Preliminary Development of Aquatic Turtle Aerial and eDNA Survey Methods in the Sabine River Basin

Final Report

EIH Final Report #EIH21-003
Draft submitted: January 07, 2022
Final acceptance: January 11, 2022

Prepared by the Environmental Institute of Houston at the University of Houston – Clear Lake in cooperation with the Sabine River Authority

Prepared by

Mandi Gordon[*], Senior Biologist, Principal Investigator
Jason Nagro, Graduate Research Assistant

Environmental Institute of Houston
University of Houston-Clear Lake
2700 Bay Area Blvd.
Houston, TX 77058
[*]correspondence to gordon@uhcl.edu; 281-283-3794

Additional Project Personnel

Dr. Marc Mokrech, GIS Specialist
Dr. Jenny Oakley, Associate Director-Research
Dr. George Guillen, Director

Prepared in cooperation with and for the Sabine River Authority of Texas

Bill Kirby, Natural Resources Manager
12777 North Hwy. 87
Orange, Texas, 77630

Recommended citation: Gordon, M.L., J. Nagro, M. Mokrech, J. Oakley, and G. Guillen. 2021. Preliminary development of aquatic turtle aerial and eDNA survey methods in the Sabine River Basin. Final Report (Report No. EIH21-003). Prepared for the Sabine River Authority (Contract No. 2021-001). 29 pages.

Table of Contents

List of Figures

List of Tables

ACKNOWLEDGEMENTS

We would like to thank our project manager, Bill Kirby, for his feedback on the project design and implementation. He was also integral in establishing contact with private landowners throughout the Sabine River basin during site selection. Additionally, we thank Bills Landing RV Park, Cedar Cove RV Park, Mr. Fleetwood, B. Glenn, B. Hardisom, S. Tatum, and D. Williams for allowing access to their properties and assistance with field logistics.

Internal support for the project was provided by numerous on campus departments including the Office of Sponsored Programs (esp. N. Devino and T. Rahman), Travel Office, Accounts Payable (esp. R. Pineda), the Office of Information and Technology, University Facilities Management, Physical Plant, and Marketing and Communications. Our Business Administrator, Patti Koch, worked tirelessly through restrictions, force majeure, and a global pandemic to ensure that supplies were procured, personnel were paid, and coordinated many tasks with internal and external departments.

All sampling was conducted under TPWD Scientific Collection Permit SPR-0504-383, TPWD Aerial Wildlife Monitoring Permit M-2885, and UHCL Institutional Animal Care and Use Committee Protocol 0320.001.R0. sUAS flights were conducted by FAA Pilot #4465149. Funding was provided by the Sabine River Authority under contract 2021-001.

EXECUTIVE SUMMARY

The southeastern United States represents one of the most ecologically diverse habitats in the world and in recent decades has exhibited overall declines in amphibian and reptile populations. The Sabine River Basin is home to numerous aquatic turtle species, including some under review for listing as an endangered species under the Endangered Species Act (ESA), specifically, the western chicken turtle (*Deirochelys reticularia miaria*; WCT). The Environmental Institute of Houston at the University of Houston–Clear Lake (EIH-UHCL) is currently conducting surveys for the WCT and is testing the use of environmental DNA (eDNA), small unmanned aerial system (sUAS), and binocular assisted visual survey (BAVS) techniques to detect WCT throughout their historic range. During the summer of 2020, EIH-UHCL was approached by the Sabine River Authority (SRA) to gather preliminary data for aquatic turtle species residing within the basin, with emphasis on the WCT. Objectives for this baseline assessment include: 1) assessing turtle species composition at selected sites using visual survey methods, 2) evaluating the viability of aerial imagery from sUAS platforms as a method for documenting species presence, and 3) determining the presence or absence of WCT at select sites via eDNA sampling.

To achieve an even distribution of survey areas, the basin was subdivided into three regions: Region 1 (Lake Tawakoni and Lake Fork), Region 2 (Toledo Bend Reservoir) and Region 3 (the Orange Canals). To maximize detection of aquatic turtles, sites were established in habitats with previously documented turtle activity, slow or non-flowing water, and large areas with open canopy. General site characteristics, water quality variables, environmental conditions, and riparian cover were documented during each sampling event. At all sites, BAVS were performed concurrently with sUAS surveys. High-resolution aerial visible and thermal spectrum video imagery were captured using a DJI Mavic 2 Enterprise Dual platform. To determine the presence of WCT, eDNA samples were collected via two water matrix types: ambient and resuspended.

From March through July 2021, 16 sampling events were conducted. No WCT were detected via eDNA samples. Monthly precipitation rates during the study were greater than normal with highest departures from normal occurring in April, May, and July, which may explain the lack of positive detections from eDNA samples. Overall, 1,585 individuals were observed via BAVS and visible spectrum sUAS video imagery. Slider turtles (*Trachemys* sp.) were most commonly observed, suggesting that individuals from this genus may be most prevalent within these areas of the Sabine River basin. Overall, sUAS allowed for more observations across all taxonomic levels, suggesting that this method allows for better enumeration and identification of turtle species than traditional linear visual surveys. During sUAS surveys, 128 individuals exhibited a reaction to the platform. Turtles were observed basking or swimming prior to reacting and, though turtles had similar response rates to sUAS flights, basking turtles were more likely to react. Turtles were most frequently observed reacting after the platform had passed. This low number of observed reactions and delayed reaction period suggests that turtles may not be as reactive to sUAS platforms as originally thought. No species were identifiable to taxonomic level using thermal spectrum sUAS imagery, though preliminary review of this imagery suggests that this spectrum may be helpful in discerning behavioral and/or habitat associations.

Data collected via this study directly supplements the ongoing aquatic turtle surveys that EIH-UHCL is conducting for the Texas Comptroller of Public Accounts, and provides baseline data necessary for overall basin-wide turtle surveys in future years. Here, we describe the results of this preliminary assessment and make recommendations for future assessments of aquatic turtles in the Sabine River basin.

INTRODUCTION AND BACKGROUND

The southeastern United States represents one of the most ecologically diverse habitats in the world (Stein 2002). In recent decades, this region has exhibited declines in aquatic amphibian and reptile populations (Gibbons et al. 2000; Ceballos and Fitzgerald 2004; Prestridge et al. 2011). Riverine, lacustrine, and wetland alteration has led to fragmentation of formerly expansive habitats while urban and agricultural sprawl have entirely removed wetland or aquatic habitat in many areas (Semlitsch and Bodie 2003; Quesnelle et al. 2015). This may be particularly detrimental to certain turtle populations due to their dependency on, and movement between, these habitat types (Ryberg et al, 2017; Chyn et al. 2020).

The Sabine River basin is home to numerous aquatic turtle species, including some currently under review for listing under the Endangered Species Act (ESA) (Table 1). Specifically, a petition to list the Western Chicken Turtle (WCT; *Deirochelys reticularia miaria*) under the ESA has been submitted to the U.S. Fish and Wildlife Service with significant 90-Day Findings (Greenwald et al. 2010; USFWS 2011). As of this report, the WCT is an endangered species in Missouri and is a species of greatest conservation need in Louisiana and Oklahoma, but holds no legal protection status in Texas (Buhlmann et al. 2008; Holcomb et al. 2015; ODWC 2016). The Environmental Institute of Houston at the University of Houston–Clear Lake (EIH-UHCL) is currently conducting surveys for the WCT funded by Texas Comptroller of Public Accounts (herein referred to as "Comptroller"; Contract No. 20-6997BG). This ongoing assessment is testing use of environmental DNA (eDNA), small unmanned aerial system (sUAS), and binocular assisted visual survey (BAVS) methods as part of a suite of novel and traditional methods to detect WCT throughout their historic range.

Table 1 Full list of taxonomic and common names for aquatic turtle species anticipated in Sabine River basin (from Dixon 2013; Hibbitts and Hibbitts 2016; Texas Turtles 2021; names verified using Bonett et al. 2017).

Taxonomic Name	Common Name
Chelydra serpentina	Snapping Turtle
Macrochelys temminckii†	Alligator Snapping Turtle
Chrysemys dorsalis	Southern Painted Turtle
Deirochelys reticularia miaria†	Western Chicken Turtle
Graptemys pseudogeographica kohnii	Mississippi Map Turtle
Graptemys sabinensis	Sabine Map Turtle
*Malaclemys terrapin littoralis**	Texas Diamond-backed Terrapin
Pseudemys concinna concinna	Eastern River Cooter
*Terrapene ornata**	Ornate Box Turtle^
*Terrapene carolina triunguis**	Three-toed Box Turtle^
Trachemys scripta elegans	Red-eared Slider
Kinosternon flavescens	Yellow Mud Turtle
Kinosternon subrubrum hippocrepis	Mississippi Mud Turtle
Sternotherus carinatus	Razor-backed Musk Turtle
Sternotherus odoratus	Eastern Musk Turtle
Apalone mutica	Smooth Softshell
Apalone spinifera pallida	Pallid Spiny Softshell

†Currently under consideration for inclusion under the Endangered Species Act
*Present within the Sabine River basin, but not anticipated in this study due to different habitat preferences
^Phylogeny currently under review; terminology may not reflect current nomenclature (Martin et al. 2013).

OBJECTIVES AND CONSERVATION BENEFITS

During the summer of 2020, EIH-UHCL was approached by the Sabine River Authority (SRA) of Texas to develop a plan to gather baseline data for aquatic turtle species residing within the Sabine River basin, with emphasis on the WCT. Data collected via this work with the SRA will directly supplement the ongoing work that EIH-UHCL is conducting related to the WCT, and will provide baseline data necessary for basin-wide turtle surveys in future years. The proposed research aims to address the following objectives:

1. Assess turtle species composition at select sites using visual survey methods (BAVS)
2. Evaluate the viability of aerial imagery from small unmanned aerial systems platforms (sUAS) as a method for documenting aquatic turtle species presence
3. Determine presence or absence of WCT at select sites using environmental DNA (eDNA)

By combining these methods, EIH-UHCL will be able to assess baseline aquatic turtle species composition within the Sabine River basin. This study provides data for future conservation and restoration efforts as well as insight for more robust future assessments.

METHODS

Site Selection

In order to generate an even distribution of survey locations, the basin was subdivided into three regions: Region 1 (upper basin), Region 2 (middle basin), and Region 3 (lower basin) (Figure 1). Two locations were selected in Region 1, each associated with the major reservoirs (Lake Fork and Lake Tawakoni), and one site each in Regions 2 (Toledo Bend reservoir) and 3 (the SRA Orange canal system) (Table 2). Sites were established in habitats with previous records of turtle activity, containing slow-moving or non-flowing water, and large areas with open canopy to maximize the efficiency of the proposed sampling methods.

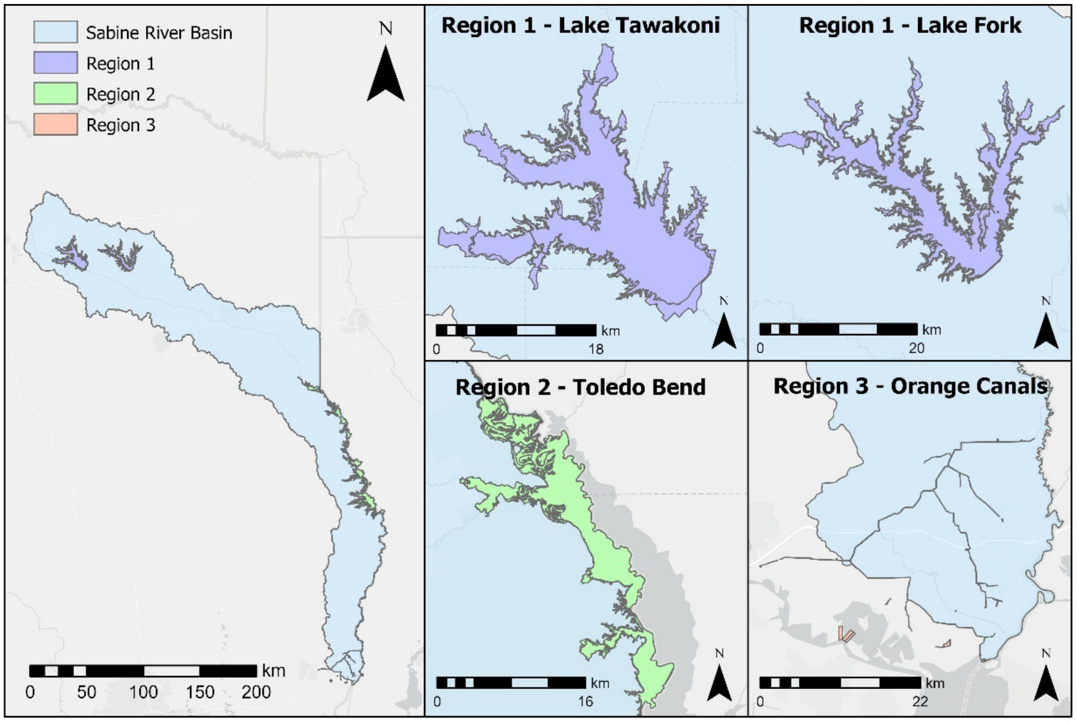


Figure 1 Distribution of Sabine River basin regions used for site selection.

Table 2 General site information for preliminary aquatic turtle surveys in the Sabine River basin. Wetted Survey Area calculated as total surface area of wetted area surveyed during binocular assisted visual surveys (BAVS) and small unmanned aerial surveys (sUAS).

Site ID	**Latitude**	**Longitude**	**County**	**Overall Habitat Description**	**Wetted Survey Area** (ha)
SRA01	32.88670	-95.91906	Rains	Lake	1.596
SRA02	32.94013	-95.48139	Anderson	Lake	6.135
SRA03	31.82184	-93.90734	Shelby	Emergent Wetland	4.848
SRA04	30.05751	-93.87947	Orange	Emergent Wetland	1.404

General Site, Water Quality, and Habitat Data Collection

General site characteristics including GPS coordinates, sample date, waterbody name, visit number, county, and overall habitat type were recorded during each sampling event. Site coordinates (NAD83) were recorded at the water's edge using a handheld GPS unit. All habitat and water quality data collection were associated with this point (herein referred to as the "assessment point"; Figure 2).

Water quality variables were recorded using a multiparameter sonde suspended at half the total depth (m) and included: collection time, measurement depth (m), temperature (°C), specific conductance (µS/cm), dissolved oxygen (percent and mg/L), and pH (standard units). Water transparency (or "water clarity") was measured using a 1.2 m Secchi tube. Turbidity (NTU) was recorded via a 125 mL grab sample analyzed with a portable LaMotte™ 2020we Turbidimeter. Water quality, clarity, and turbidity were taken adjacent to the assessment point.

Environmental conditions, site photographs, and riparian canopy cover were documented from the assessment point. Condition data were recorded following methods outlined in the Texas Commission on Environmental Quality's (TCEQ) Surface Water Quality Monitoring manual (TCEQ 2012) and included current weather, percent cloud cover, water surface state, water odor, wind intensity, water color, and days since last significant rainfall. Days since last "significant" rainfall were calculated based on daily accumulated precipitation rates recorded by weather stations closest to the sites (http://www.wunderground.com). "Significance" levels varied by site, but were generally set to > 0.10" of total accumulation for the day. Due to increased precipitation during the 2021 sampling season, monthly precipitation rates presented as a departure from normal levels were extracted from the National Oceanographic and Atmospheric Administration's (NOAA) Advanced Hydrologic Prediction Service (AHPS) [accessed 22 December 2021]. Normal levels are derived from the Parameter-elevation Regressions on Independent Slopes Model (PRISM) climate mapping system and are based on a 30-year dataset (1981-2010) (https://water.weather.gov/precip/).

Riparian cover was visually divided into three layers (> 5 m, 0.5-5 m, and < 0.5 m vegetation height). Dominant vegetation type, percent cover of dominant vegetation, and percent cover of all vegetation were recorded as two-dimensional aerial coverage for each layer (visualization plot extending 5 m in each direction from the assessment point; Figure 2). Overall percent canopy cover was calculated using a spherical crown convex densiometer (Mills and Stevenson 1999).

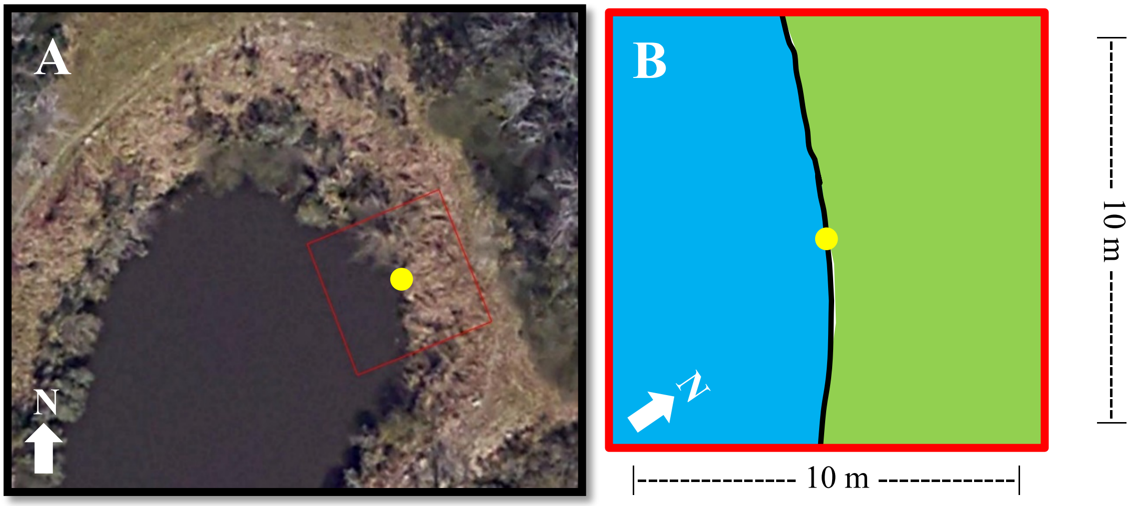


Figure 2 Example of 10 x 10 m site and habitat assessment plot. *Left image*: GoogleEarth aerial imagery with plot (red square) around assessment point (yellow circle). *Right image*: example of site assessment plot with approximately 50% water coverage (blue) and 50% vegetation (green).

Binocular Assisted Visual Surveys (BAVS)

At all sites, binocular assisted visual surveys (BAVS) were performed concurrently with sUAS surveys. Field personnel established a stationary location along the boundary of a waterbody and conducted surveys by scanning along a 180° plane extending from one bank to the other (facing the water) using binoculars. For each observation of aquatic fauna, time, distance (m) from the survey point using a range finder, bearing (°) from the survey point, species (recorded to lowest taxonomic level), number of individuals observed, and behavior or activity of the individual(s) were recorded. During sUAS surveys, behavioral response to sUAS unit was recorded.

Small Unmanned Aerial System (sUAS) Surveys

High-resolution aerial visible and thermal spectrum video imagery were captured using a DJI Mavic 2 Enterprise Dual (Figure 3). Flights were performed by a Part 107 certified remote Pilot in Command (PIC) following Federal Aviation Administration (FAA) regulations and conducted under permit with landowner permission.

Figure 3 DJI Mavic 2 Enterprise Dual small unmanned aerial system (sUAS) platform used to conduct aquatic turtle surveys in the Sabine River Basin.

Prior to field flights, planned transect flight paths were generated via the DJI Pilot application (iOS v1.1.5). Flights were conducted during daylight hours and we attempted to conduct flights at times when the sun not at an extreme angle in order to avoid impacts of glare to the sUAS imagery. Flights were canceled, suspended, or rescheduled during times of heavy rain, high winds (> 15 mph), and/or high heat (> 100°F) in order to avoid risk of damaging the platform. Automated flight paths (not controlled by the pilot, but monitored remotely) were determined based on current environmental conditions and in a manner that allowed the pilot to maintain line of sight of the sUAS at all times. Manual flights (controlled by the pilot at all times) were conducted at sites where a plot transect was inefficient. Additionally, areas with high turtle activity or ideal habitat (e.g. multiple basking locations or shallow water) were targeted by the PIC for observations. Flights were performed at 1 m/s at a target altitude of 5 m with a -90° gimble (camera angle). All flights started and ended from the safe launch zone and mission lengths were dependent upon surface area of the survey zone and battery life (~20 minutes).

Visible and thermal spectrum videos were analyzed using the VLC Media Player, a cross-platform multimedia player developed by the VideoLAN non-profit organization (https://www.videolan.org/). This free to download, open-source software allows the data analyst to zoom in, slow down play back speed, and extract snippets or clips of video imagery. Data recorded for each observation were similar to that for BAVS and included: time stamp, location in image, species (recorded to lowest taxonomic level), number of individuals observed, and behavior or activity. If a turtle reacted to the sUAS unit, the level of reaction was scored on a scale between 0-7 with 0 being least reactive and 7 being most reactive (Table 3). If a reaction was indeterminable from video analysis, a score of "Unk" (unknown) was recorded.

Table 3 Reactivity score, descriptions, and examples of reaction types observed during small unmanned aerial systems (sUAS) video analysis.

Reaction Score	Reaction Type	Examples
0	No reaction	No reaction
1	Reacted but did not submerge	Followed with head, slight movement
2	Submerged but did not retreat	Submerged but stayed at surface or resurfaced
3	Submerged after sUAS passed	Went under after drone passed
4	Submerged after sUAS approached	Went under after sUAS had been over, went under while sUAS was zooming in or out
5	Slow submergence and retreat	Went under slowly
6	Submerged and retreated; swam away	Submergence was not drastic; swam away, to cover, or out of view
7	Quickly retreated	Went under quickly, made a large splash during submergence
Unk	Unknown	Unknown reaction, submerged before entering frame, ripples in edges of frame

Environmental DNA Surveys

Environmental DNA (eDNA) surveys targeted at detecting WCT were conducted during all site visits. Specific details of eDNA sampling methods are provided in the interim reports for the WCT study funded by the Texas Comptroller of Public Accounts (Gordon et al. 2020; Gordon et al. 2021). In summary, two water-matrix samples were collected (ambient and re-suspended sediment) at four equidistant (10m) locations in 20-40 cm water along the waterline and composited prior to filtering. For each sample, water depth (cm) and type were recorded. Between sample sites, gear (including waders and personal equipment) were decontaminated using a 10% bleach solution and allowed to dry to avoid cross contamination between locations. Additionally, samples were stored on ice in separate coolers prior to filtering to minimize possibility of cross contamination via ice-melt water. All sample bottles were soaked in 50% bleach solution and allowed to completely dry before reuse in the field.

Water-matrix samples were filtered within 72 hours of collection. Filtering was performed in a dedicated lab space at UHCL. At the beginning of each filtering day, blank samples were collected using DI water and a sterile, pre-loaded 0.45 µm cellulose nitrate (CN) filter. Water-matrix samples were filtered using a 3.0 µm CN filter. Glass filter apparatus' and equipment reused between samples were soaked in 50% bleach, rinsed, and allowed to dry. Filters were placed in individual Whirl-Paks pre-loaded with desiccant beads and stored at 4°C until they were shipped to Tangled Bank Conservation (TBC; Asheville, NC) for polymerase chain reaction (PCR) analysis using WCT primers (Siler et al. 2020). After PCR analysis, samples with a minimum of two replicate amplifications were deemed "positive" indicators of WCT presence, while single amplifications were labeled as "potential" indicators of WCT presence.

Statistical Analyses

Data were compiled in Microsoft Excel and Google Sheets. Statistical analyses were performed using SigmaPlot v14.5 (Systat Software Inc.) with significance values set at $p = 0.05$. Values are presented as average ± 1 standard error (SE) with range in parentheses. Data were checked for normality and equal variance. For parametric data, significant groupings for One-Way and Two-Way ANOVA's (F-score) were tested via the Holm-Sidak method (Holm 1979). For non-parametric data (non-normal or equal variance), significant groupings for Kruskal-Wallis One-Way ANOVA on Ranks (H) were tested via Dunn's Method (Dunn 1964). All regressions were performed as 2nd Order Polynomial Regressions (R^2).

RESULTS

From March through July 2021, 16 sampling events were conducted across 4 sites (Figure 4). Across all sites, ambient and resuspended eDNA samples were collected at an average depth of 0.435 m ± 0.0362 (0.23 – 0.71 m) ($N = 32$). No WCT were detected via eDNA samples. Concurrent BAVS and sUAS flights averaged 71.6 min ± 6.44 (16 – 126 min) and 20.88 min ± 3.291 (2.5 – 45.5 min), respectively (Table 4).

Monthly precipitation rates during the study were higher than normal with the highest departures from normal (> 8" of accumulated monthly precipitation) occurring in April, May, and July (Figure 5). For all events, average percent cloud cover was 48.6% ± 0.85, though over half of all surveys were conducted under 0% ($n = 4$) or > 95% cloud cover ($n = 6$). Average days since last "significant" rainfall (e.g. > 0.1") was 3.0 days ± 0.70 (< 1 – 11 days), though 4 surveys were conducted on days when significant rainfall occurred. Average significant rainfall amounts (based on daily accumulation) were 0.726" ± 0.1752 (0.21 – 2.42"). During all sampling events, water surface state classification ranged from 1 (glass-like, $n = 10$) to 2 (small ripples, $n = 6$).

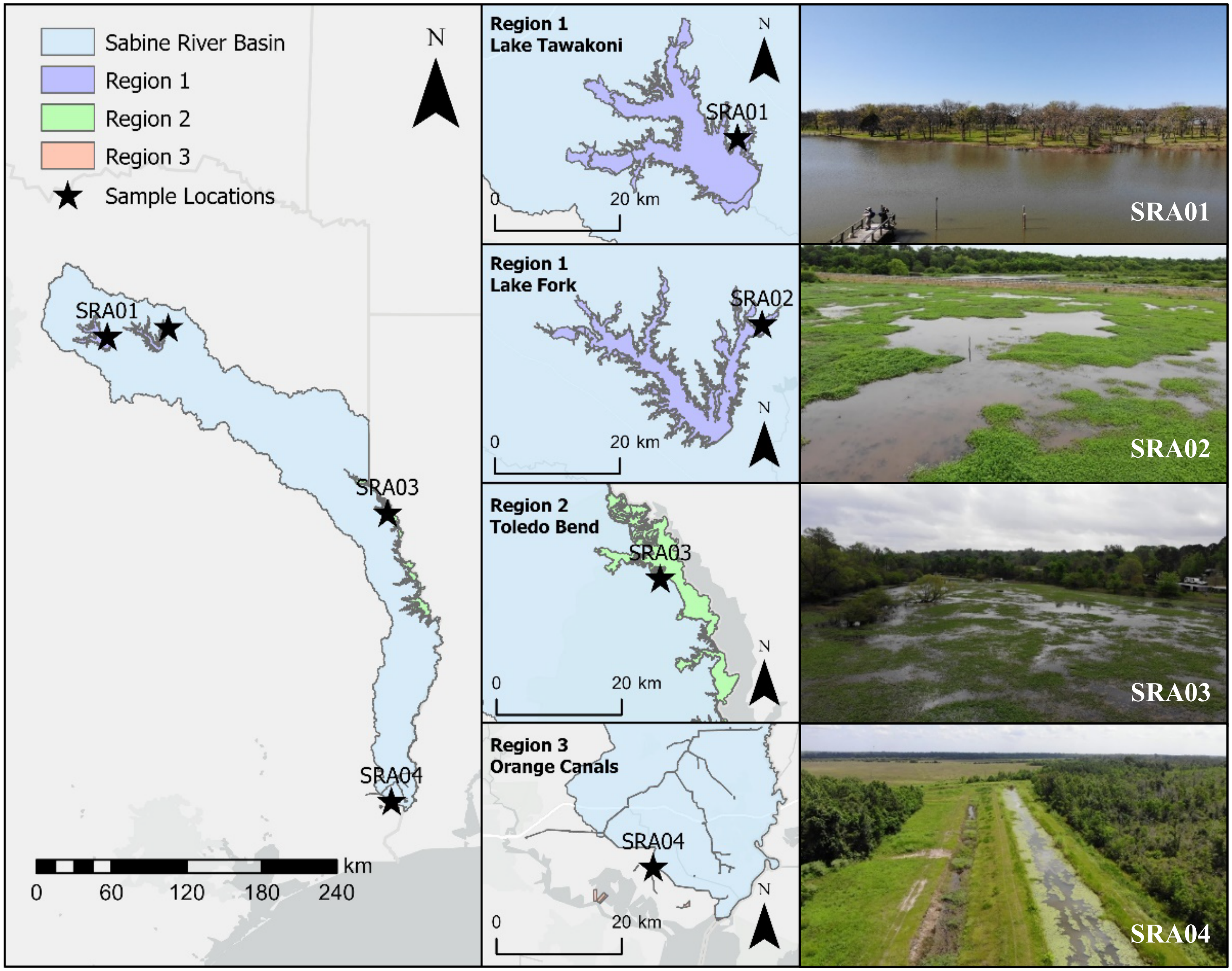


Figure 4 Survey locations within the Sabine River Basin. Sites indicated by stars; static field images on right taken using DJI Mavic 2 Enterprise Dual platform.

Table 4 Survey effort for concurrent small unmanned aerial systems (sUAS) flights and binocular assisted visual surveys (BAVS). Total duration for sUAS video footage calculated based on length of each visible and thermal spectrum file (duration same for both spectrums). Cumulative duration of BAVS surveys presented with number of surveyors noted in parentheses.

Site ID	**Date**	**Visible Spectrum Video Length** (min)	**Thermal Spectrum Video Length** (min)	**Cumulative Duration of Video Footage**	**Cumulative BAVS Duration** (min)
SRA01	03/24/2021	13.8	13.8	27.5	48 (2)
	05/05/2021	15.2	15.2	30.5	54 (2)
	06/08/2021	14.3	14.3	28.6	78 (3)
	07/13/2021	18.6	18.6	37.3	104 (2)
SRA02	03/23/2021	45.5	45.5	91.0	126 (2)
	05/05/2021*	2.5	2.5	5.1	60 (2)
	06/08/2021	34.6	34.6	69.3	75 (2)
	07/13/2021	39.8	39.8	79.6	73 (2)
SRA03	04/07/2021	20.0	20.0	39.9	82 (2)
	05/18/2021*	6.4	6.4	12.7	16 (2)
	06/24/2021	25.9	25.9	51.8	70 (2)
	07/27/2021	32.7	32.7	65.4	76 (2)
SRA04	04/05/2021	32.0	32.0	64.0	100 (2)
	05/17/2021**	0.0	0.0	0.0	43 (2)
	06/24/2021	15.8	15.8	31.6	74 (2)
	07/26/2021	16.9	16.9	33.8	66 (2)
	Total	334		668	1,145
	Average ± 1SE	20.88 ± 3.291		789.8 ± 273.84	71.6 ± 6.44

*sUAS flight duration shortened due to inclement weather and field conditions

**sUAS flight cancelled due to inclement weather

Air and water temperatures increased (air: $R^2 = 0.7390$, water: $R^2 = 0.8517$, $p < 0.001$) while dissolved oxygen (D.O.) levels decreased (D.O. mg/L: $R^2 = 0.5364$, $p = 0.007$; D.O. percent: $R^2 = 0.4209$, $p = 0.029$) during the course of the study (Figure 6). While water clarity and turbidity did not differ significantly between sampling events, turbidity was greater at SRA04 than at SRA02 and SRA03 (One-way ANOVA, $F_{3,12} = 6.622$, $p = 0.007$) (Figure 6). Median specific conductance was lower at SRA04 (Kruskal-Wallis One-Way ANOVA on ranks, H = 8.493, $p = 0.037$) (Figure 6) while pH was higher at SRA01 than SRA02 and SRA03 (One-Way ANOVA, $F_{3,12} = 5.640$, $p = 0.012$) (Figure 6). Total depth at SRA04 was greater than at all other sites (One-Way ANOVA, $F_{3,12} = 9.382$, $p = 0.002$) (Figure 6).

Average densiometer-derived percent canopy cover was 32.1% ± 7.38 (0 – 86.8%) while average estimated cover of the upper (> 5 m), middle (0.5 – 5m), and lower (< 0.5 m) canopies within the assessment area were 4.7% ± 2.86 (0 – 45%), 46.1% ± 4.99 (20 – 90%), and 72.2% ± 3.45 (45 – 90%), respectively. Cover estimates were greater at the lowest canopy layer (< 0.5 m vegetation height) (Two-Way ANOVA, $F_{2,47} = 108.625$, $p < 0.001$) while average densiometer-derived cover was highest at SRA03 and lowest at SRA02 (One-Way ANOVA, $F_{3,15} = 4.076$, $p = 0.033$) (Figure 7).

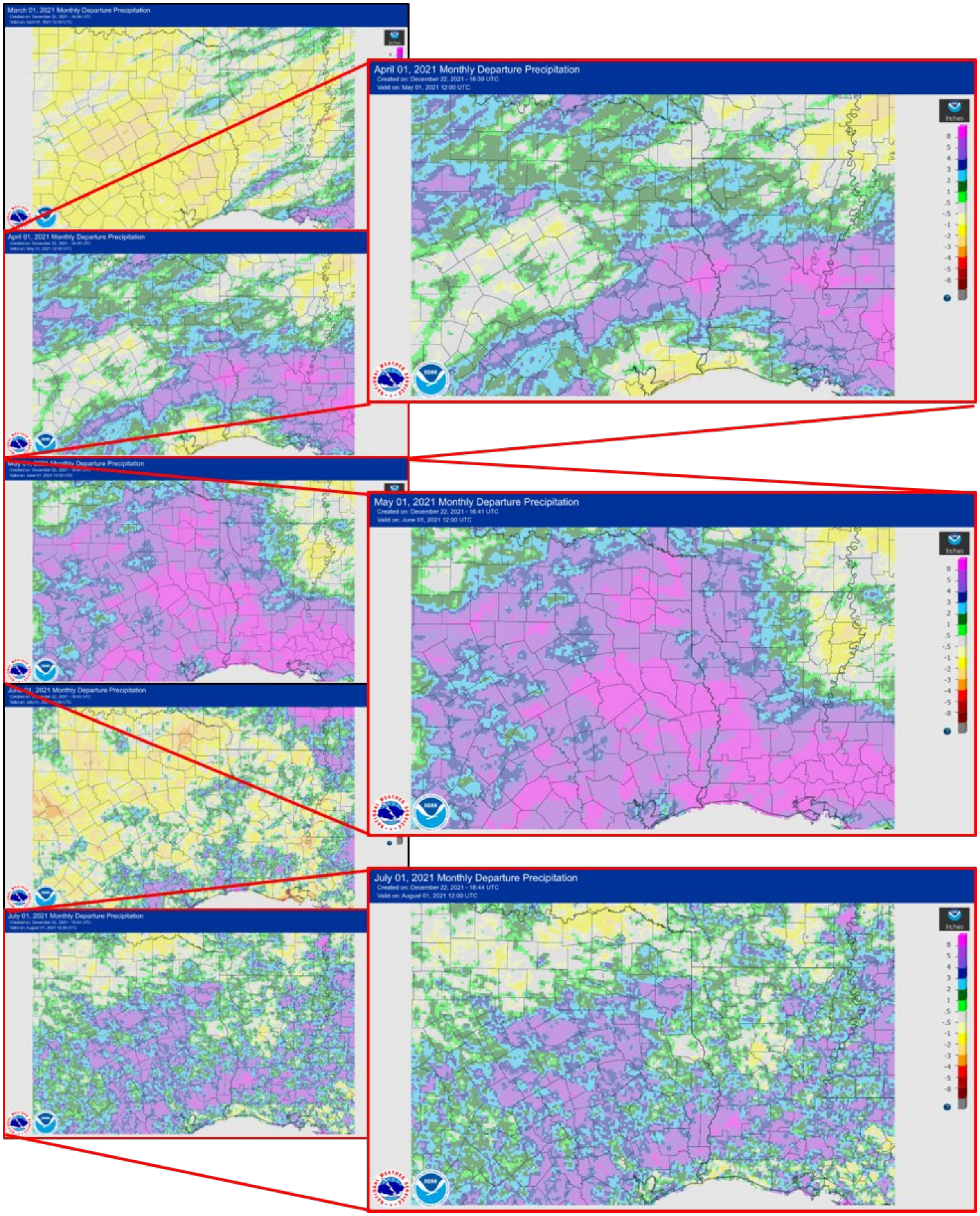


Figure 5 Monthly accumulated precipitation presented as a departure from normal levels from the National Oceanographic and Atmospheric Administration (NOAA) Advanced Hydrologic Prediction Service (AHPS) [accessed 22 December 2021]. Normal levels are derived from the Parameter-elevation Regressions on Independent Slopes Model (PRISM) climate mapping system and are based on a 30-year dataset (1981-2010). Highest departures from normal (> 8” accumulated precipitation) occurred in April, May and July of the current study.

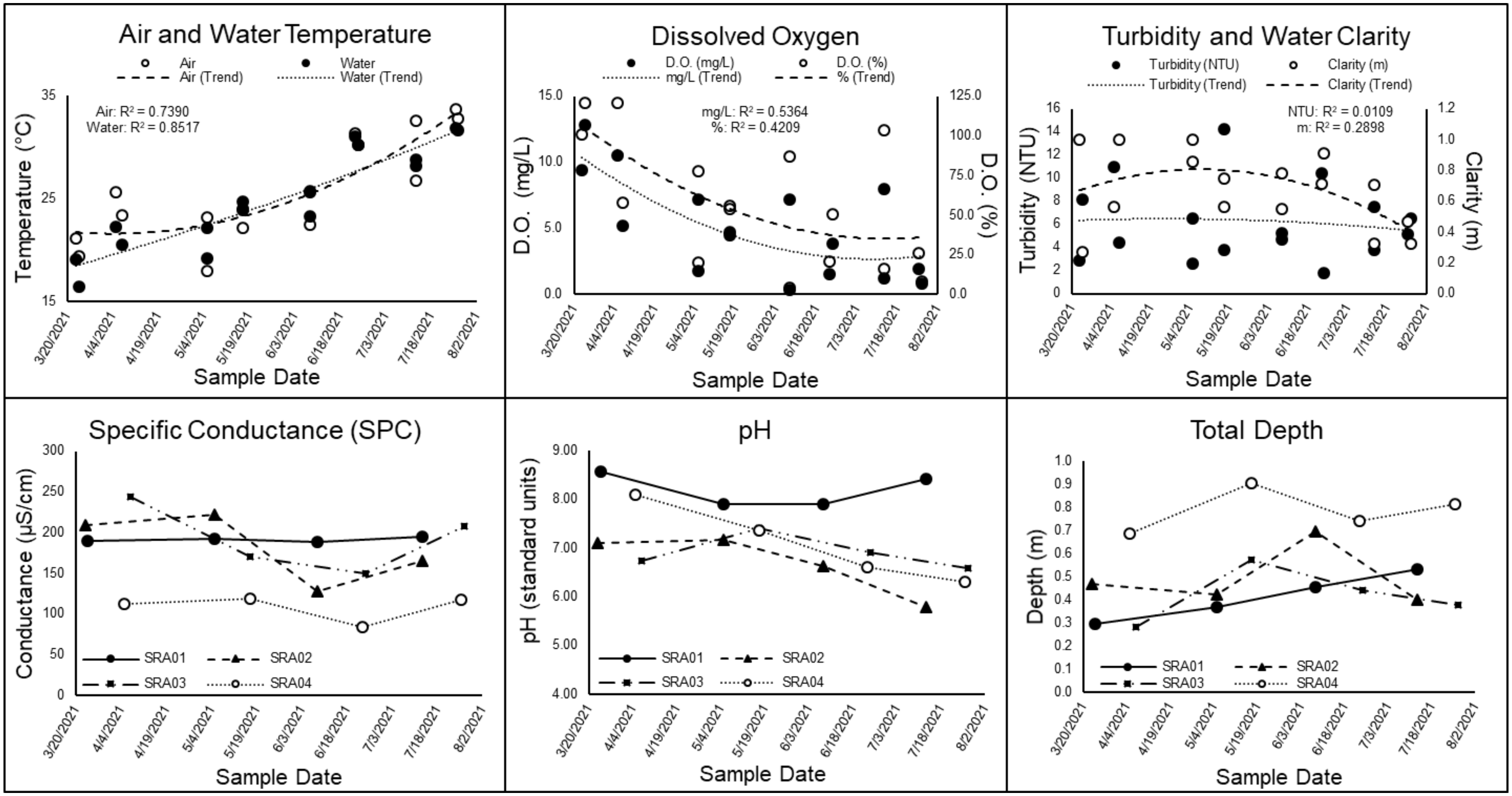


Figure 6 Water quality, turbidity, clarity, and depth. Air and water temperatures increased (air: $R^2 = 0.7390$, water: $R^2 = 0.8517$, $p < 0.001$) while dissolved oxygen (D.O.) levels decreased (D.O. mg/L: $R^2 = 0.5364$, $p = 0.007$; D.O. percent: $R^2 = 0.4209$, $p = 0.029$) following a seasonal pattern during the course of the study. Turbidity was greater at SRA04 than at SRA02 and SRA03 (One-way ANOVA, $F_{3,12} = 6.622$, $p = 0.007$). Specific conductance was lower at SRA04 (Kruskal-Wallis One-way ANOVA on ranks, H = 8.493, $p = 0.037$) while pH was higher at SRA01 than SRA02 and SRA03 (One-way ANOVA, $F_{3,12} = 5.640$, $p = 0.012$). Total depth at SRA04 was greater than at all other sites (One-way ANOVA, $F_{3,12} = 9.382$, $p = 0.002$).

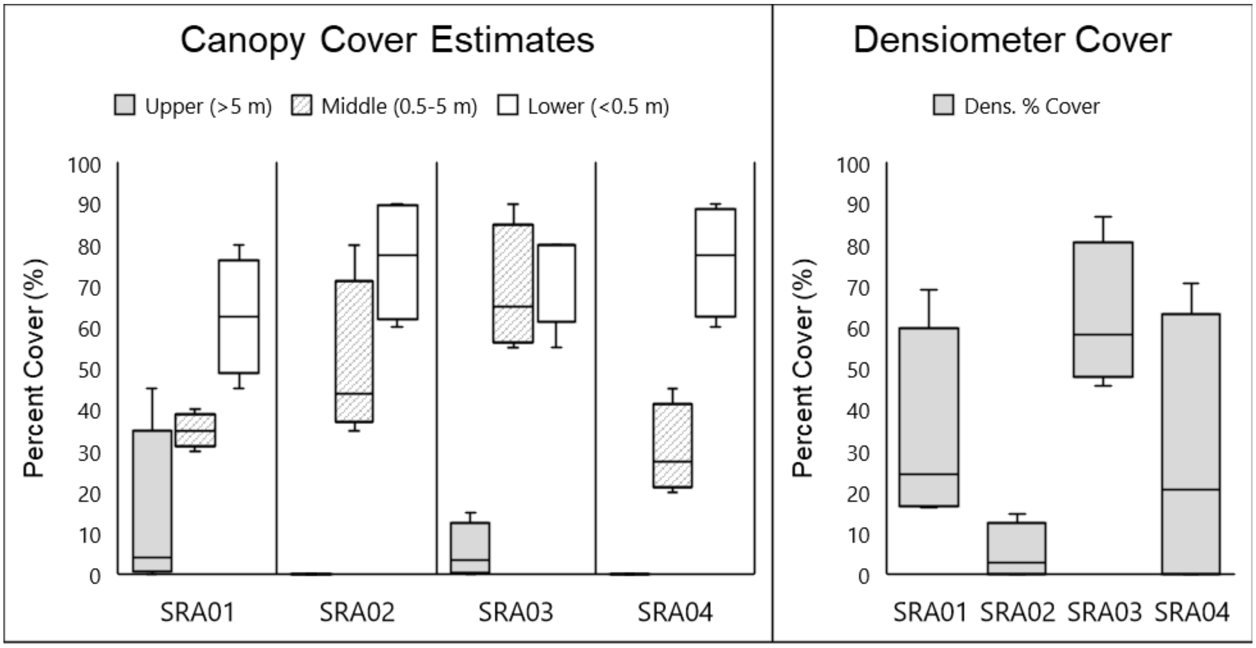


Figure 7 Estimated and densiometer-derived canopy covers. Median cover estimates were lowest in the upper canopy layer (>5 m) and highest in the lower canopy layer (< 0.5 m) at all sites (Two-way ANOVA, $F_{2,47} = 108.625$, $p < 0.001$). Densiometer cover was highest at SRA03 and lowest at SRA02 (One-way ANOVA, $F_{3,15} = 4.076$, $p = 0.033$).

Overall, 1,585 individuals were observed via BAVS and visible spectrum sUAS video imagery (Table 5 and Figure 8). Both BAVS and sUAS were successful in identifying two species: *Trachemys scripta elegans* (red-eared slider) and *Chelydra serpentina* (common snapping turtle). *Pseudemys concinna* (river cooter) were identifiable to species level via BAVS, while *Apalone spinifera* (spiny softshell) were identifiable to species level via sUAS. Slider turtles (*Trachemys* sp.) were most commonly observed via both methods (total observations = 1,064) while sUAS allowed for more overall observations across all taxonomic levels ($n = 1{,}077$). Unknown turtles made up approximately 25% of all observations (relative abundance = 0.2416) (Table 5). Overall proportion of all taxonomic levels observed were highest via sUAS except for *P. concinna* and *T. scripta elegans* (Figure 8).

During sUAS surveys, 128 individuals exhibited a reaction to the sUAS platform. Only *Trachemys* sp., *Apalone* sp., and unknown turtles exhibited a response to the sUAS platform and turtles were observed basking ($n = 63$) or swimming ($n = 65$) prior to reacting (Figure 9). Basking and swimming turtles had similar response rates to sUAS flights (12.3% and 11.5%, respectively), though basking turtles were more likely to react (Two-Way ANOVA, $F_{1,70} = 9.959$, $p = 0.002$) (Figure 9). The highest proportion of turtles observed had no reaction to the sUAS platform (88.1%) (Figure 10). Turtles were most frequently observed reacting after the platform had passed (score #4: $n = 43$), though no significant differences were detected between reaction score or species (Two-Way ANOVA, $p > 0.05$) (Figure 10). No species were identifiable to taxonomic level via thermal spectrum sUAS imagery (Figure 11). Examples of other species observed via sUAS surveys can be found in Appendix A (Appendix Figure A.1).

Table 5 List of species observed for binocular assisted visual surveys (BAVS) and visible spectrum small unmanned aerial systems (sUAS) video imagery. Relative abundance calculated as total number of observations for a species divided by the sum of all species counts. Species listed in order of highest to lowest relative abundance.

Common Name	Lowest Taxonomic Level	BAVS	sUAS	Total	Relative Abundance
Unknown slider	*Trachemys* sp.	256	808	1,064	0.6713
Unknown turtle	Unknown turtle	150	233	383	0.2416
Red-eared slider	*Trachemys scripta elegans*	99	9	108	0.0681
North American softshell turtle	*Apalone* sp.	1	19	20	0.0126
Common snapping turtle	*Chelydra serpentina*	1	2	3	0.0019
Map turtle	*Graptemys* sp.		3	3	0.0019
Spiny softshell	*Apalone spinifera*		2	2	0.0013
Musk turtle	*Kinosternon* sp.		1	1	0.0006
River cooter	*Pseudemys concinna*	1		1	0.0006
	Total (*N*)	508	1,077	1,585	
	# Taxonomic Groups Documented (*s*)	6	8	9	
	# Species Reported to Specific Epithet (S)	3	3	4	

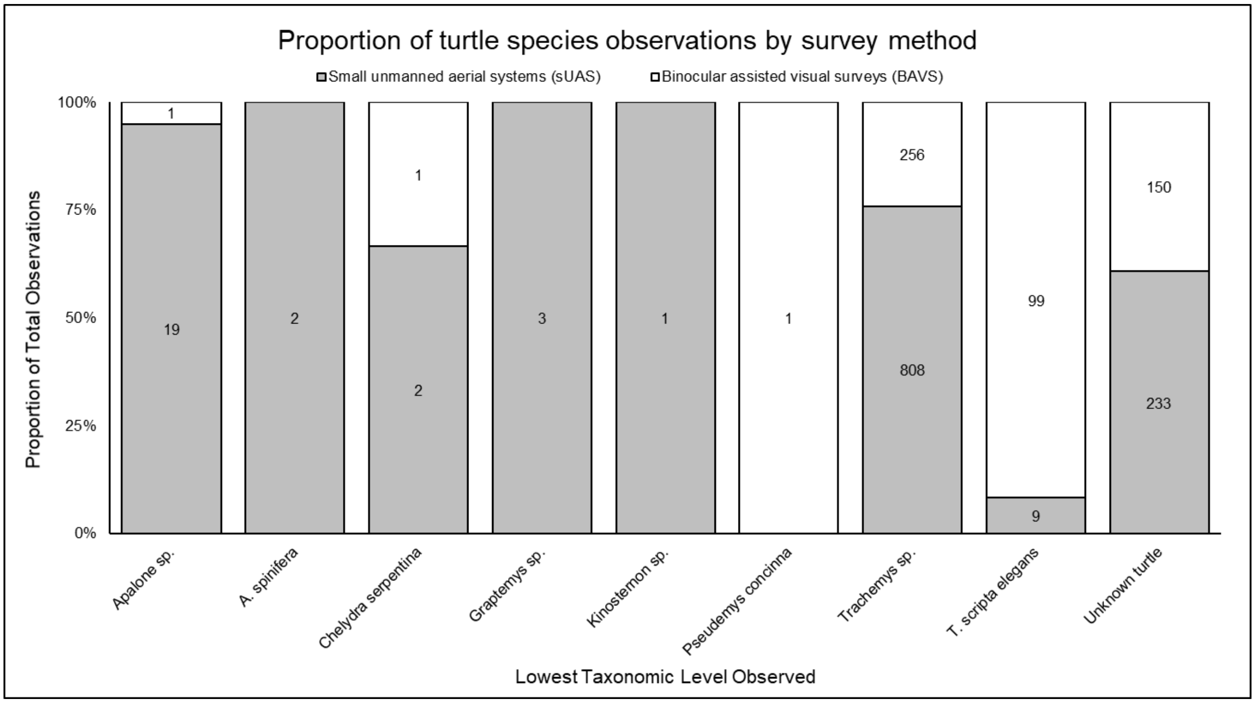


Figure 8 Proportion of turtle species observed for each survey method reported to the lowest taxonomic level observed. Number of observations presented in bar sections.

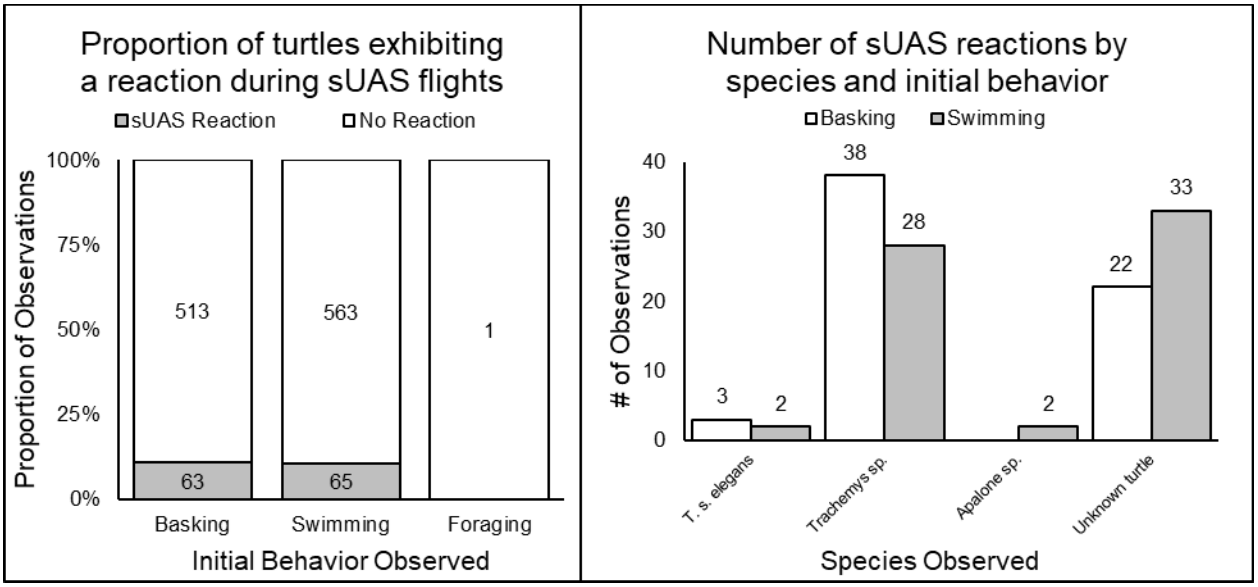
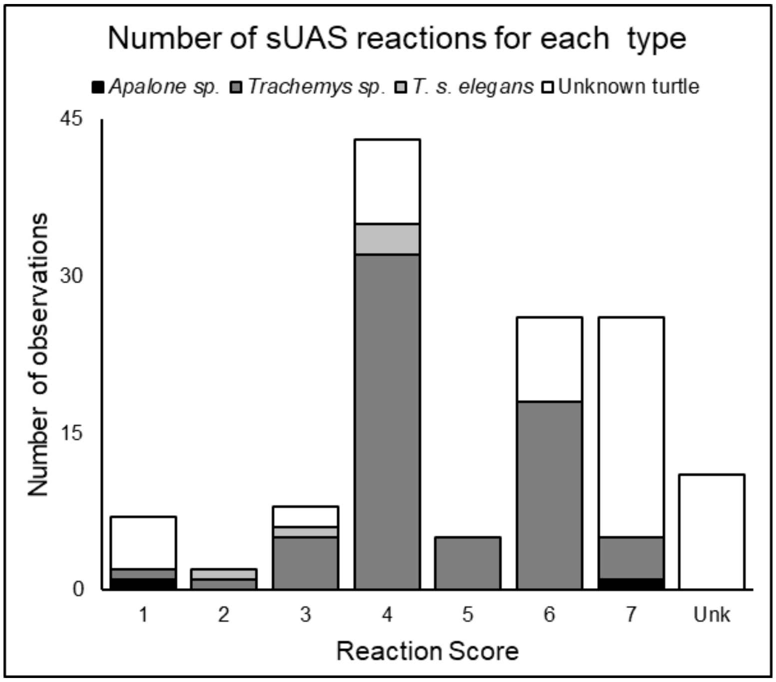


Figure 9 Proportion of aquatic turtles exhibiting a reaction to small unmanned aerial systems (sUAS) flights based on initial behavior observed (left) and number of sUAS reactions for each species by initial behavior observed (right). Number of observations presented within and above bar sections. No significant difference was detected between initial behavior type or species and number of observed reactions (Two-Way ANOVA, $p > 0.05$).



Reaction Score	# of Reactions	Percent of Total (%)
0	949	88.1
1	7	0.6
2	2	0.2
3	8	0.7
4	43	4.0
5	5	0.5
6	26	2.4
7	26	2.4
Unk	11	1.0
Total	1,077	

Figure 10 Count of reaction scores for turtles exhibiting a reaction to small unmanned aerial systems (sUAS) platform (left) and overall proportion of aquatic turtle observations for each reaction category (right). No significant difference was detected between count for each reaction score or count for each species exhibiting reactions (Two-Way ANOVA, $p > 0.05$).

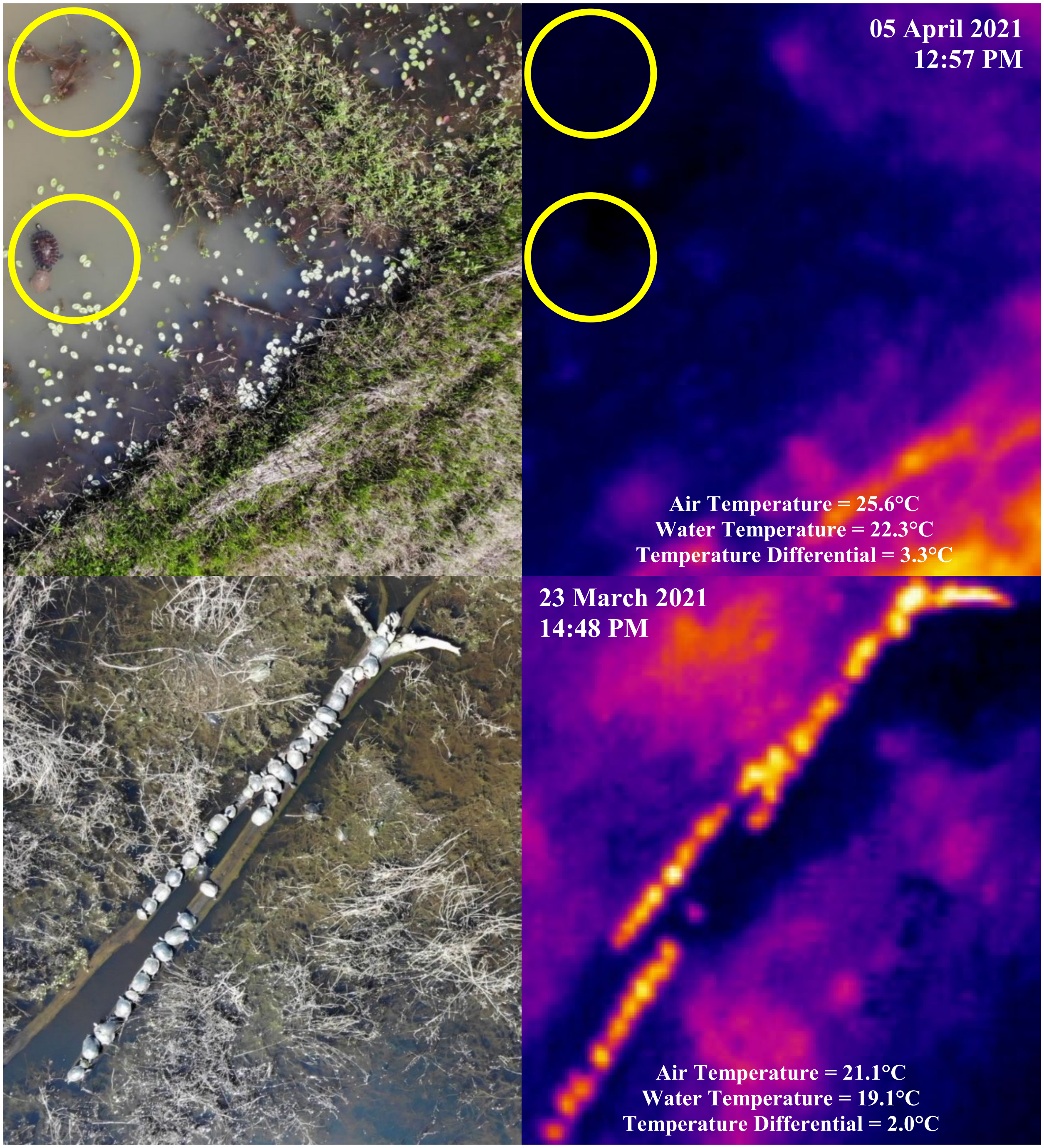


Figure 11 Comparison of simultaneous visible (left) and thermal (right) video imagery collected via the DJI Mavic 2 Enterprise Dual sUAS platform. Top row: swimming sliders (*Trachemys* sp.) present in visible spectrum but not thermal spectrum imagery (yellow circles). Bottom row: turtles basking on fallen log present in both imagery types. Temperature differential calculated as difference between air and water temperatures at time of sUAS survey (°C).

DISCUSSION

Though sites were distributed throughout the Sabine River basin (Figure 4), most water quality variables followed similar trends across all sites (Figure 6). While most variables followed expected seasonal trends (e.g. air and water temperature increased, dissolved oxygen levels declined, and turbidity levels stayed relatively constant at all sites as the season progressed) (Boyd 2019), specific conductance was lowest at the site within the lower reaches of the watershed (SRA04; Figure 6). This site also represents the deepest location sampled during the study, with water depths consistently nearing 1.0 m. Water in this area is directly pulled from the Sabine River and pumped through the sample area so it does not receive runoff or discharge. Variation in water quality may be due to natural settling of compounds, general construction, and day-to-day operation of the canal system especially since this site is located within a man-made environment (B. Kirby, pers. comm.). Alternatively, pH was highest at the site within the upper-most regions of the watershed (SRA01; Lake Tawakoni), though it generally declined over the course of the study as was expected (Figure 6) (Boyd 2019). Most of the survey area experienced above normal rainfall when compared to the 30-year normal (Figure 5). This increase in precipitation may have caused deviations from expected water quality levels. Ultimately, increased rainfall may have affected all survey methods in ways discussed later in this section.

Preliminary eDNA surveys for WCT did not yield positive detections at sites surveyed in this study, though eDNA sampling has been successful in detecting WCT during other studies (Gordon et al. 2020, 2021; Siler et al. 2020). Because the goal of this preliminary assessment was to evaluate eDNA and sUAS sampling methods across the entirety of the basin, we targeted areas where it was most likely to observe all aquatic turtle species potentially present within the Sabine River basin (Table 1), e.g. aquatic habitat along reservoirs with basking areas, open water, and open canopy for sUAS flights. The habitat sampled in this preliminary assessment may not be most representative of that utilized by WCT (Buhlmann et al., 2008; Ryberg et al., 2017), though it does represent ideal habitat for primarily basking and surface swimming aquatic turtle species (Ernst and Lovich 2009). Additionally, 2021 was a record-setting wet year with precipitation levels deviating > 8” from 30-year normal levels for three of the four months surveys were conducted. Increased precipitation rates, and associated water level rises, may impact persistence of genetic material due to dilution, increased levels of inhibiting compounds, alterations to water quality which may affect eDNA residency rates, or make areas where WCT would be more prevalent difficult to access (Barnes and Turner, 2016; Seymour et al., 2018; Stewart, 2019). In recent years, a promising body of research has grown evaluating the use of eDNA for multi-species models, or metabarcoding (McClenaghan et al., 2020; McColl-Gausden et al., 2020; Wang et al., 2021). Though this eDNA metabarcoding and modeling has been applied mostly within the fisheries realm, it’s applicability to aquatic turtles and other long-term aquatic residences should be evaluated in future assessments.

Overall field effort for sUAS was nearly half that of BAVS (Table 4), though number of species reported to the species level was the same between survey methods (Table 5). Each survey method yielded an observation of at least one species not confirmed by the other, though sUAS identified more taxonomic groups overall than BAVS (Table 5). Though only 4 of the 14 expected species were observed between both methods (Table 1 and Table 5), sUAS video imagery documented over twice as many individuals as BAVS alone (Figure 8 and Table 5). Additionally, individuals observed via sUAS exhibited low reaction rates to the platform as it approached, flew over, and flew past them (Figure 9 and Figure 10). These results suggest that sUAS may be a viable method for detection, identification, and enumeration of aquatic turtle species within the Sabine River basin and concur with results from other surveys focusing on assessments of marine turtles and turtle populations outside the U.S. (Bevan et al., 2018; Biserkov and Lukanov 2017, Rees et al. 2018, Schofield et al. 2019). Our results also show that sUAS can survey larger areas in a shorter amount of time with minimal disturbances to an individual's natural behavior(s). It should be noted that not all species were observed with a single method, so continued pairing of concurrent survey methods is recommended until a method is proven to be effective over others or intercalibration for detection efficiency of methods is confirmed.

Previous research with birds and mammals has shown that canopy cover can negatively impact sUAS imagery and that surveys evaluating non-arboreal species should focus on large areas with less heterogenous and decreased canopy cover (Corcoran et al. 2019, Corcoran 2021). For this study, canopy cover estimates were consistent across all sites (Figure 7) and estimated percent cover was lowest in the upper-most canopy layer (> 5 m vegetation height). Densiometer-derived canopy cover values were lowest at SRA02 (Lake Fork) but were consistent across all other sites. All sites provided ample line-of-sight and spatial area for flights to be conducted. Alternatively, canopy within the middle layer (0.5-5 m vegetation height), the most impactful to linear visual surveys such as BAVS, was near 50% cover (Figure 7). During BAVS, a surveyor's visibility can be limited by conditions (glare, shadows, etc.), vegetation or structures, distance to the individual being observed, etc. Surveyors are limited in the number of individuals that can be accurately identified and recorded while juggling equipment during surveys, at times during physically taxing conditions (e.g. extreme heat, excessive perspiration, long hours, eye-fatigue, etc.). Conversely, sUAS footage can be reviewed in a more controlled environment, out of the elements, and with the luxury of being able to stop the footage or image analysis to zoom in or rewind something that may have been missed initially. Our results show that using sUAS to fly over visual impediments allows for documentation of data that might be missed from a ground level perspective (e.g. a turtle basking on the opposite side of an emergent bush or shrub).

A major limitation of aerial surveys is environmental conditions, especially inclement weather. During the course of the project, one sUAS survey had to be cancelled while two other surveys ended prematurely due to inclement weather (Table 4). Even on days when precipitation levels may be limited, variables such as cloud cover, wind speed, and glare can negatively affect a surveyor's ability to observe individuals, either through BAVS or sUAS flights. During the course of this study, accumulated precipitation levels were greater than the 30-year average and average percent cloud cover was near 50% (Figure 5). This increase in accumulated precipitation and subsequent inclement weather conditions may explain some of the variability in our data, especially in cases where individuals could not be identified to a lower taxonomic level. Increased cloud cover, decreased water clarity, and altered water surface state have been shown to be beneficial in reducing an individual's behavioral reaction the sUAS platform (Giles et al., 20219). Since most flights were conducted on days when at least some cloud cover was present, the sUAS platform may have been "masked" by clouds or shadows, limiting the response of individuals as the platform approached. This may be why we observed such low reaction rates during the course of the study, though it should be noted that the unit was also flying at a reduced altitude, so "masking" may not have been possible.

Surveys studying small bodied organisms have shown that individuals smaller than 5-20 cm^2 require lower flight elevations in order to gather data > 1-4 pixels (Tait et al., 2021). This limitation was anticipated at the onset of the assessment and flight elevation was reduced to 5 m in order to mitigate this factor. Additionally, surveys were conducted at the slowest speed allowable by the sUAS unit, which reduced overall survey area due to battery life but increased quality of the imagery captured. Even with these methodology alterations, most individuals were identified as "unknown turtles", though sUAS imagery was generally more useful in identifying a greater proportion and more individuals to genus than BAVS (Figure 8 and Table 5). Additionally, we did not employ a polarizing filter during sUAS surveys, which may have reduced occurrence of reflections and glare (Raoult et al., 2020), because at the time of the study no filters were commercially available for this particular unit. Finally, advances in sUAS technology have allowed for increased potential of data gathering via multispectral imagery, including RGB- and near-infrared. These platforms are costlier and are being utilized in the ongoing Comptroller assessment for a comparison of sUAS efficacy across multiple spectra. Based on our findings from this assessment, we recommend that future surveys using sUAS for evaluation of aquatic turtles continue to refine survey methods (especially in relation to survey speed and platform elevation), evaluate effectiveness of polarizing or other filters as they become available, and investigate application of data from additional spectra.

Though thermal imaging has proven useful in terrestrial and marine surveys of mammals and birds (Chretien et al., 2016; Corcoran et al., 2019; Liu et al., 2015; Oishi et al., 2018; Seymour et al., 2017; Witczuk et al., 2017), to our knowledge, this is the first application of thermal imaging to aquatic freshwater turtle surveys. We were unable to discern species and, in some cases, locations of individuals via thermal imagery, as was anticipated due to the ectothermic nature of aquatic turtles. Thermal imaging failed to detect turtles that were swimming, but, in many cases, individuals were visible to the thermal sensor when dry and out of the water basking (Figure 11). Previous surveys utilizing thermal sUAS imaging suggest conducting surveys at times when contrast between the heat signatures of the target animals and target environment are highest (Corcoran, 2021; Lhoest et al., 2015; Longmore et al., 2017). Because efficacy of thermal sensing is unknown for aquatic turtles, we conducted flights at varying times and in varying conditions in order to evaluate potential optimal sensing conditions. Interestingly, we found that when surveys were conducted earlier in the day, later in the season, and at a higher temperature differential, thermal signatures were not visible for swimming individuals. Conversely, when surveys were conducted later in the day, earlier in the season, and at a lower temperature differential, thermal signatures were observable for basking individuals (Figure 11). The most likely explanation for this difference in thermal detectability is likely due to time spent basking or absorbing solar energy. It could be hypothesized that an individual whom recently spent time basking may be visible on the thermal spectrum for a determinate amount of time while swimming before water temperature reduced the temperature differential between the body and surrounding environment. Conversely, it could also be hypothesized that an individual whom recently climbed onto a structure to bask may not be immediately observable on the thermal spectrum due to the lower differential between body and surrounding environment. Future surveys utilizing thermal imagery for detection of aquatic turtles should continue to evaluate impacts of behavioral and environmental factors to efficacy of thermal imaging. Though we are unable to make a recommendation at this time, we are continuing to evaluate the efficacy of thermal imaging through our Comptroller study and will be able to make future recommendations at the culmination of that project.

Aside from information gained specific to aquatic turtles, our data show that sUAS has the potential to be used for other fauna, habitat, or multi-species population assessments. Our sUAS surveys demonstrate minimal disturbance not only upon the aquatic turtle species that were targeted by the survey, but other wildlife with mammals, birds, and fish exhibiting minimal interest in the platform as flights took place (Appendix Figure A.1). However, there are strict requirements to flying sUAS that need to be considered before missions can be carried out successfully and safely. Proper training, licensing, and understanding of the regulations set forth by the FAA and state (specifically TPWD) need to be assessed before flight. Additionally, there are limitations to using sUAS technology such as battery life and environmental conditions (weather, available air space, etc.). This makes proper pre-flight site assessment key for gathering inclusive datasets. Ultimately, if proper understanding of sUAS technology is utilized by the pilot, efficient datasets can be collected in a way beneficial to future wildlife management and conservation.

CONCLUSIONS

- Accumulated precipitation levels were increased in 2021, which may have caused for deviations from expected water quality levels and affected all survey methods, especially in relation to identifying individuals to the lowest taxonomic level.
- Preliminary eDNA surveys for WCT did not yield positive detections, though eDNA sampling has been successful in detecting WCT for other studies.
- sUAS can survey larger areas in less time versus BAVS, though not all species were observed via sUAS.
- Individuals exhibited low reaction rates to the sUAS platform, though this may be an artifact of environmental conditions “masking” the unit.
- Using sUAS to fly over visual impediments allows for documentation of data that might be missed from a ground level perspective (i.e. via BAVS).

SUGGESTIONS FOR FUTURE SURVEYS

Though this survey has provided baseline data for aquatic turtle surveys in the Sabine River Basin, more information is needed for a complete basin-wide inventory and assessment.

- Future assessments utilizing eDNA sampling techniques specific to the WCT should expand efforts to ephemeral wetlands and areas not necessarily associated with reservoirs for maximum detection potential and may want to evaluate modifications to sampling techniques based on localize precipitation rates.
- For surveys utilizing eDNA, expansion to multi-species or metabarcoding analyses may help elucidate additional species via detection of genetic material.
- Continued inclusion of concurrent survey methods is necessary to identify all species presence to the lowest taxonomic group, though sUAS shows promise in being able to identify more individuals and a greater number of lower taxonomic groups.
- Future surveys using sUAS for evaluation of aquatic turtles should continue to refine survey methods (especially in relation to survey speed and platform elevation), evaluate effectiveness of polarizing or other filters, and include imagery from additional spectral bands, such as multispectral and near-infrared
- Future surveys utilizing thermal imagery for detection of aquatic turtles should continue to evaluate impacts of behavioral and environmental factors to efficacy of imaging

Literature Cited

Barnes, M.A. and C.R. Turner. 2016. The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics* 17: 1-17.

Bevan, E. S. Whiting, T. Tucker, M. Guinea, A. Raith, and R. Douglas. 2018. Measuring behavioral responses of sea turtles, saltwater crocodiiles, and crested terns to drone disturbance to devine ethical operating thresholds. *PLoS ONE* 13(3): e0194460

Biserkov, V.Y. and Lukanov, S.P. 2017. Unmanned Aerial Vehicls (UAVs) for Surveying Freshwater Turtle Populations: Methodology Adjustment. *ACTA Zoologica Bulgarica* 10: 161-163.

Bonett, R.M., J. Boundy, F.T. Burbrink, B.I. Crother, K. de Quieroz, D.R. Frost, R. Highton, J.B. Iverson, E.L. Jockusch, F. Kraus, K.L. Krysko, A.D. Leache, E.M. Lemmon, R.W. McDiarmid, J.R. Mendelson III, P.A. Meylan, T.W. Reeder, S. Ruane, and M.E. Seidel. 2017. Scientific and standard English names of amphibians and reptiles of North America north of Mexico, with comments regarding confidence in our understanding: 8th edition. J.J. Moriarty (ed.). Society for the Study of Amphibians and Reptiles, Shoreview, Minnesota. 104 pp.

Boyd, C.E. 2019. Water quality: An introduction (2nd edition). Springer International, Heidelberg, Switzerland. 374 pp.

Buhlmann, K.A., Gibbons, J.W., Jackson, D.R., 2008. *Deirochelys reticularia* (Latreille 1801) - Chicken Turtle. *Chelonian Research Monographs* 5: 014.011-014.016.

Ceballos, C.P. and Fitzgerald, L.A. 2004. The trade in native and exotic turtles in Texas. *Wildlife Society Bulletin* 32: 881-892.

Chretien, L.-P., J. Theau, and P. Menard. 2016. Visible and thermal infrared remote sensing for the detection of white-tailed deer using an umanned aerial system. *Wildlife Society Bulletin* 40(1): 181-191.

Chyn, K., Lin, T.-E., Wilkinson, D.P., Tracy, J.L., Lawing, A.M., and Fitzgerald, L.A. 2020. Fine-scale roadkill risk models: Understanding the intersection of wildlife and roads. *Biodiversity and Conservation*. https://doi.org/10.1007/s10531-20-02083-6.

Corcoran, E., Denman, S., Hanger, J., Wilson, B., and Hamiton, G. 2019. Automated detection of koalas using low-level aerial surveillance and machine learning. *Nature*. 9(3208): 1-9.

Corcoran, E.M. 2021. Monitoring and modeling vulnerable wildlife populations using remotely piloted aircraft systems and machine learning. Queensland University of Technology, Brisbane City, Queensland, Australia, Ph.D. Dissertation. 208 pp.

Dunn, O.J. 1964. Multiple comparisons using rank sums. *Technometrics* 6(3): 241-252.

Ernst., C.E. and Lovich, J.E. 2009. Turtles of the United States and Canada. John Hopkins University Press, Baltimore, Maryland, USA. 827 pp.

Gibbons, J.W., Scott, D.E., Ryan, T.J., Buhlmann, K.A., Tuberville, T.D., Metts, B.S., Greene, J.L., Mills, T., Leiden, Y., Poppy, S., Winne, C.T. 2000. The Global Decline of Reptiles: Déjà Vu Amphibians. *BioScience* 50: 653-666.

Giles, A.B., P.A. Butcher, A.P. Colefax, D.E. Pagendam, M. Mayjor, and B.P. Kelaher. 2019. Responses of bottlenose dolphins (*Tursiops* spp.) to small drones. *Marine and Freshwater Ecosystems* 31: 677-684.

Gordon, M.L., J.W. Oakley, J. Nagro, D. DeChellis, J. Welch, J.J. Apodaca, and G.J. Guillen. 2020. Distribution and habitat association of western chicken turtles in Texas, Interim Report FY20-21.

(Report No. EIH20-003). Prepared for the Texas Comptroller of Public Accoutns (Contract 20-6997BG). 31 pp.

Gordon, M.L., J. Nagro, D. DeChellis, J. Oakley, J.J. Apodaca, L. Speight, and G. Guillen. 2021. Distribution and habitat association of western chicken turtles in Texas, Interim Report FY21-22. (Report No. EIH21-004). Prepared for the Texas Comptroller of Public Accoutns (Contract 20-6997BG). 45 pp.

Greenwald, D.N., Curry, T., Alabama Rivers Alliance, Clinch Coalition, Dogwood Alliance, Gulf Restoration Network, Tennessee Forests Council, and West Virginia Highlands Conservancy. 2010. Petition to list 404 aquatic, riparian and wetland species from the southeastern United States as threatened or endangered under the Endangered Species Act. Center for Biological Diversity, Portland, OR. 1145 pp.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* 6(2): 65-70

Holcomb, S.R., Bass, A.A., Reid, C.S., Seymour, M.A., Lorenz, N.F., Gregory, B.B., Javed, S.M., Balkum, K.F. 2015. Louisiana Wildlife Action Plan. *In*: Fisheries, L.D.o.W.a. (Ed.), Baton Rouge, Louisiana, p. 705 pp.

Lhoest, S., J. Linchant, S. Quevauvillers, C. Vermeulen, and P. Lejeune. 2015. How many hippos (HOMHIP): Algorithm for automatic counts of animals with infra-red thermal imagery from UAV. *The Interational Archives of Photogrammetry, Remote Sensing and Spatial Information Sciences* XL-3/W3: 355-362.

Liu, C., Y. Chen, and H. Wen. Supporting the annual international black-faced spoonbill census with a low-cost unmanned aerial vehicle. *Ecological Informatics* 30: 170-178.

Longmore, S.N., R.P. Collins, S. Pfeifer, S.E. Fox, M. Mulero-Pazmany, F. Bezombes, A. Goodwin, M. de Juan Ovelar, J.H. Knapen, and S.A. Wich. Adapting astronomical source detection software to help detect animals in thermal images obtained by unmanned aerial systems. *International Journal of Remote Sensing* 38: 2623-2638.

Martin, B.T., N.P. Bernstein, R.D. Birkhead, J.F. Kouki, S.M. Mussmann, and J.S. Placyk Jr. 2013. Sequence-based molecular phylogenetics and phylogeography of the American box turtles (Terrapene spp.) with support from DNA barcoding. *Molecular Phylogenetics and Evolution* 68: 119-134.

McClenaghan, B., Z.G. Compson, and M. Hajibabaei. 2020. Validating metabarcoding-based biodiversity assessments with multi-species occupancy models: A case study using coastal marine eDNA. PLoS ONE 15(3): e0224119.

McColl-Gausden, E.F., A.R. Weeks, R.A. Coleman, K.L. Robinson, S. Song, T.A. Raadik, and R. Tingley. 2020. Multispecies models reveal that eDNA metabarcoding is more sensitive than backpack electrofishing for conducting fish surveys in freshwater streams. *Molecular Ecology* 30: 3111-3126.

Mills, K.E. and Stevenson, N.J. 1999. Riparian vegetation. In: Bain, M.B. and Stevenson, N.J., eds. Aquatic habitat assessment: common methods. American Fisheries Society, Bethesda, Maryland. Pgs. 125-133.

Oklahoma Department of Wildlife Conservation (ODWC), 2016. Okalhoma Comprehensive Wildlife Conservation Strategy: A Strategic Consevation Plan for Oklahoma's Rare and Declining Wildlife, in: Conservation. ODWC, Oklahoma City, OK. 422 pp.

Oishi, Y., H. Oguma, A. Tamura, R. Nakamura, and T. Matsunaga. 2018. Animal detection using thermal images and its required observation conditions. *Remote Sensing* 10: 1050. doi: 10.3390/rs10071050.

Prestridge, H.L., Fitzgerald, L.A., Hibbitts, T.J. 2011. Trade in Non-Native Amphibians and Reptiles in Texas: Lessons for Better Monitoring and Implications for Species Introduction. *Herpetological Conservation and Biology* 6: 324-339.

Quesnelle, P.E., Lindsay, K.E., Fahrig, L. 2015. Relative effects of landscape-scale wetland amount and landscape matrix quality on wetland vertebrates: a meta-analysis. *Ecological Applications* 25: 812-825.

Rees, A.F., Avens, L., Ballorain, K., Bevan, E., Broderick, A.C., Carthy, R.R., Christianen, M.J.A., Duclos, G., Heithaus, M.R., Johnston, D.W., Mangel, J.C., Paladino, F., Pendoley, K., Reina, R.D., Robinson, N.J., Ryan, R., Sykora-Bodie, S.T., Tilley, D., Varela, M.R., Whitman, E.R., Whittock, P.A., Wibbels, T., Godley, B.J. 2018. The potential of unmanned aerial systems for sea turtle research and conservation: a review and future directions. *Endangered Species Research* 35: 81-100.

Raoult, V., A.P. Colefax, B.M. Allan, D. Cagnazzi, N. Castelblanco-Martinez, D. Ierodiaconou, D.W. Johnston, S. Landeo-Yauri, M. Lyons, V. Pirotta, G. Schofield, and P.A. Butcher. 2020. Operational protocols for the use of drones in marine animal research. *Drones* 4: 64. doi: 10.3390/drones4040064

Ryberg, W., Wolaver, B., Prestridge, H., Labay, B., Pierre, J., Costley, R., S, A., Bowers, B., Hibbitts, T. 2017. Habitat Modeling and Conservation of the Western Chicken Turtle (*Deirochelys reticularia miaria*). *Herpetological Conservation and Biology* 12(2): 307-320.

Schofield, G., K.A. Katselidis, M.K.S. Lilley, R.D. Reina and G.C. Hays. 2019. Detecting elusive aspects of wildlife ecology using drones: new insights on the mating dynamics and operation sex ratios of sea turtles. *Functional Ecology* 31(12): 2310-2319.

Semlitsch, R.D. and Bodie, J.R. 2003. Biological Criteria for Buffer Zones around Wetlands and Riparian Habitats for Amphibians and Reptiles. *Conservation Biology* 17: 1219-1228.

Seymour, A.C., J. Dale, M. Hammill, P.N. Halpin, and D.W. Johnston. 2017. Automated detection and enumeration of marine wildlife using unmanned aircraft systems (UAS) and thermal imagery. *Nature Scientific Reports* 7: 45127. doi: 10.0138/srep45127.

Seymour, M., I. Durance, B.J. Cosby, E. Ransom-Jones, K. Deiner, S.J. Ormerod, J.K. Colbourne, G. Wilgar, G.R. Carvalho, M. de Bruyn, F. Edwards, B.A. Emmett, H.M. Bik, and S. Creer. 2018. Acidity promotes degradation of multi-species environmental DNA in lotic mesocosms. *Communications Biology* 1(4).

Siler, C.D, Freitas, E.S., Yuri, T., Souza, L, and Watters, J.L. 2020. Development and validation of four environmental DNA assays for species of conservation concern in the south-central United States. *Conservation Genetics Resources*. https://doi.org/10.1007/s12686-020-01167-3.

Stewart, K.A. 2019. Understanding the effects of biotic and abiotic factors on sources of aquatic environmental DNA. *Biodiversity and Conservation* 28: 1-19.

Texas Commission on Environmental Quality (TCEQ). 2012. Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods. TCEQ RG-415, Austin, TX. 202 pp.

Texas Turtles. 2021. Texas Turtles. Online resource: https://www.texasturtles.org/. Accessed 22 December 2021.

U.S. Fish and Wildlife Service (USFWS). 2011. Endangered and Threatened Wildlife and Plants; Partial 90-Day Finding on a Petition to List 404 Species in the Southeastern United States as Endangered or Threatened with Critical Habitat. pp. 59836-59862

Wang, S., Z. Yan, B. Hanfling, X. Zheng, P. Wang, J. Fan, and J. Li. 2021. Methodology of fish eDNA and its applications in ecology and environment. *Science of the Total Environment* 755: 142622.

Witczuk, J., S. Pagacz, A. Zmarz, and M. Cypel. 2017. Exploring the feasibility of unmanned aerial vehicles and thermal imaging for ungulate surveys in forests – preliminary results. *International Journal of Remote Sensing* 39: 5504-5521.

Appendix A – Examples of non-Aquatic Turtle Species Observations

Appendix Figure A.1 Examples of other species observed during small unmanned aerial systems (sUAS) surveys. Also visible are different vegetation and habitat types (algae in top left, lily pads in top right, emergent vegetation in bottom right).

Appendix B – List of Abbreviations

BAVS	Binocular assisted visual surveys
CN	Cellulose nitrate
eDNA	Environmental DNA
EIH	Environmental Institute of Houston
ESA	Endangered Species Act
F	Test value returned as part of the One- and Two-Way ANOVA
FAA	Federal Aviation Administration
GPS	Global Positioning System
H	Test value returned as part of the Kruskal-Wallis One-Way ANOVA on Ranks
NTU	Nephelometric Turbidity Units
PCR	Polymerase chain reaction
TBC	Tangled Bank Conservation, Inc.
TPWD	Texas Parks and Wildlife Department
R^2	Statistical value returned in regressions
SE	Standard error
SRA	Sabine River Authority of Texas
sUAS	Small unmanned aerial system
UHCL	University of Houston-Clear Lake
USFWS	United States Fish and Wildlife Service
WCT	Western Chicken Turtle