San Felipe Springs Golf Course, San Felipe Creek, Val Verde County (Site 27); and (C) Fort Clark Springs, Headwater Pond, Kinney County (Site 29). The colors of the corresponding classes are indicated on the left.

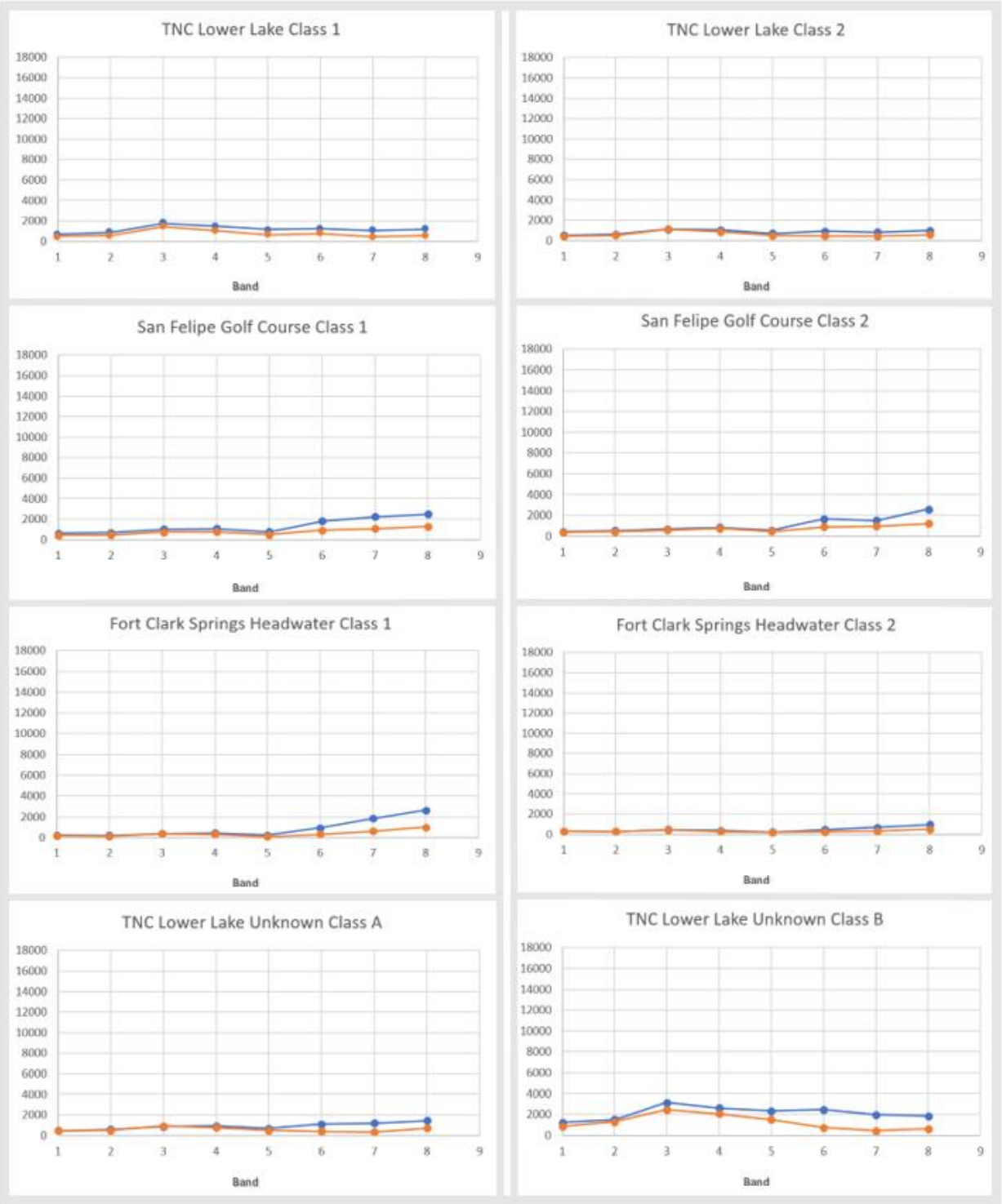

Figure 29. Spectral signatures from areas of open water at a subset of study sites. This was composed of Class 1 (on the left) and Class 2 (on the right) which were the result of an unsupervised classification. Additionally, two classes that were unique to TNC Independence Creek Preserve, Lower Lake, Terrell County (Site 5; on the bottom) fall into this category. Blue indicates the mean spectral value of each band and orange indicates the mode spectral value.

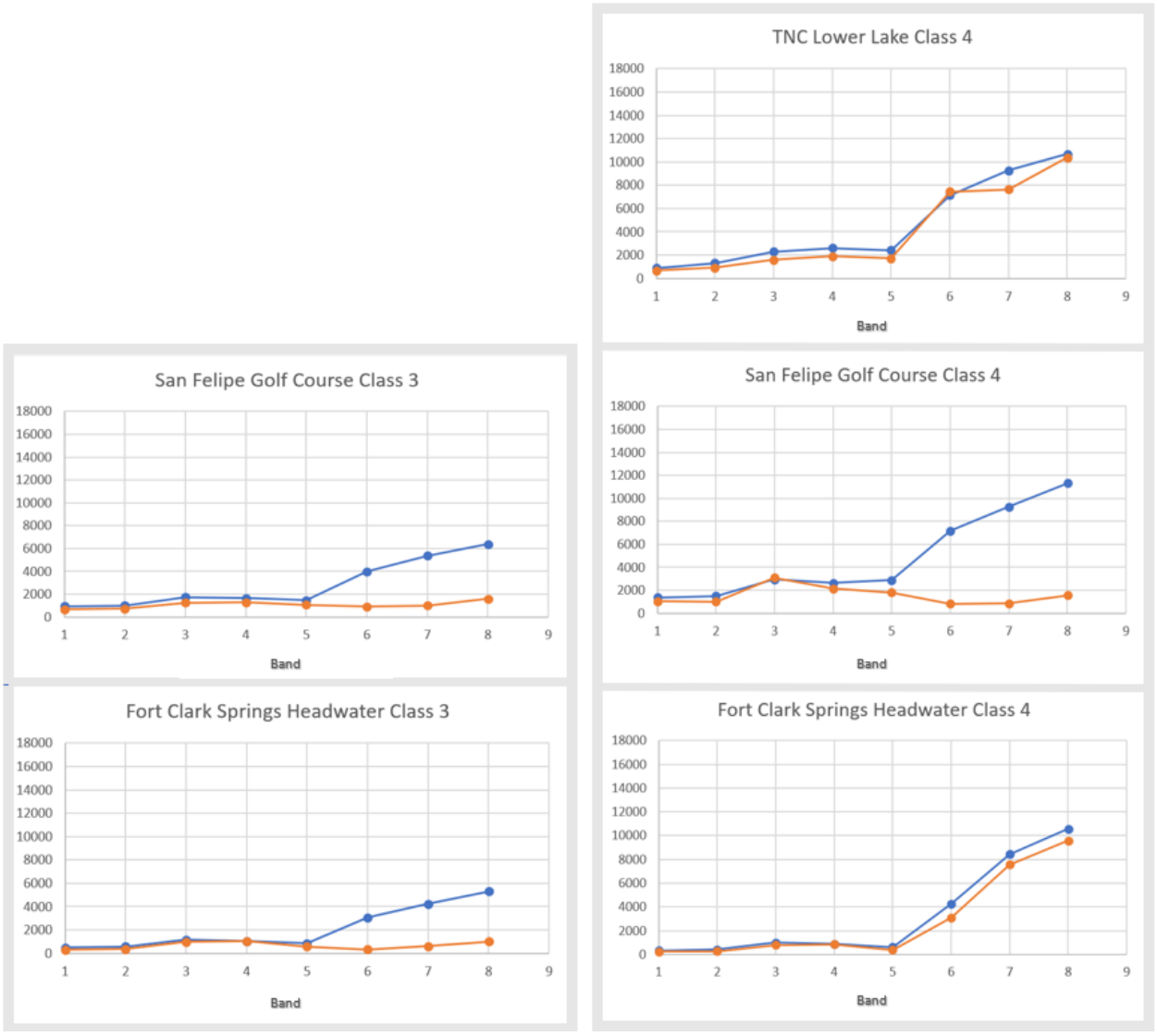

Figure 30. Spectral signatures from areas with various types of vegetation at a subset of study sites. This was composed of Class 3 (on the left) and Class 4 (on the right) which were the result of an unsupervised classification. TNC Independence Creek Preserve, Lower Lake, Terrell County (Site 5) did not have a class that matched the Class 3 values. Blue indicates the mean spectral value of each band and orange indicates the mode spectral value.

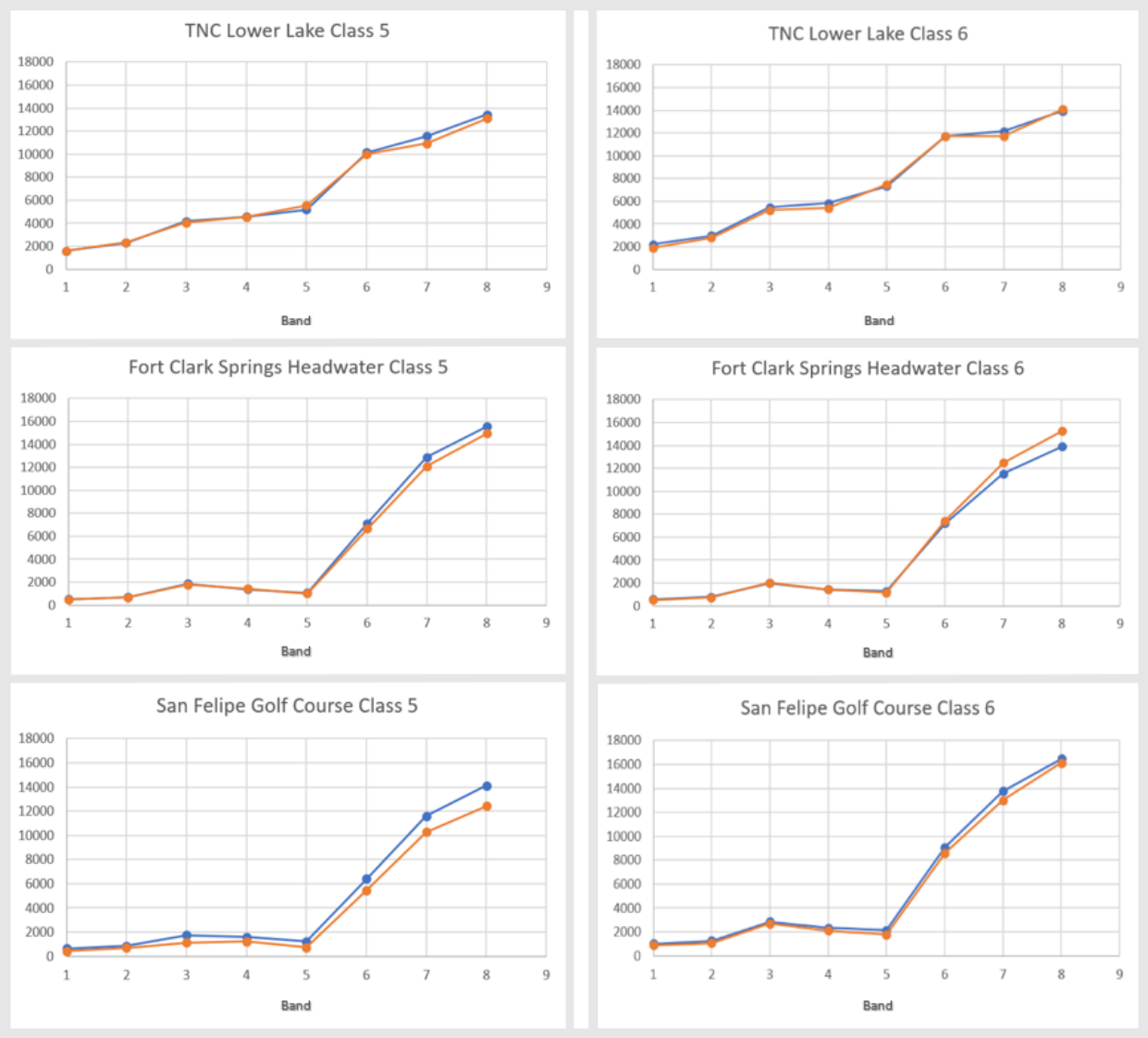

Figure 31. Spectral signatures from areas with grass at a subset of study sites. This was composed of Class 5 (on the left) and Class 6 (on the right) which were the result of an unsupervised classification. Blue indicates the mean spectral value of each band and orange indicates the mode spectral value.

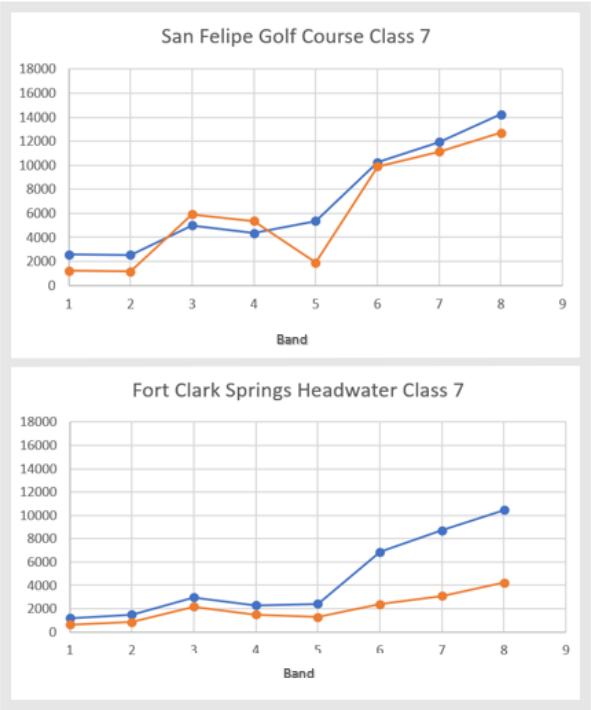

Figure 32. Spectral signatures from areas of rock and soil at a subset of study sites. This composed Class 7 which was the result of an unsupervised classification. TNC Independence Creek Preserve, Lower Lake, Terrell County (Site 5) did not have a class that matched this class. Blue indicates the mean spectral value of each band and orange indicates the mode spectral value.

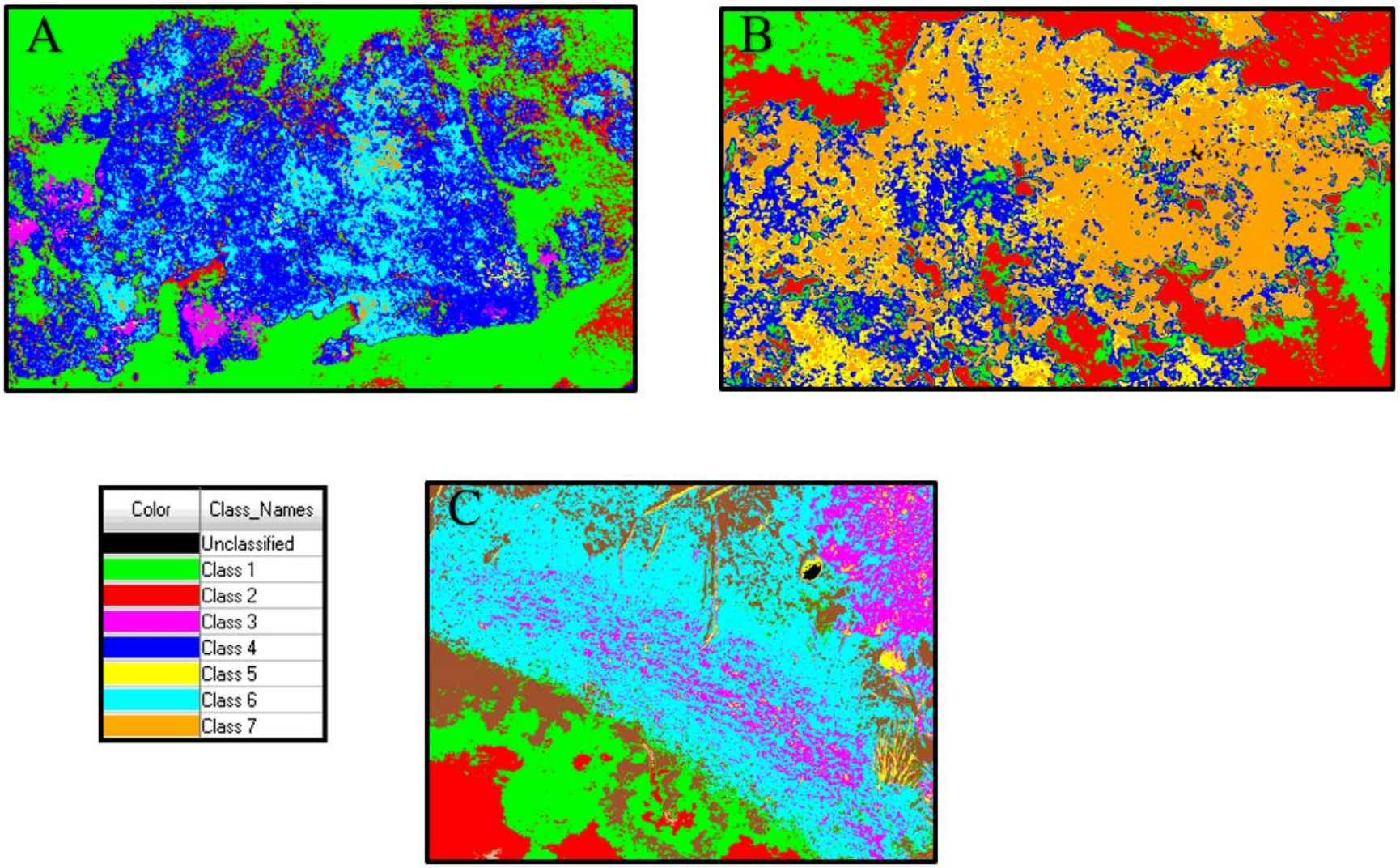

Figure 33. Examples of algal mats from thematic images of three study sites (A) Del Rio, San Felipe Springs Golf Course, San Felipe Creek, Val Verde County (Site 27); (B) Fort Clark Springs, Headwater Pond, Kinney County (Site 29); and (C) TNC Independence Creek Preserve, Lower Lake, Terrell County (Site 5) that resulted from unsupervised classifications. The colors of the corresponding classes are indicated on the left.

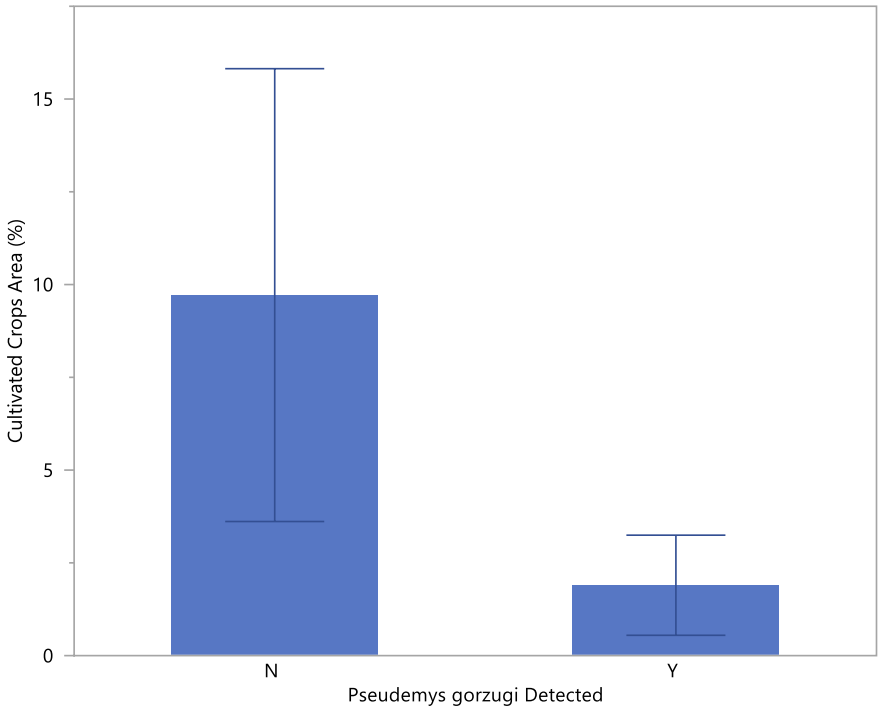

Figure 34. Mean cultivated crop area percentage (± 1 SE) for the 250 m buffer of sites where *Pseudemys gorzugi* were detected (Y) and were not detected (N) throughout this study.

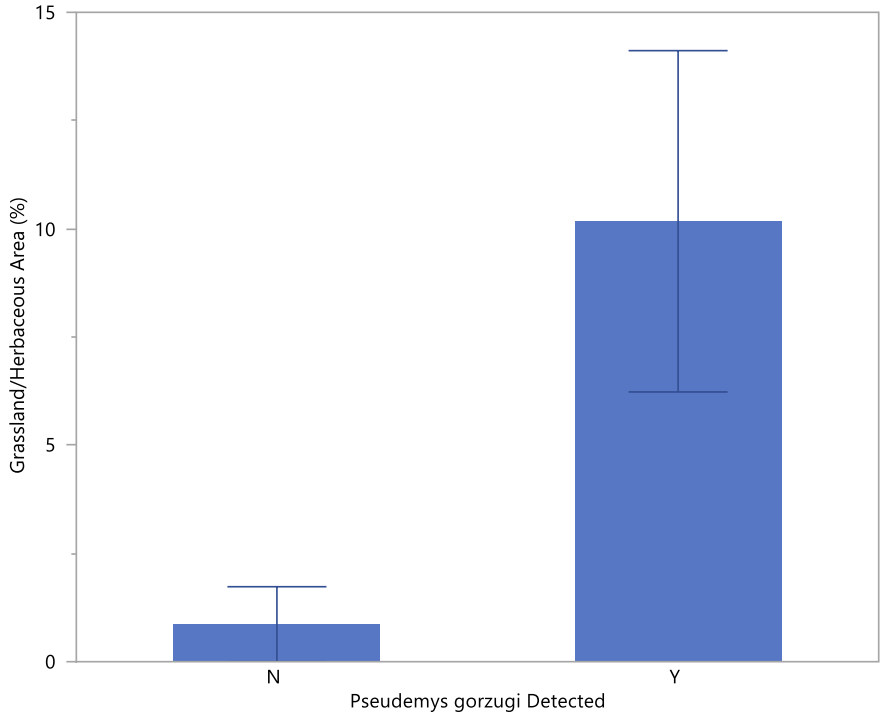

Figure 35. Mean grassland/herbaceous area percentage (± 1 SE) for the 100 m buffer of mainstem sites where *Pseudemys gorzugi* were detected (Y) and were not detected (N) throughout this study.

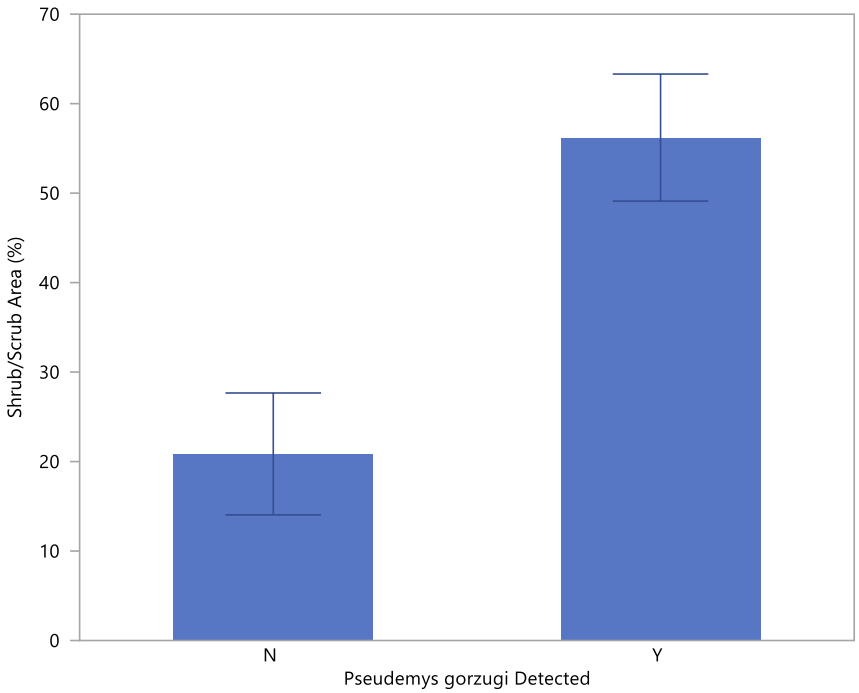

Figure 36. Mean shrub/scrub area percentage (± 1 SE) for the 1000 m buffer of mainstem sites where *Pseudemys gorzugi* were detected (Y) and were not detected (N) throughout this study.

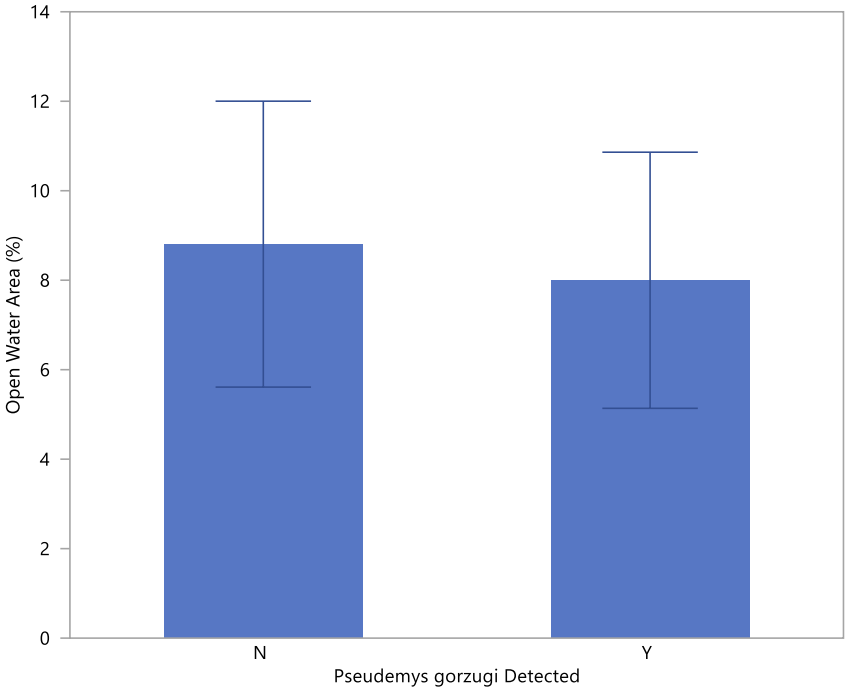

Figure 37. Mean open water area percentage (± 1 SE) for the 1000 m buffer of mainstem sites where *Pseudemys gorzugi* were detected (Y) and were not detected (N) throughout this study.

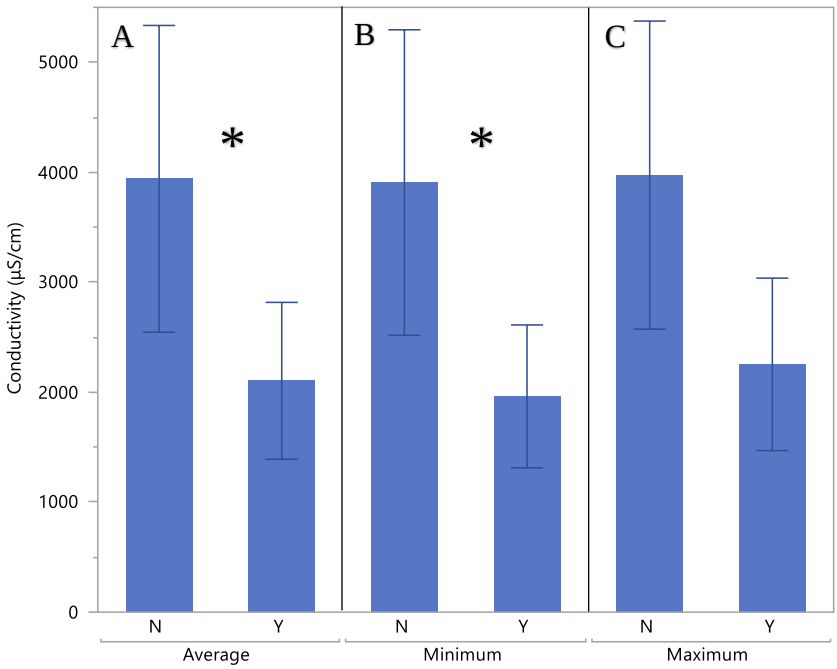

Figure 38. Average (A), mean (± 1 SE) minimum (B), and mean (± 1 SE) maximum (C) conductivity values measured at *Pseudemys gorzugi* survey sites. Sites where *P. gorzugi* was detected are indicated with a Y and sites where *P. gorzugi* was never detected are indicated with an N. Asterisk indicates a significant difference between the two groups (α = 0.05).

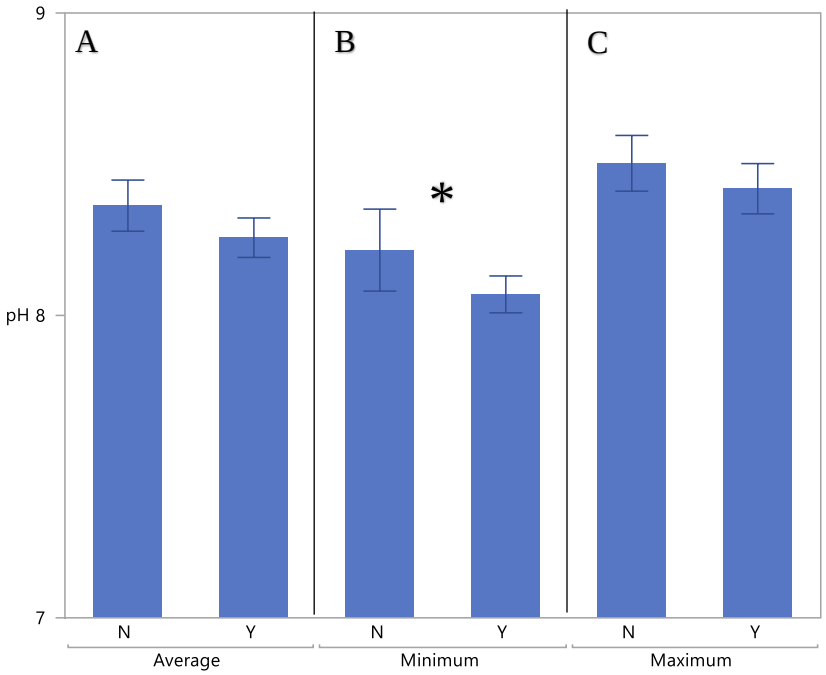

Figure 39. Average (A), mean (± 1 SE) minimum (B), and mean (± 1 SE) maximum (C) pH values measured at *Pseudemys gorzugi* survey sites. Sites where *P. gorzugi* was detected are indicated with a Y and sites where *P. gorzugi* was never detected are indicated with an N. Asterisk indicates a significant difference between the two groups (α = 0.05).

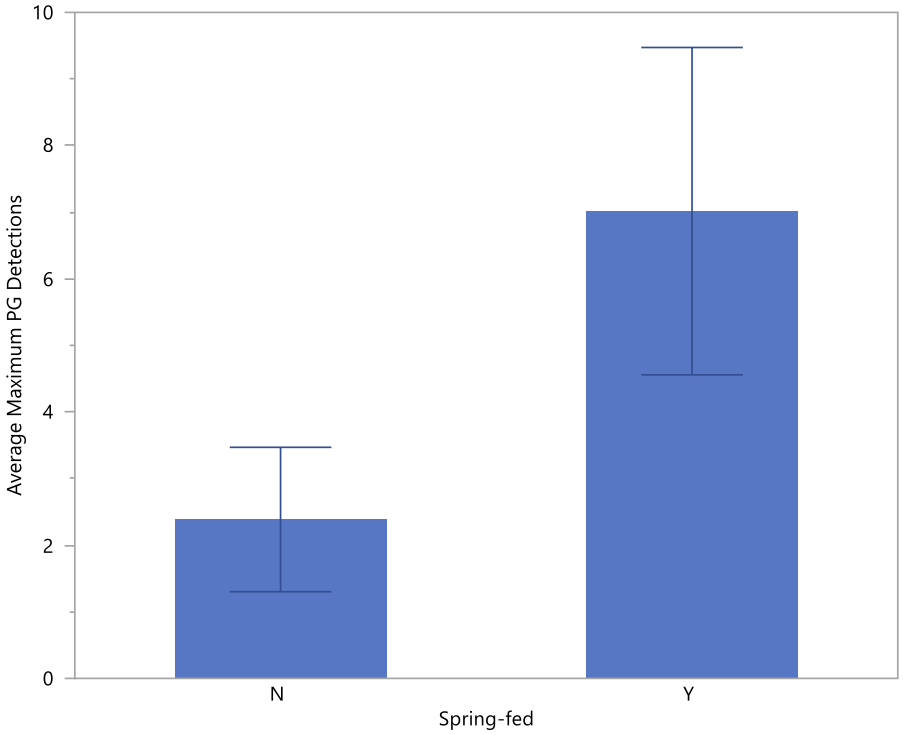

Figure 40. Average (± 1 SE) maximum *Pseudemys gorzugi* (PG) detections in spring-fed (Y) and non-spring-fed (N) survey sites.

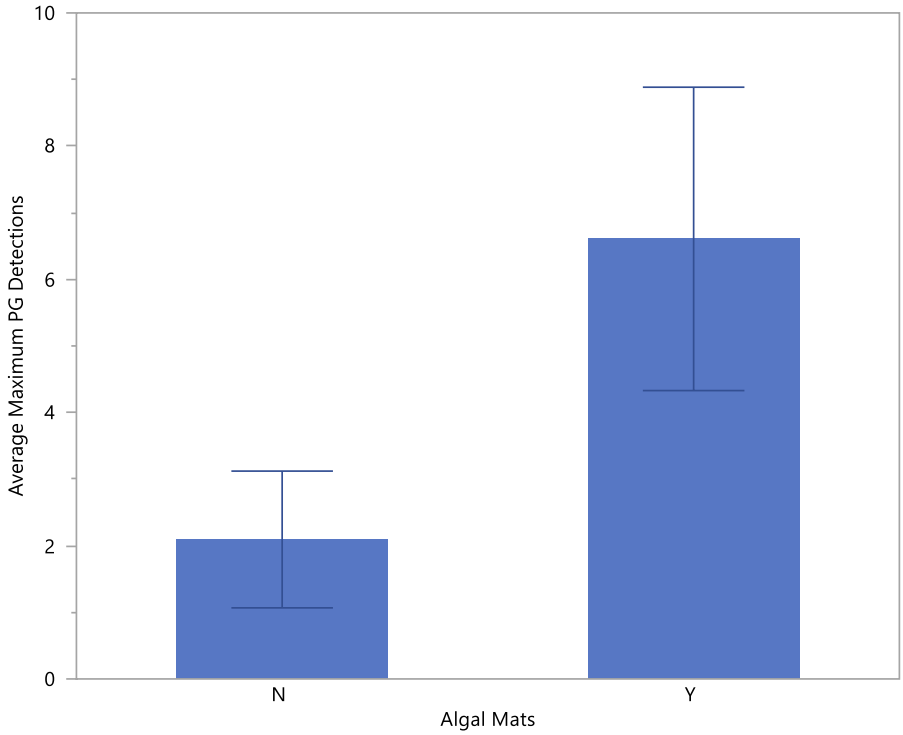

Figure 41. Average (± 1 SE) maximum *Pseudemys gorzugi* (PG) detections in survey sites with algal mats (Y) and without algal mats (N).

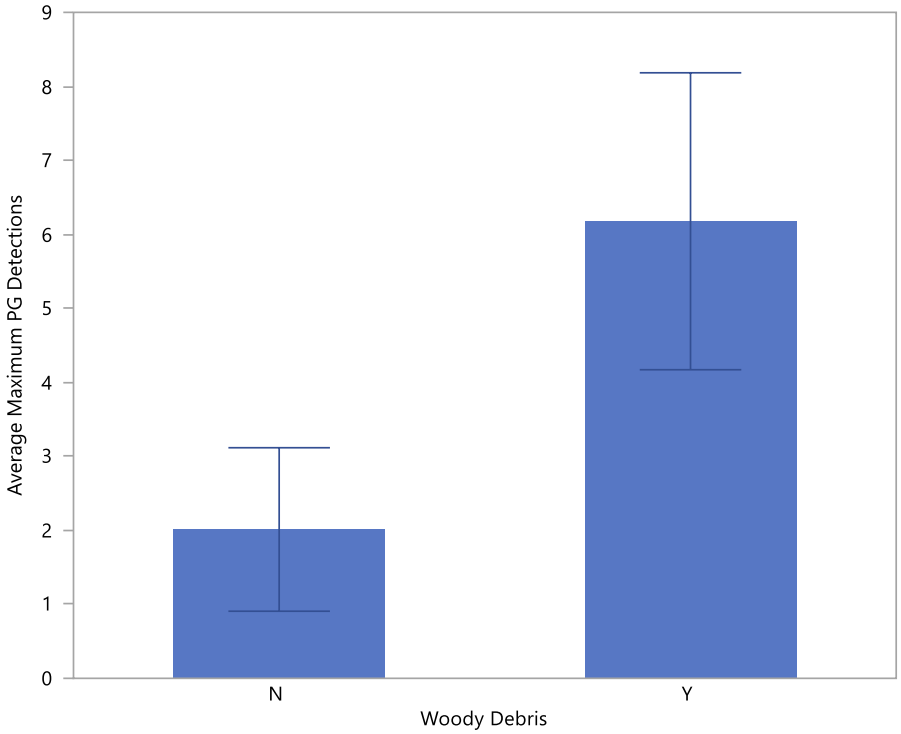

Figure 42. Average (± 1 SE) maximum *Pseudemys gorzugi* (PG) detections in survey sites with woody debris (Y) and without woody debris (N).

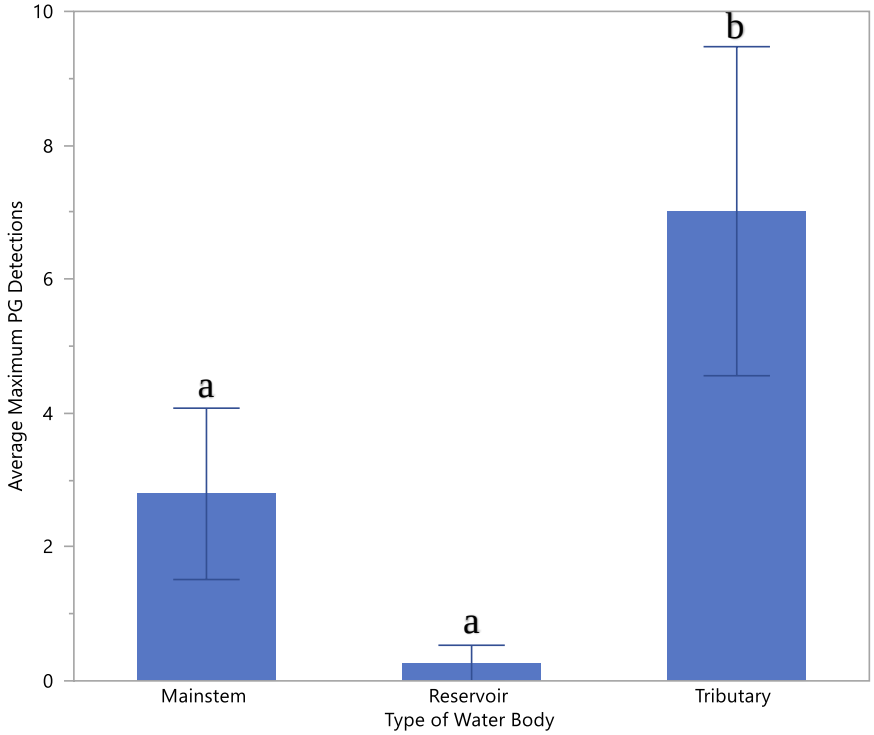

Figure 43. Average (± 1 SE) maximum *Pseudemys gorzugi* (PG) detections in mainstem, reservoir, and tributary survey sites. Letters indicate groupings from Wilcoxon multiple comparisons tests (α = 0.05).

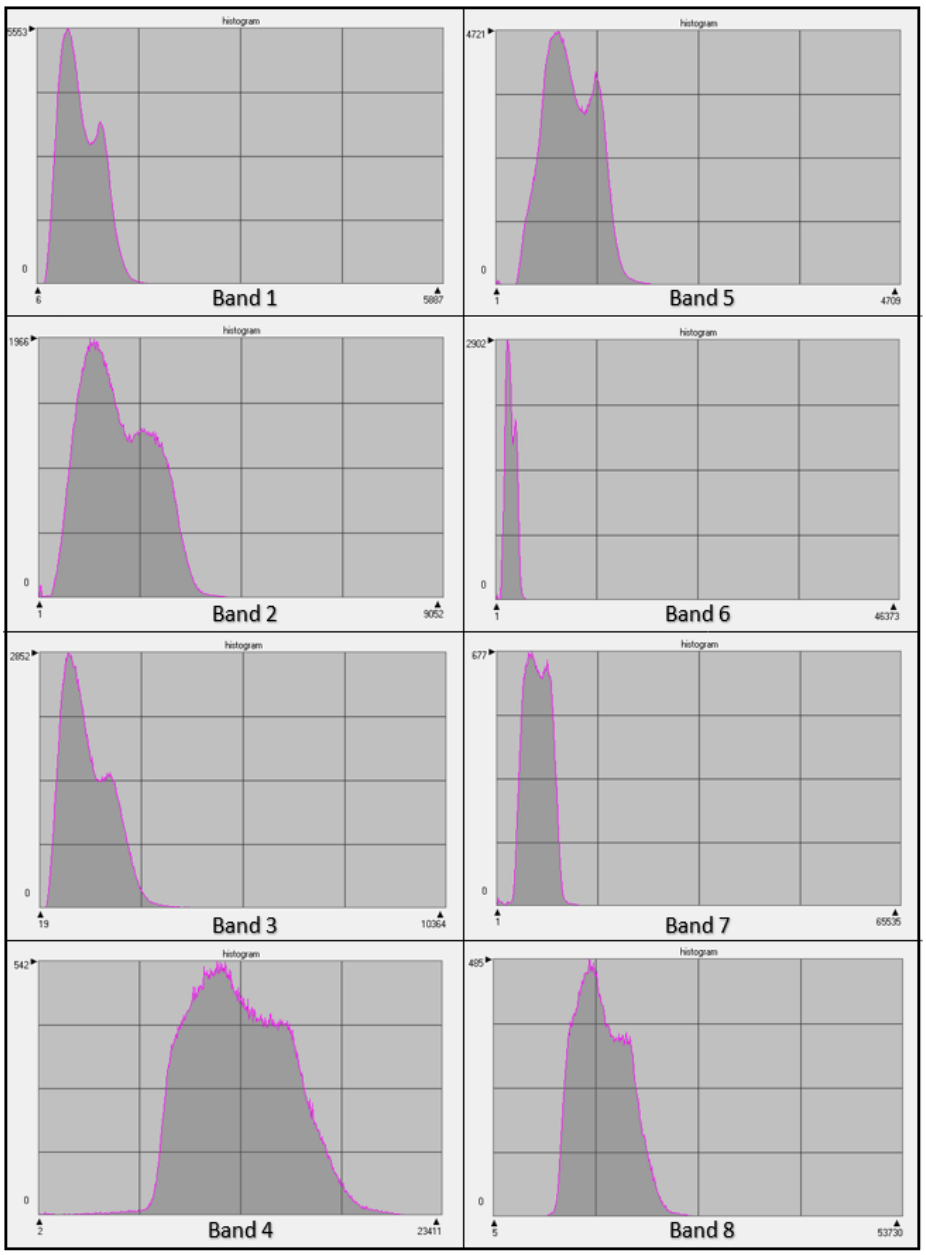

Figure 44. Histograms from each multispectral band depicting the distribution of pixel spectral values from Class 3. This resulted from an unsupervised classification at Del Rio, San Felipe Springs Golf Course, San Felipe Creek, Val Verde County (Site 27).

Table 9. The number of study sites composing each percent area interval per land class at the 100 m buffer size for sites where *Pseudemys gorzugi* was detected.

Area	Open Water	Developed	Forest	Shrub/ Scrub	Grassland/ Herbaceous	Wetlands	Barren Land (Rock/Sand/Clay)	Cultivated Crops
0–20%	27	22	41	22	39	27	43	42
21–40%	12	12	2	8	1	6	0	0
41–60%	4	5	0	8	2	7	0	1
61–80%	0	1	0	3	1	3	0	0
81–100%	0	3	0	2	0	0	0	0

Table 10. The number of study sites composing each percent area interval per land class at the 100 m buffer size for sites where *Pseudemys gorzugi* was not detected.

Area	Open Water	Developed	Forest	Shrub/ Scrub	Grassland/ Herbaceous	Wetlands	Barren Land (Rock/Sand/Clay)	Cultivated Crops
0–20%	11	12	16	10	17	12	17	16
21–-40%	2	3	2	2	1	3	1	0
41–60%	4	1	0	2	0	2	0	0
61–80%	0	2	0	2	0	0	0	2
81–100%	1	0	0	2	0	1	0	0

Table 11. The number of study sites composing each percent area interval per land class at the 250 m buffer size for sites where *Pseudemys gorzugi* was detected.

Area	Open Water	Developed	Forest	Shrub/ Scrub	Grassland/ Herbaceous	Wetlands	Barren Land (Rock/Sand/Clay)	Cultivated Crops
0–20%	37	28	42	15	40	29	43	41
21–40%	4	6	1	6	3	12	0	1
40–60%	1	1	0	9	0	2	0	1
61–80%	1	6	0	11	0	0	0	0
81–100%	0	2	0	2	0	0	0	0

Table 12. The number of study sites composing each percent area interval per land class at the 250 m buffer size for sites where *Pseudemys gorzugi* was not detected.

Area	Open Water	Developed	Forest	Shrub/ Scrub	Grassland/ Herbaceous	Wetlands	Barren Land (Rock/Sand/Clay)	Cultivated Crops
0–20%	13	14	17	7	17	12	18	16
21–40%	3	1	1	2	1	5	0	0
41–60%	1	2	0	4	0	1	0	0
61–80%	1	1	0	2	0	0	0	1
81–100%	0	0	0	3	0	0	0	1

Table 13. The number of study sites composing each percent area interval per land class at the 500 m buffer size for sites where *Pseudemys gorzugi* was detected.

Area	Open Water	Developed	Forest	Shrub/ Scrub	Grassland/ Herbaceous	Wetlands	Barren Land (Rock/Sand/Clay)	Cultivated Crops	Pasture/ Hay
0–20%	39	27	43	11	39	36	43	41	43
21–40%	1	5	0	10	4	7	0	1	0
41–60%	3	2	0	5	0	0	0	1	0
61–80%	0	4	0	8	0	0	0	0	0
81–100%	0	5	0	9	0	0	0	0	0

Table 14. The number of study sites composing each percent area interval per land class at the 500 m buffer size for sites where *Pseudemys gorzugi* was not detected.

Area	Open Water	Developed	Forest	Shrub/ Scrub	Grassland/ Herbaceous	Wetlands	Barren Land (Rock/Sand/Clay)	Cultivated Crops	Pasture/ Hay
0–20%	14	14	18	7	17	14	18	16	18
21–40%	2	1	0	1	1	4	0	0	0
41–60%	2	2	0	3	0	0	0	0	0
61–80%	0	1	0	3	0	0	0	2	0
81–100%	0	0	0	4	0	0	0	0	0

Table 15. The number of study sites composing each percent area interval per land class at the 1000 m buffer size for sites where *Pseudemys gorzugi* was detected.

Area	Open Water	Developed	Forest	Shrub/ Scrub	Grassland/ Herbaceous	Wetlands	Barren Land (Rock/Sand/Clay)	Cultivated Crops	Pasture/ Hay
0–20%	37	31	43	8	38	41	43	40	43
21–40%	3	3	0	9	5	1	0	1	0
41–60%	3	5	0	7	0	1	0	0	0
61–80%	0	1	0	1	0	0	0	2	0
81–100%	0	3	0	18	0	0	0	0	0

Table 16. The number of study sites composing each percent area interval per land class at the 1000 m buffer size for sites where *Pseudemys gorzugi* was not detected.

Area	Open Water	Developed	Forest	Shrub/ Scrub	Grassland/ Herbaceous	Wetlands	Barren Land (Rock/Sand/Clay)	Cultivated Crops	Pasture/ Hay
0–20%	17	10	18	8	18	18	18	16	17
21–40%	1	1	0	1	0	0	0	1	1
41–60%	0	1	0	4	0	0	0	0	0
61–80%	0	5	0	1	0	0	0	1	0
81–100%	0	1	0	4	0	0	0	0	0

Table 17. Average (avg.), maximum (max.), and minimum (min.) values of water quality parameters collected throughout the survey with a water quality sonde. Water temperature (water temp.), pH, dissolved oxygen (DO), conductivity (cond.), and oxidation-reduction potential (ORP) were measured. Sites that resulted in a positive *Pseudemys gorzugi* detection through drone, visual, trapping, and/or eDNA surveys at any point throughout the survey period are designated with a Y in the PG detected column. Sites that never resulted in a *P. gorzugi* detection are indicated with an N in this column. Site numbers correspond to those used in Table 1.

Site #	PG Detected	Avg. Water Temp. (°C)	Max. Water Temp. (°C)	Min. Water Temp. (°C)	Avg. pH	Max. pH	Min. pH	Avg. DO (mg/L)	Max. DO (mg/L)	Min. DO (mg/L)	Avg. Cond. (µS/cm)	Max. Cond. (µS/cm)	Min. Cond. (µS/cm)	Avg. ORP (mV)	Max. ORP (mV)	Min. ORP (mV)
1	Y	24.9	29.0	19.1	8.7	9.22	7.94	8.96	1.98	6.27	23197	26000	20590	186.2	242.7	153.5
2	N	26.5	32.8	20.2	7.72	9.1	6.34	9.38	11.76	7	19630	19820	19440	221.1	340.3	101.8
3	N	28.9	28.9	28.9	8.29	8.29	8.29	11.53	11.53	11.53	12260	12260	12260	219.8	219.8	219.8
4	N	26.2	26.2	26.2	7.9	7.9	7.9	6.64	6.64	6.64	10750	10750	10750	214.8	214.8	214.8
5	Y	29.9	32.0	28.3	7.88	8.41	7.34	6.14	12.05	0.23	783	785	781	123.0	123	-333.9
7	Y	26.1	26.1	26.1	8.01	8.01	8.01	11.74	11.74	11.74	922	922	922	88.6	88.6	88.6
9	Y	33.6	33.6	33.6	7.9	7.9	7.9	-	-	-	6060	6060	6060	124.7	124.7	124.7
10	Y	28.3	28.5	28.4	8.26	8.51	8	8.22	8.35	8.09	1037	1038	1035	87.5	99.2	75.7
11	Y	25.5	29.3	20.7	8.41	8.51	8.3	7.32	7.34	7.3	11440	11440	11440	61.2	113	9.46
13	Y	23.2	26.8	19.5	8.23	8.27	8.19	7.42	7.96	6.87	4760	5270	4250	121.3	123.9	118.6
14	Y	28.4	30.0	26.2	8.27	8.42	7.97	9.1	10.92	7.27	453	469	436	116.7	220.9	9.6
15	N	26.2	27.0	24.8	8.16	8.43	7.89	10.87	12.54	9.2	464	470	457	117.0	222.1	11.9
16	Y	26.5	29.8	22.2	8.34	8.42	8.27	8.5	8.9	8.1	454	462	445	148.4	248.3	93.5
17	N	28.3	28.4	28.4	8.34	8.34	8.34	6.95	6.95	6.95	1338	1338	1338	164.4	164.4	164.4
18	Y	29.7	30.4	29.1	8.28	8.38	8.17	6.96	7.16	6.76	1156	1434	877	119.9	122.8	117
20	Y	29.3	30.5	28.2	8.12	8.32	7.91	8.52	9.82	7.22	2367	2600	2134	140.3	180.8	99.8
21	Y	29.2	32.2	26.7	8.5	8.79	8.24	8.29	9.77	6.91	575	598	556	122.9	160.9	77.4
22	Y	31.4	31.4	31.4	8.29	8.29	8.29	5.54	5.54	5.54	1052	1052	1052	101.8	101.8	101.8
23	Y	29.7	30.1	28.4	8.46	8.57	8.29	7.99	8.74	7.53	979	1080	868	109.6	117	100.1
24	Y	24.4	25.9	22.1	8.4	8.44	8.36	3.87	5.07	2.67	1032	1094	969	13.8	187.3	-159.8
25	Y	27.3	27.2	27.0	8.1	8.5	7.69	6.47	7.89	5.04	1019	1042	996	133.5	143.7	123.3
26	Y	24.8	28.2	21.1	8.21	8.36	8	7.32	8.06	6.14	1047	1117	1004	185.1	236.3	130.6
27	Y	27.2	28.4	26.0	7.84	7.94	7.78	4.03	11.68	0.2	583	640	550	163.1	182.8	143.4
29	Y	25.4	26.3	22.7	7.75	8.12	7.29	8.66	10.47	6.84	433	503	430	205..2	252.8	175.3

30	Y	23.1	23.1	23.1	7.9	7.9	7.9	8.64	8.64	8.64	442	442	442	203.6	203.6	203.6
31	Y	21.4	21.4	21.4	7.77	7.77	7.77	-	-	-	505	505	505	184.9	184.9	184.9
32	Y	26.1	28.9	24.0	8.28	8.34	8.2	9.54	10.46	8.9	436	440	432	179.6	214.4	121.2
34	Y	21.0	21.0	21.0	7.87	7.87	7.87	-	-	-	505	505	505	181.3	181.3	181.3
35	Y	24.8	25.0	20.3	8.34	8.77	7.97	7.8	8.12	7.48	443	507	431	217.6	279.6	172.9
36	Y	17.5	17.5	17.5	7.81	7.81	7.81	-	-	-	452	452	452	132.3	132.3	132.3
37	Y	19.4	19.4	19.4	8.24	8.24	8.24	-	-	-	518	518	518	105.8	105.8	105.8
38	N	18.2	18.2	18.2	7.79	7.79	7.79	-	-	-	1748	1748	1748	134.3	134.3	134.3
39	N	18.5	18.5	18.5	8.35	8.35	8.35	-	-	-	986	986	986	106.6	106.6	106.6
40	Y	20.3	20.3	20.3	8.01	8.01	8.01	-	-	-	1777	1777	1777	130.1	130.1	130.1
41	N	20.6	20.6	20.6	8.26	8.26	8.26	-	-	-	-	-	-	83.0	83	83
42	Y	23.5	28.3	18.8	8.24	8.43	8.04	7.31	7.56	7.05	1748	2430	1065	109.0	118.3	99.7
43	Y	32.9	33.4	19.1	8.65	8.79	8.51	9.64	10.73	8.55	895	901	889	122.9	144.7	101
44	Y	29.2	29.5	20.7	9.76	10.48	9.03	7.78	7.78	7.78	1528	1592	1463	91.0	129.6	52.4
45	N	34.8	35.0	34.6	8.83	9	8.65	9.42	9.43	9.4	1360	1427	1293	126.4	134.8	118
46	N	31.4	31.9	31.0	8.85	9.03	8.67	8.85	9.92	7.78	1160	1289	1030	112.0	128.1	95.9
47	Y	27.4	30.6	21.6	8.25	8.49	8.11	6.78	7.43	5.96	896	990	804	127.0	174.8	86.9
48	N	27.4	32.0	22.8	8.29	8.39	8.18	7.08	7.44	6.72	889	964	813	133.4	157.4	109.4
49	Y	26.9	30.0	24.0	8.02	8.17	7.87	2.49	4.62	0.36	1033	1079	987	138.5	175.1	101.8
50	N	30.2	30.3	30.3	8.55	8.55	8.55	7.8	7.8	7.8	924	924	924	153.6	153.6	153.6
51	N	29.8	31.3	28.1	8.86	8.94	8.74	9.2	10.3	8.02	998	1022	986	76.7	111.1	53.6
52	N	29.7	32.5	28.0	8.6	8.76	8.38	8.37	10.02	7.76	983	985	982	78.0	104.5	61.2
53	Y	31.1	31.1	31.1	9.02	9.02	9.02	9.57	9.57	9.57	992	992	992	94.2	94.2	94.2
54	Y	28.3	29.1	20.0	8.39	8.66	7.69	6.31	9.12	1.94	982	993	909	12.2	129	-210.8
58	N	24.8	24.8	24.8	8.33	8.33	8.33	9.85	9.85	9.85	6910	6910	6910	132.1	132.1	132.1
59	Y	29.8	32.2	27.7	8.49	8.53	8.45	7.79	7.93	7.65	1118	1134	1101	393.5	580	207
60	N	28.4	28.4	28.4	8.33	8.33	8.33	6.24	6.24	6.24	1346	1346	1346	530.0	530	530
61	N	30.9	32.5	29.3	8.72	8.76	8.68	5.75	11.5	0.008	1297	1349	1245	81.6	169.7	-6.6

Table 18. Average (avg.), maximum (max.), and minimum (min.) values of water quality parameters collected throughout the survey with water quality test strips. Nitrate (NO_2^-), nitrite (NO_3^-), ammonia (NH_3), alkalinity, and hardness were measured. Sites that resulted in a positive *Pseudemys gorzugi* detection through drone, visual, trapping, and/or eDNA surveys at any point throughout the survey period are designated with a Y in the PG column. Sites that never resulted in a *P. gorzugi* detection are indicated with an N in this column. Site numbers correspond to those used in Table 1.

Site #	PG	Avg. NO_2^- (ppm)	Max. NO_2^- (ppm)	Min. NO_2^- (ppm)	Avg. NO_3^- (ppm)	Max. NO_3^- (ppm)	Min. NO_3^- (ppm)	Avg. NH_3 (ppm)	Max. NH_3 (ppm)	Min. NH_3 (ppm)	Avg. Alkalinity (ppm)	Max. Alkalinity (ppm)	Min. Alkalinity (ppm)	Avg. Hardness (ppm)	Max. Hardness (ppm)	Min. Hardness (ppm)
1	Y	0	0	0	0	0	0	0.12	0.25	0	63	120	30	425	425	425
2	N	0	0	0	0	0	0	0.13	0.25	0	40	40	40	425	425	425
3	N	0	0	0	0	0	0	0	0	0	80	80	80	425	425	425
4	N	0	0	0	0	0	0	0	0	0	80	80	80	425	425	425
5	Y	0	0	0	0	0	0	0	0	0	210	240	180	338	425	250
7	Y	2	2	2	0	0	0	0	0	0	240	240	240	425	425	425
9	Y	0	0	0	0	0	0	0	0	0	240	240	240	425	425	425
10	Y	0	0	0	0	0	0	0	0	0	200	240	160	425	425	425
11	Y	0	0	0	0	0	0	0.25	0.5	0	180	180	180	425	425	425
13	Y	1	1	1	0.15	0.15	0.15	0.25	0.25	0.25	180	180	180	425	425	425
14	Y	2	2	2	0	0	0	0	0	0	190	220	160	425	425	425
15	N	2	2	2	0	0	0	0.25	0.25	0.25	240	240	240	425	425	425
16	Y	0	0	0	0	0	0	0	0	0	240	240	240	338	425	250
17	N	0	0	0	0	0	0	0.25	0.25	0.25	210	210	210	400	400	400
18	Y	0	0	0	0	0	0	0.08	0.15	0	240	240	240	425	425	425
20	Y	0	0	0	0	0	0	0	0	0	200	240	160	413	425	400
21	Y	0	0	0	0	0	0	0.08	0.25	0	200	240	180	308	425	250
22	Y	0	0	0	0	0	0	0.1	0.1	0.1	180	180	180	400	400	400
23	Y	0	0	0	0	0	0	0	0	0	133	160	120	425	425	425
24	Y	0.5	1	0	0	0	0	0.13	0.25	0	130	160	100	425	425	425
25	Y	0.5	1	0	0	0	0	0.13	0.25	0	160	160	160	425	425	425
26	Y	0	0	0	0	0	0	0	0	0	220	240	180	425	425	425
27	Y	0.67	2	0	0	0	0	0	0	0	240	240	240	400	425	350

29	Y	1.67	3	0	0	0	0	0.13	0.25	0	223	240	210	308	425	250
30	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	Y	2	2	2	0	0	0	0	0	0	240	240	240	250	250	250
32	Y	1.5	3	0	0	0	0	0.23	0.25	0.2	240	240	240	313	425	200
34	Y	2	2	2	0	0	0	0	0	0	240	240	240	425	425	425
35	Y	1.67	2	1	0	0	0	0.08	0.25	0	230	240	210	325	425	250
36	Y	-	-	-	-	-	-	-	-	-	180	180	180	250	250	250
37	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	N	-	-	-	-	-	-	-	-	-	180	180	180	425	425	425
39	N	-	-	-	-	-	-	-	-	-	180	180	180	250	250	250
40	Y	-	-	-	-	-	-	-	-	-	-	-	-	425	425	425
41	N	-	-	-	-	-	-	-	-	-	240	240	240	250	250	250
42	Y	1.5	3	0	0.05	0.1	0	0.23	0.25	0.2	160	180	140	424	425	423
43	Y	1	1	1	0	0	0	0	0	0	195	230	160	338	425	250
44	Y	1	1	1	0	0	0	0	0	0	140	180	100	338	425	250
45	N	0	0	0	0	0	0	0	0	0	240	240	240	400	425	375
46	N	0	0	0	0	0	0	0	0	0	240	240	240	425	425	425
47	Y	0	0	0	0	0	0	0.13	0.25	0	180	180	180	425	425	425
48	N	0	0	0	0	0	0	0	0	0	-	-	-	425	425	425
49	Y	0	0	0	0	0	0	0.25	0.25	0.25	180	180	180	425	425	425
50	N	0	0	0	0	0	0	0	0	0	120	120	120	425	425	425
51	N	0	0	0	0	0	0	0	0	0	117	150	80	338	425	250
52	N	0	0	0	0	0	0	0.08	0.25	0	137	180	150	363	375	350
53	Y	0	0	0	0	0	0	0.25	0.25	0.25	100	100	100	425	425	425
54	Y	0.25	1	0	0	0	0	0.06	0.25	0	145	180	100	308	425	250
58	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59	Y	0	0	0	0	0	0	0	0	0	120	120	120	425	425	425
60	N	2	2	2	0	0	0	0	0	0	-	-	-	-	-	-
61	N	2	2	2	0	0	0	0	0	0	180	180	180	425	425	425

APPENDIX A

APPENDIX A

SITE CHARACTERIZATION USED FOR CATEGORICAL METHODOLOGY COMPARISONS

Appendix A. Categories used to classify sites for the categorical methodology comparisons performed in Chapter II. This characterization was used to determine whether differences in mean turtle detections, *Pseudemys gorzugi* detections, or identification percentages occurred for the drone, visual, and trapping methodologies under certain habitat types and conditions. The categories examined were *P. gorzugi* (PG) detected, spring-fed, waterbody type, turbidity, flow, connectivity, algal mat presence, woody debris presence, tree presence, and shoreline vegetation presence. For the waterbody type column M = mainstem, T = tributary, and R = reservoir. For turbidity, L = low, M = mid-level, and H = high. For all other categories Y = yes and N = no.

Site #	Latitude	Longitude	PG Detected	Spring-fed	Waterbody Type	Turbidity	Flow	Connectivity	Algal Mats	Woody Debris	Trees	Shoreline Vegetation
1	30.90516	-101.88083	Y	N	M	L	Y	Y	Y	Y	Y	Y
2	30.78851	-101.83502	N	N	M	L	Y	Y	Y	Y	Y	N
3	30.71808	-101.80954	N	N	M	M	Y	Y	N	N	Y	Y
4	30.65960	-101.77022	N	N	M	M	Y	Y	N	N	Y	Y
5	30.46955	-101.80131	Y	Y	T	L	Y	Y	Y	Y	Y	N
6	30.46736	-101.80181	Y	Y	T	L	Y	Y	Y	Y	Y	N
9	30.45259	-101.71940	Y	N	M	M	Y	Y	N	N	Y	Y
10	30.45026	-101.73124	Y	Y	T	L	Y	Y	N	N	Y	N
11	30.44767	-101.72119	Y	N	M	M	Y	Y	Y	Y	Y	Y
13	30.13120	-101.57450	Y	N	M	M	Y	Y	N	N	Y	Y
14	29.89387	-100.99561	Y	Y	T	L	Y	Y	Y	Y	Y	Y
15	29.88591	-100.99292	N	Y	T	L	Y	Y	Y	Y	Y	Y

16	29.88385	-100.99397	Y	Y	T	L	Y	Y	Y	Y	Y	Y
17	29.80829	-101.54893	N	N	M	H	Y	Y	N	N	Y	Y
18	29.80564	-101.55088	Y	N	M	H	Y	Y	N	N	Y	Y
19	29.80343	-101.56750	Y	Y	T	H	Y	Y	N	Y	Y	N
20	29.70431	-101.36667	Y	N	M	H	Y	Y	N	N	N	N
21	29.57490	-100.97809	Y	N	R	L	N	Y	N	N	N	N
23	29.52420	-101.17585	Y	N	R	M	N	Y	N	N	N	N
24	29.44737	-101.05667	Y	N	M	L	Y	Y	N	N	Y	Y
25	29.42455	-101.04118	Y	N	M	L	Y	Y	N	N	Y	Y
26	29.37719	-101.01348	Y	N	M	L	Y	Y	N	N	Y	Y
27	29.37029	-100.88526	Y	Y	T	L	Y	Y	Y	Y	Y	N
29	29.30944	-100.42125	Y	Y	T	L	Y	Y	Y	Y	Y	N
30	29.29043	-100.42386	Y	Y	T	M	Y	Y	N	N	Y	N
31	29.28034	-100.42076	Y	Y	T	L	Y	Y	Y	Y	Y	Y
42	28.70416	-100.51046	Y	N	M	M	Y	Y	Y	Y	Y	N
43	28.70294	-100.51089	Y	N	M	M	Y	Y	N	N	Y	Y
44	28.70146	-100.50979	Y	N	M	M	N	N	N	N	N	N
45	27.54447	-99.44098	N	N	R	M	N	Y	N	N	Y	Y
46	27.53861	-99.43475	N	N	R	M	N	Y	N	N	N	Y
47	27.52372	-99.52431	Y	N	M	H	Y	Y	N	N	Y	Y
48	27.49835	-99.51674	N	N	M	H	Y	Y	N	N	Y	Y
49	27.33117	-99.51195	Y	N	M	H	Y	Y	N	N	Y	Y
50	27.04330	-99.44496	N	N	M	M	Y	Y	N	N	Y	Y
51	26.58179	-99.15259	N	N	R	M	N	Y	N	N	N	N
52	26.54608	-99.17093	N	N	M	M	Y	Y	N	N	Y	Y
53	26.53233	-99.15546	Y	N	M	M	Y	Y	Y	Y	Y	Y
54	26.51429	-99.11662	Y	N	M	M	Y	Y	N	Y	Y	Y
59	26.16934	-98.36742	Y	N	M	M	Y	Y	Y	Y	Y	Y
60	25.85462	-97.37676	N	N	M	H	Y	Y	N	N	Y	Y
61	25.85008	-97.39865	N	N	M	H	Y	Y	N	N	Y	Y

APPENDIX B

APPENDIX B

PERMITS AND REQUIREMENTS FOR DRONE SURVEYS

Studies that utilize drone surveys must secure additional permits and meet specific requirements in addition to the sampling permits that are required for general scientific studies. Regulations are subject to change and may differ due to location, so it is important to be knowledgeable of specific requirements for your sampling area and stay up to date as legislation changes. As drones are a novel technology, regulations and legislation are changing frequently, with several changes occurring throughout the course of this study. However, these are the requirements that we needed to meet in order to conduct drone surveys for turtle species in southwestern Texas.

All drone pilots must obtain a Remote Pilot License, as required under federal law by the Federal Aviation Administration, when operating a drone in a non-recreational manner (Federal Aviation Association, 2020). This requires passing a written aeronautical knowledge exam. Upon passing the exam a Temporary Airman Certificate is issued, which gives you temporary permission to pilot a drone until your Remote Pilot License arrives. The Remote Pilot License is valid until two years after the issue date. Additionally, the drone must be registered through the FAA, which can be accomplished online, or by paper, for a fee of five dollars. Through this process you receive a registration number for your drone, which must be visibly displayed on the aircraft at all times. Registration is valid for three years (Federal Aviation Association, 2019).

Additionally, it is important to abide by no-fly zones and care should be taken to ensure that sampling areas do not fall within these zones. If sampling areas must be conducted in no-fly zones, you can request that the FAA issue you a Certificate of Waiver or Authorization. This can be accomplished in Low Altitude Authorization and Notification Capability (LAANC), an automated system that evaluates your request against airspace data sources with the capability of offering approval in near-real time (Federal Aviation Association, 2018). If the airport is not equipped with LAANC, or your request is denied, you can submit a request through DroneZone, FAA's online system. Through this system you submit the details of your flight request and a FAA employee evaluates your flight to determine if a waiver can be issued. As my LAANC request was denied, I submitted a request through DroneZone to obtain a waiver to fly at Lake Casa Blanca International State Park, Casa Blanca Lake, near El Ranchito pavilion (Site 45; Figure 3; Table 1) and Lake Casa Blanca International State Park, Casa Blanca Lake, fishing pier (Site 46; Figure 3; Table 1). I found my assigned FAA contact eager to assist in obtaining a waiver, and he was able to help me receive one within a reasonable time frame. Requirements of the waiver included notifying Air Traffic Control fifteen minutes prior to the flight and upon its completion. This process in my experience, was fairly straightforward and did not create any challenges.

Federal agencies provided some of the greatest resistance to drone surveys for this study, with the USFWS denying permission to conduct drone flights on their land, and the National Park Service requiring a separate permitting process for the drone aspect of the study. This permitting process is often lengthy, generally taking several months, and requiring permission from the sampling site, the regional director, and the national aviation manager. An Operator Safety Plan detailing our safety precautions for the proposed flight was required to be developed

and submitted as well. In our experience this proved to be a slow process, with drone permission being granted several months after the process was initiated.

Whether on government or private land, in the state of Texas, Texas Parks and Wildlife Department requires that an Aerial Wildlife and Exotic Animal Management Permit be obtained when photographing wildlife (AMW Permits, 2020). Applications are submitted online though their online portal, Texas Wildlife Information Management Services (TWIMS). After receiving a permit, Landowner Authorizations (LOAs) must be obtained from the landowner of each proposed drone flight location. Information about the landowner, their property, and a map of the proposed flight is submitted though the portal, and an email is generated to the landowner informing them of the proposed flight and requesting their permission. If they consent to the flight, the drone pilot is notified. Additionally, all conducted drone flights must be logged and submitted to TPWD through the portal on a quarterly basis. This proved to be a time and labor-intensive process, with many landowners hesitant about offering permission through the portal, despite verbal and written permission outside of the system.

While many factors influence the experiences each pilot will have when obtaining their drone-related permits, there are a few steps pilots can take to make this process smoother. Ensure that you are fully aware and up to date on the regulations in each of your sampling areas well before sampling is scheduled to begin. Start the permit application process as early as possible to increase the likelihood of having permits issued before your sampling begins. Be prepared for the possibility of permit denial, and seek alternate sampling sites, such as nearby private land, in case you are denied a permit. In our experience private property owners were the most open to drone surveys. Finally, be prepared for the time and labor requirements of obtaining and maintaining permits and allocate time and personnel accordingly.

References

AWM Permits. (2020). *AWM Aerial Wildlife Management Permits.* Retrieved March 2, 2020, https://tpwd.texas.gov/business/permits/land/wildlife_management/aerial_wl_management/.

Federal Aviation Association. (2018). *Flying Drones Near Airports (Controlled Airspace) – Part 107. United States Department of Transportation*. Retrieved March 15, 2020, https://www.faa.gov/uas/commercial_operators/part_107/.

Federal Aviation Association. (2019). *Register Your Drone*. United States Department of Transportation. Retrieved March 15, 2020, https://www.faa.gov/uas/getting_started/register_drone/.

Federal Aviation Association. (2020). *Become a Drone Pilot*. United States Department of Transportation. Retrieved March 15, 2020, https://www.faa.gov/uas/commercial_operators/become_a_drone_pilot/.

APPENDIX C

APPENDIX C

PREPARATION INSTUCTIONS FOR MODIFIED LONGMIRE BLOOD STORAGE BUFFER

Modified Longmire Blood Storage Buffer	pH = 8.0	1 L
Dissolve the following in ca. 900 mL ddH_2O:		
12.114 g TRIS MW = 121.14		0.1 M
37.224 g EDTA Disodium Dihydrate MW = 372.24		0.1 M
10 g SDS MW = 288.38		1% w/v
Adjust pH to 8.0. Add ddH_2O to 1 L total volume of solution. Do not autoclave. Store at room temperature.		

APPENDIX D

APPENDIX D

SAMPLING DATES FOR ENVIRONMENTAL DNA ANALYSIS

Site #	Date	County	Site	Latitude	Longitude
1	18 May 2019	Pecos	Pecos River, at US Hwy 190 crossing	30.90516	-101.88083
3	18 May 2019	Pecos	Pecos River, at I-10 crossing	30.71808	-101.80954
4	18 May 2019	Pecos	Pecos River, at TX Hwy 290 crossing	30.65960	-101.77022
5	5 June 2019	Terrell	TNC Independence Creek Preserve, Lower Lake	30.46955	-101.80131
7	7 June 2019	Terrell	TNC Independence Creek Preserve, raceway below Upper Lake	30.46736	-101.80181
10	6 June 2019	Terrell	Independence Creek, at County Road crossing	30.45026	-101.73124
11	5 June 2019	Crockett	Pecos River, 0.3 river km upstream of confluence with Independence Creek	30.44767	-101.72119
13	6 June 2019	Val Verde	Pecos River, at Pandale crossing	30.13120	-101.57450
14	20 July 2019	Val Verde	TNC Dolan Falls Preserve, Devils River, upstream of confluence with Dolan Creek	29.89387	-100.99561
16	20 July 2019	Val Verde	TNC Dolan Falls Preserve, Devils River, Dolan Falls	29.88385	-100.99397
18	22 June 2019	Val Verde	Rio Grande, near Langtry	29.80564	-101.55088
20	23 June 2019	Val Verde	Pecos River, near confluence with Rio Grande	29.70431	-101.36667
21	21 June 2019	Val Verde	Lake Amistad, Rough Canyon	29.57490	-100.97809
22	21 June 2019	Val Verde	Lake Amistad, along Spur 406	29.54023	-101.01623

23	21 June 2019	Val Verde	Lake Amistad, Box Canyon	29.52420	-101.17585
24	2 October 2019	Val Verde	Rio Grande, spillway below Amistad Dam	29.44737	-101.05667
25	21 August 2019	Val Verde	Rio Grande, weir below Amistad Dam	29.42455	-101.04118
26	2 October 2019	Val Verde	Rio Grande, near Lugo property	29.37719	-101.01348
27	31 July 2019	Val Verde	Del Rio, San Felipe Springs Golf Course, San Felipe Creek	29.37029	-100.88526
29	26 June 2019	Kinney	Fort Clark Springs, Headwater Pond	29.30944	-100.42125
32	29 June 2019	Kinney	Fort Clark Springs, Las Moras Creek, upstream of golf pro shop	29.29043	-100.42386
35	31 July 2019	Kinney	Fort Clark Springs, Las Moras Creek, Buzzard Roost	29.28034	-100.42076
36	11 March 2019	Val Verde	Sycamore Creek, at US Hwy 277 crossing	29.25473	-100.75216
37	11 March 2019	Kinney	Pinto Creek, at US Hwy 277 crossing	29.18898	-100.70340
38	11 March 2019	Maverick	Tequesquite Creek, at US Hwy 277 crossing	29.06453	-100.63899
39	11 March 2019	Maverick	Irrigation canal along US Hwy 277, near Las Moras Creek	29.00785	-100.63817
40	11 March 2019	Maverick	Quemado Creek, along US Hwy 277	28.92578	-100.61490
41	10 March 2019	Maverick	Elm Creek, near US Hwy 277	28.77016	-100.49828
42	9 March 2019	Maverick	Eagle Pass Golf Course, spillway into Rio Grande	28.70416	-100.51046
43	29 June 2019	Maverick	Rio Grande, along Eagle Pass Golf Course	28.70294	-100.51089

44	1 July 2019	Maverick	Eagle Pass Golf Course, settling pond along Rio Grande	28.70146	-100.50979
45	6 September 2019	Webb	Lake Casa Blanca International State Park, near El Ranchito pavillion	27.54447	-99.44098
47	6 September 2019	Webb	Rio Grande, Laredo, near water treatment center	27.52372	-99.52431
49	5 September 2019	Webb	Rio Grande, near El Cenizo	27.33117	-99.51195
50	7 July 2019	Zapata	Rio Grande, near San Ygancio	27.04330	-99.44496
51	4 September 2019	Starr	Falcon State Park, Falcon Lake	26.58179	-99.15259
52	7 July 2019	Starr	Rio Grande, spillway below Falcon Dam	26.54608	-99.17093
53	6 July 2019	Starr	Rio Grande, near Chapeno	26.53233	-99.15546
54	6 July 2019	Starr	Rio Grande, near Salineño	26.51429	-99.11662
58	12 March 2019	Hidalgo	Bentsen-Rio Grande Valley State Park, La Parido Banco	26.17906	-98.38716
59	24 September 2019	Hidalgo	Rio Grande, near National Butterfly Center	26.16934	-98.36742
61	24 September 2019	Cameron	Rio Grande, near TNC Southmost Preserve Office	25.85008	-97.39865

BIOGRAPHICAL SKETCH

Amy P. Bogolin graduated in 2014 from Le Moyne College in Syracuse, New York with two Bachelor of Science degrees, one in Biology and the other in Environmental Science Systems. After graduating, she worked a handful of seasonal positions, working as a Kettle Pond Water Quality Monitor in Cape Cod, Massachusetts, a Desert Tortoise Ecology Intern in Henderson, Nevada, and two seasons as a Wildlife Technician in Knoxville, Tennessee studying the effects of prescribed fire on Eastern Box Turtles. Through these positions Amy found her love for working with wildlife, in particular with turtles and tortoises. In 2018, she decided to continue her education entering into graduate school at the University of Texas Rio Grande Valley in Brownsville, Texas to earn a Master of Science degree in Agricultural, Environmental, and Sustainability Sciences, which she completed in May 2020. Her passion for turtles has also led her to become a regular volunteer at Sea Turtle Inc. Amy hopes to enter into the workforce fulltime as a wildlife biologist and hopes to find more opportunities to pursue her passion for turtles. Amy can be reached at amybogolin@gmail.com.